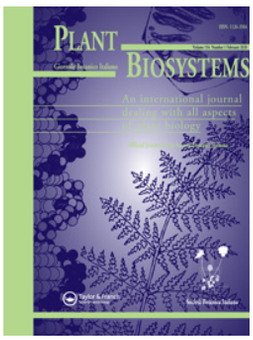

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Morphological and molecular characterization of sweet, grain and forage sorghum (*Sorghum bicolor* L.) genotypes grown under temperate climatic conditions

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ABSTRACT

In the present study, we used 12 genotypes of sorghum originated from different countries (five sweet, four grain and three forage). These different genotypes and types of sorghum were evaluated for the agro-morphological traits that are associated with the estimated sugar and bioethanol yield to estimate their phenotypic diversity. Analysis of variance showed significant differences between different types of sorghum for all the evaluated traits. Sweet sorghum genotypes, however, showed better performance with respect to all studied traits than the other genotypes. A positive significant correlation was observed between plant height, leaf number, leaf area, biomass yield, cane and bagasse yields, and the predicted bioethanol yield. Both, cluster and principal component analysis were performed to group the genotypes according to their agro-morphological and molecular similarity coefficients. For analytical approaches, the Iranian grain and forage genotypes clustered separately from the other genotypes. The clustering patterns obtained from the molecular dominant markers had higher discriminatory power than using morphological characters to separate sweet genotypes from the forage and grain sorghum ones. The results clearly indicated that sweet sorghum can be grown in Germany and maintains its superiority in biomass production and sugar yield over grain and forage sorghum types.

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Sorghum bicolor; genetic diversity; biomass; sugar; brix degree; ethanol

Introduction

Sorghum (*Sorghum bicolor* L.) is the fifth most important cereal crop in the world due to its multi-purpose economically important yields such as food, fodder, bio-fuel and other industrial uses (Tesfaye 2017). The C4 photosynthesis aspect of sorghum gives this plant a high efficiency in terms of biomass production in a relatively short time per generation and superior performance in arid and semi-arid areas (Ramu et al. 2009; Maiti and Satya 2014; Irving 2015). The multi-purpose yield of sorghum divides it into different categories: grain, forage and sweet sorghum, in which a wide range of genotypic and phenotypic diversity is found (Kong et al. 2000). However, different types of sorghum can be distinguished based on their stem-related yield and the grain quantity and quality (Vietor and Miller 1990).

Sweet sorghum has thicker stems used as a primary sink tissue for the synthesized sugar during flowering before the being translocated to seeds during seed formation (Rao et al. 2009), which makes the stem tissues a valuable source of easily fermented sugars. Thus, the Environmental Protection Agency (EPA) has approved sweet sorghum as an advanced bioenergy feedstock, as it is highly tolerant to drought, requires lower fertilizer inputs than corn, and has lower greenhouse gas emissions on a life-cycle basis (Ben-Iwo et al. 2016). In temperate areas (e.g. Germany), sweet sorghum has been considered as source of raw material for 2nd generation of bioethanol and lignocellulosic feedstock. In spite of these impressive advantages, the genetic base for breeding sorghum lines adapted to temperate climate, has remained narrow (Windpassinger et al. 2015), and only looks back on a short breeding history (Braconnier et al. 2011). Consequently, it is necessary to obtain information about the potential of this type of sorghum in Germany.

To effectively devise sorghum breeding programs in Germany, information on adaptation to low temperature environments, on genetic diversity, and on genetic relationships between sorghum accessions is essential to allow selection of parents with desirable traits. Sorghum is amenable to different molecular breeding approaches that can be used to support and consolidate any breeding scheme (Govindaraj et al. 2015). Although agro-morphological traits can be studied and quantified to assess the potential genetic diversity (Rohman et al. 2004; Grenier et al. 2004; Ritter et al.

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Table 1. List of sorghum genotypes used in the study along with their name, place of collection, pedigree and some of the most important characters.

Sr. No.	Genotype	Origin	Pedigree	Characters
1	ICSV25274	India	DSV4 X SSV84	Sugar type, tolerant to downy mildew
2	ICSSH25	India	ICSA675 X ICSV574	Sugar type, early maturity
3	SSV84	India	SSV84	Sugar type, tolerance to shoot fly, stem borer and leaf disease
4	ICSV574	India	DSV4 X SSV84	Sugar type, high sugar yield and Brix
5	ICSSH30-11-ADP	India	Elite line derived from ICSSH30 hybrid (ICSA724 X SSV74)	Sugar type, late maturity, tall, high sugar
6	Razinieh	Syria	Landrace improved by bulk method	Grain type, early maturity, for fodder and fiber
7	Payam	Iran	Landrace improved by pedigree method	Grain type, early maturity, tolerance to lodging, dwarf
8	Kimia	Iran	FGS X LGS9	Grain type, medium maturity, dwarf, tolerance to lodging, for fodder
9	Sepideh	Iran	FGS X LGS20	Grain type, medium maturity, dwarf, tolerance to lodging, for fodder and human feeding
10	Speed-feed	Australia	Grain sorghum X Sudan grass	Forage type, early maturity, for fiber and fodder
11	Pegah	Iran	Early orange X LFS56	Forage type, late maturity, tall, tolerance to lodging, for fodder and fiber, high sugar
12	KFS2	Iran	As9 X LFS	Forage type, tall, medium maturity

2007; Barro et al. 2010), variation of environmental conditions, and the late expression of particular traits are considerable drawbacks of this approach (Smith and Smith 1992). Molecular markers combined with agro-morphological trait analyses under environmental conditions of the target region is recommended as strategy to maximize the efficiency of sorghum breeding programs (Sunil et al. 2011). DNA markers like Random Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeats (ISSR) can be efficiently used to detect genetic diversity and its correlations with agro-morphological traits of interest. Both types of dominant markers are easily applied, relatively cheap and do not require previous knowledge about the genome sequence (Izzatullayeva et al. 2014). Moreover, both marker types have been successfully used in different sorghum studies (Alhajturki et al. 2011; Aruna et al. 2012; Tadesse and Feyissa 2013).

The objectives of this study were (i) to examine the performance of sweet, grain and forage sorghum genotypes under temperate climatic conditions of Germany, (ii) to estimate the genetic diversity of these cultivars based on molecular markers and agro-morphological parameters related to biofuel productio, and (iii) to explore new sources of genetic diversity coming from ancient landraces originating in Syria and Iran.

Materials and methods

Plant materials

Twelve *S. bicolor* genotypes were used in this study. These genotypes were chosen and grouped according to their use in agricultural practice: Sweet sorghum (ICSV25274, ICSSH25, SSV84, ICSV574 and ICSSH30-11-ADP), grain sorghum (Payam, Kimia, Sepideh and Razinieh) and forage sorghum (Pegah, KFS2 and Speed-feed) (Table 1). All sweet sorghum genotypes originated from India. ICSSH30-11-ADP is an elite F_7 line developed by pedigree selection from the F_2 generation of ICSSH30 hybrid. The genotype Razinieh is a Syrian landrace that was improved by bulk breeding method to enhance its biomass and grain productivities (Alhajturki et al. 2011, 2012). While ICSSH22 is a hybrid cultivar. The most grain and forage sorghum genotypes were generated in Iran. The forage sorghum genotype Speed-feed was developed in Australia by crossing grain sorghum and Sudan grass (*Sorghum* × *drummondii*).

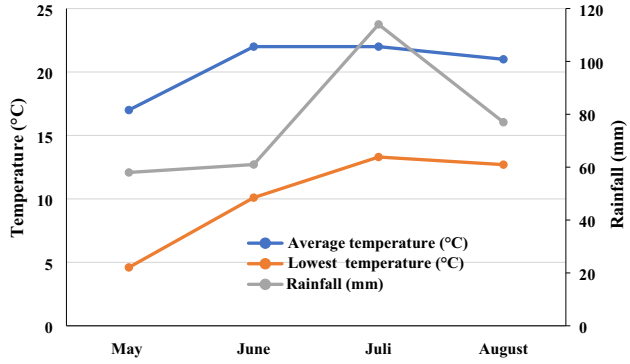

Figure 1. Temperature and rainfall measured during the summer season 2017 (May–August) in Karlsruhe, Germany.

Evaluation of phenotypic diversity

For agro-morphological characterization, seedlings were planted in an experimental field at the Botanical garden, Karlsruhe Institute of Technology, Karlsruhe, Germany in summer (May–August) 2017. The city of Karlsruhe is located in the Rhine Valley, Southeast of Germany (latitudes: 49°0′N and 13N, longitude: 8°22′48.00″E), with an average elevation of 119 m above sea level. The climate is temperate oceanic with temperatures ranging from around −1 °C in winter to 26 °C during summer. Temperature and rainfall were measured for the duration of the experiment, and monthly average values are presented in Figure 1. For the experiment, we used a randomized complete block design (RCBD) with three-independent replications and five samples per replicate. Seedlings were planted on 15 May in plots of 2.5 × 2 m length with a plant density of 20 plants/m^2. The plants were rainfed with three time-point supplementary irrigation: at sowing time, after germination of the seeds, and at 10 days seedling age. Sorghum plants were harvested at dough stage (the seeds are soft and immature but fully formed), as recommended by previous studies (Undersander et al. 1990). This stage provides the optimum concentration of stored sugar in the stem sink tissues.

During the month of August, five random plants located in the center of a plot were harvested from each replicate,

recording green leaf area (cm^2), plant height (cm), leaf number, fresh biomass yield (t/ha), cane yield (t/ha), bagasse yield (t/ha), brix degree and juice yield (kl/ha). In order to quantify the juice yield, and to measure the brix degree of the juice, we used a conventional cane crusher to crush the canes. Stem sugar percentage was estimated using brix degree and a regression equation developed in the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) (Reddy et al. 2005). Brix degree was recorded with a hand-held refractometer (Model PAL, Atago Co. Ltd., Tokyo, Japan) for each individual cane. The theoretical ethanol yield was estimated as 40 l per ton of cane yield (Dayakar et al. 2004). Sugar yield (t/ha) and percentage was estimated using formulas of Reddy et al. (2005):

$$\text{Sugar \%} = (\text{Brix}^{\circ} \text{ at dough stage} \times 0.8746) + 0.1516$$

$$\text{Sugar yield (t/h)} = (\text{Sugar \%}/100) \times \text{Juice yield (kl/ha)}$$

The variance of each trait was analyzed for all genotypes (ANOVA). The standard error was calculated using PROC ANOVA in SAS (SAS Institute Inc. 1996). The coefficient of variation (CV %) and the least significant difference (LSD) were calculated to assess the stability of each genotype in the given environment. To estimate the degree of linear association between the studied traits, the simple correlation coefficient (R^2) was computed by using the standard formula of Pearson (1895). The significance of correlation coefficients was tested at n-2 degrees of freedom on a't' table from Fisher and Yates at 5% and 1% significance levels.

Data values were standardized using Minitab 17 (Minitab Ltd., Coventry, UK), and Euclidean distance matrix was generated according to Sneath and Sokal (1973) with STATISTICA (StatSoft, Inc. 2003). General agglomerative hierarchical clustering was conducted with Ward's minimum variance method and subsequently used to plot a dendrogram. The principal component analysis (PCA) was applied to plot the relationship between distance matrix elements with respect to their first two principal components, using Minitab 17.

Evaluation of genotypic diversity

Extraction of genomic DNA

Fresh leaf tissue was used to extract DNA from three plants of each genotype using the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. DNA quantity and quality was determined photometrically (Nano Drop ND-100m peqlab) and visually by agarose gel electrophoresis (1% agarose gel with 5% v/v SYBR safe dye).

RAPD amplification

Twenty-four RAPD primers were screened to determine which can be used to detect reproducible polymorphisms (Table 2). PCR was carried out in a reaction volume of 10 µl containing 50 ng of genomic DNA, reaction buffer (Thermopol, NEB) including $MgSO_4$ (2 mM), 200 µM dNTPs (NEB), 200 nM of primer and 0.1 units Taq DNA polymerase (NEB). The standard RAPD cycling profile (Williams et al. 1990) was used: 45 cycles of initial denaturation at 94 °C for 1 min, annealing at 36 °C for 1 min and extension at 68 °C for 2 min. A final extension step of 5 min at 68 °C was added. For the separation and visualization of RAPD populations, a 1% agarose gel (see previous section) was used. Additionally, we used a 100-bp DNA ladder (NEB) to estimate the size of DNA fragments.

Table 2. List of RAPD and ISSR primers used in the current study.

Primer name	Sequence (5′–3′)	NAF	NPF	PPB (%)
GLJ-09	TGAGCCTCAC	11	8	72.73
PRKAT-09	CCGTTAGCGT	7	5	71.43
A7	GAAACGGGTGA	8	5	62.50
PKAT-06	CCGTCCCTGA	12	8	66.67
OPK-7	AGCGAGCAAG	3	1	33.33
PKAT-12	CTGCCTA GCC	7	2	28.57
GLA-18	AGGTGACCGT	7	2	28.57
GLC-8	TGGACCGGTG	10	4	40.00
GLC-20	ACTTCGCCAC	15	11	73.33
OPK-9(a)	CCCTACCGACA	12	8	66.67
PKAT-17	AGGGACTGCT	9	4	44.44
PKAT-2	CAGGTCTAGG	9	5	55.56
OPJ-06	TCGTTCCGCA	14	9	64.29
OPAM3	CTTCCCTGTG	13	12	92.31
OPC7	GTCCCGACG	14	10	71.43
OPAM2	ACTTGACGGG	14	10	71.43
OPAM6	CTCGGGATGT	6	4	66.67
OPC10	TGTCTGGGTG	10	7	70.00
OPAC	TCGGCCGAAT	7	2	28.57
OPC13	AAGCCTCGCT	8	6	75.00
OPC3	GGGGGTCTTT	8	5	62.50
OPDB	GCCTCTATCT	8	6	75.00
ISSR1	G(AG)8AT	13	11	84.62
ISSR2	(AC)8T	15	14	93.33
ISSR3	(CTC)5	11	5	45.45
ISSR4	(AG)8A	8	2	25.00
ISSR5	(AG)7ACC	11	7	63.64
ISSR6	(CAG)5C	19	14	73.68
ISSR7	(CAG)5G	10	1	10.00
ISSR8	G(CA)8	9	3	33.33

Note. Primer name, sequence, total number of amplified fragments (NAF), total number polymorphic fragments (NPF) and percentage of polymorphic bands (PPB).

ISSR amplification

Eight ISSR primers were screened (Table 2). PCRs were prepared as described under RAPD amplification. The ISSR cycling profile (Zietkiewicz et al. 1994; **Alhajturki** et al. 2011) was as follows: initial denaturation of 12 min at 94 °C, followed by 35 cycles of 94 °C for 30 s, annealing at 48 °C (primer 1, 2, 4 and 5) and 56 °C (primer 3, 6, 7 and 8) for 30 s, respectively, extension at 68 °C for 1 min and a final extension at 68 °C for 12 min. ISSR DNA populations were separated by agarose gel electrophoresis (1.5% agarose gel) and their size estimated using a 100-bp DNA ladder. Individual bands obtained from ISSR and RAPD were scored as absent (0) or present (1), respectively, and treated as independent characters. These data were used for a cluster analysis as described earlier with the exception that data were not standardized prior to generation of the distance matrix. The Mantel test (Mantel 1967) was performed, using the ede4 package in R (Chessel et al. 2004) software, to test the significance of the correlation between the morphological and the molecular distance matrices, considering ten thousand random permutations and a 5% significance level.

Table 3. Mean values of agro-morphological traits recorded from 12 sweet, grain and forage sorghum genotypes, their grouped overall mean values, least significant difference (LSD at $p < .05$) and coefficient of variation (CV) at dough stage under temperate climate climatic conditions.

Genotype	PHT (cm)	LN	GLA (cm^2)	FBY (t/ha)	CY (t/ha)	BY (t/ha)	B° (%)	JY (kl/ha)	SY (t/ha)	EY (kl/ha)
ICSSH30-11-ADP	294.7 b(b)	10.0 a(a)	2664.0 c(c)	77.3 a(a)	64.4 a(a)	23.1 b(b)	15.0 a(a)	33.2 a(a)	4.4 a(a)	2.6 a(a)
ICSSH25	342.0 a(a)	9.7 ab(a)	3711.7 b(b)	59.7 ab(ab)	43.8 b(bc)	17.3 c(c)	9.3 d(b)	19.8 c(ab)	1.7 b(b)	1.7 c(bc)
ICSV25274	281.0 c(b)	10.3 a(a)	3866.2 b(b)	69.6 a(a)	54.1 b(b)	29.9 a(a)	8.4 e(bc)	19.4 c(ab)	1.5 bc(bc)	2.2 b(b)
ICSV574	257.3 d(c)	9.3 b(a)	5062.6 a(a)	68.3 a(a)	52.4 b(b)	22.8 b(b)	9.6 c(b)	22.3 b(ab)	1.9 b(b)	2.1 b(b)
SSV84	254.7 d(c)	10.3 a(a)	2659.2 c(c)	71.6 a(a)	54.0 b(b)	24.4 b(b)	10.6 b(b)	18.5 c(ab)	1.8 b(b)	2.1 b(b)
Sweet	**285.9**	**9.9**	**3592.7**	**69.3**	**53.7**	**23.5**	**10.5**	**22.6**	**2.2**	**2.1**
LSD (0.05)	5.73	0.97	200.56	10.77	9.39	3.06	0.28	2.41	0.36	0.37
CV (%)	1.06	5.19	2.96	8.25	9.29	6.93	1.42	5.66	8.72	9.79
Razinieh	283.3 a(b)	9.7 a(a)	2691.2 a(c)	57.1 a(ab)	46.9 a(b)	22.9 a(b)	7.8 a(bc)	19.5 a(ab)	1.4 a(bc)	1.9 a(b)
Payam	148.0 b(d)	6.0 b(b)	1376.3 b(d)	40.0 b(c)	33.0 b(d)	18.4 a(bc)	7.0 a(cd)	15.4 b(bc)	1.01 a(bc)	1.3 b(d)
Sepideh	137.3 b(d)	5.0 b(b)	1347.3 b(d)	35.5 b(c)	28.9 b(d)	12.5 ab(d)	9.0 a(bc)	13.5 b(bc)	1.1 a(bc)	1.2 b(d)
Kimia	142.7 b(d)	5.0 b(b)	1268.2 b(d)	33.4 b(c)	27.4 b(d)	12.4 ab(d)	8.6 a(bc)	14.1 b(bc)	1.06 a(bc)	1.1 b(d)
Grain	**177.8**	**6.4**	**1670.7**	**41.5**	**34.1**	**16.6**	**8.1**	**15.6**	**1.14**	**1.4**
LSD (0.05)	32.47	1.91	552.89	13.04	10.24	6.43	2.00	2.73	0.41	0.40
CV (%)	9.14	14.92	16.56	15.73	15.05	19.48	12.36	14.34	20.10	15.05
KFS2	237.0 b(c)	9.0 a(a)	2350.8 b(c)	52.5 a(bc)	43.8 a(bc)	21.1 a(b)	10.6 a(b)	18.3 a(ab)	1.7 a(b)	1.7 a(bc)
Speed-feed	297.3 a(b)	9.0 a(a)	2913.6 a(c)	55.2 a(ab)	47.3 a(b)	18.5 ab(bc)	8.4 b(bc)	25.2 a(a)	1.9 a(b)	1.9 a(b)
Pegah	263.0 b(c)	9.0 a(a)	2970.6 a(c)	63.6 a(ab)	49.8 a(b)	18.7 a(bc)	9.1 b(bc)	25.3 a(a)	2.3 a(b)	2.0 a(b)
Forage	**265.8**	**9.0**	**2745.0**	**57.1**	**47.0**	**19.4**	**9.4**	**22.9**	**2.0**	**1.9**
LSD (0.05)	26.49	1.30	423.42	12.13	10.59	2.49	0.97	8.98	0.68	0.42
CV (%)	4.39	6.41	6.80	9.37	9.94	5.66	4.57	15.85	14.69	9.94
Overall	**244.9**	**8.5**	**2740.1**	**56.9**	**45.5**	**20.17**	**9.4**	**20.4**	**1.8**	**1.8**
LSD (0.05)	17.90	1.30	507.60	11.40	8.80	3.70	1.40	7.00	0.70	0.30
CV (%)	4.30	9.10	10.90	11.80	11.50	10.80	9.20	19.60	22.91	11.50

Note. Plant height (PHT), leaf number (LN), green leaf area (GLA), fresh biomass yield (FBY), cane yield (CY), bagasse yield (BY), brix degree (B°), juice yield (JY), sugar yield (SY) and theoretical ethanol yield (EY). The letters in the parentheses are overall mean comparison. Their grouped and overall mean values (bold).

Results and discussion

Sorghum (*S. bicolor* L. Moench) is a multiple-purpose crop used as source for food and fodder. Additionally, due to its easily fermented sugar, it is a valuable source for bioethanol and lignocellulosic feedstock production (Cifuentes et al. 2014). This study provides details of agro-morphological and molecular variability and functional correlations among 12 sweet, grain and forage sorghum genotypes collected from different countries and screened under temperate climatic conditions.

Agro-morphological descriptive analysis

The analysis of variance indicated a significant ($p < .05$) variation in all studied traits with considerable ranges in plant height (137.3–342.0 cm), leaf number (5.0–10.3), green leaf area (1268.2–5062.6 cm^2), fresh biomass yield (33.4–77.3 t/ha), cane yield (27.4–64.4 t/ha), bagasse yield (12.4–29.9 t/ha), brix degree (7–15) and juice yield (13.5–33.2 kl/ha) (Table 3). With the exception of sugar yield (22.9%), the coefficient of variation was between low and moderate, ranging from 4.3% to 19.6%. The classification was based on Burton and DeVane (1953). Furthermore, variance within each genotype was insignificant, indicating stability within replications. The overall morphological variation is thought to be based on genetic differences; thus, provides a valuable source for crop improvement and breeding (Moose and Mumm 2008).

Among the studied genotypes, it is worth noting that sweet sorghum genotype ICSSH30-11-ADP produced the highest fresh biomass yield, cane yield, brix degree, juice yield and predicted ethanol yield. Very similar results were obtained with the ICSSH30 hybrid, from which ICSSH30-11-ADP was derived (Alhajturki et al. 2012), where under the semi-arid environmental conditions of Syria, ICSSH30 produced the tallest cane and excelled also with respect to juice, sugar and ethanol yield both, under well-watered and low-moisture stress conditions. Rao et al. (2009) compared sugar and grain yield of different genotypes including ICSSH30 hybrid under humid conditions. The genotype ICSSH30 was also superior to other evaluated genotypes in rainy seasons in terms of sugar and grain yield. Although that sweet sorghum varieties are known to be superior than other sorghum types in terms of stem yield (~50 t/ha) with 22% average of brix reading in different geographical regions (Reddy et al. 2005; Alhajturki et al. 2011). Almodares and Mostafafi (2006) found that reduction of temperature and fluctuations of photoperiod can negatively affect the quantity and quality of stem yield and juice quality. However, under the specific conditions of Germany, how the low temperature during night hours can affect the accumulation of sugar in the sink (stem) has to be addressed in future studies.

Excluding the Syrian landrace Razinieh, which displayed morphological traits more similar to sweet and forage than to grain sorghum genotypes, variation within the agronomic group of grain sorghum was low. Differences between sweet and grain sorghum, as well as between forage and grain sorghum genotypes, were notable. The grain sorghum variety Kimia recorded the lowest leaf number, green leaf area, fresh biomass yield, cane yield, bagasse yield and predicted ethanol yield (1.1 l/ha). Significantly lower brix degree and sugar yield were recorded in the grain sorghum genotype Payam. The Syrian landrace Razinieh showed better performance with respect to growth, fresh biomass yield, sugar yield and ethanol productivity under temperate condition compared to semi-arid condition (Alhajturki et al. 2012). This genotype had been cultivated in Syria for a long time to help alleviate feed and food shortage during water shortage seasons and later it has undergone to a bulk breeding program to

improve the grain yield with maintaining its drought tolerance capability (Alhajturki et al. 2011).

For the genotypes of forage sorghum (Pegah, KFS2 and Speed-feed), the variety Pegah produced the highest fresh biomass yield (63.6 t/ha), cane yield (49.8 t/ha), juice yield (25.3 t/ha) and leaf area (2970.6 cm^2) compared to the other two forage varieties, KFS2 and Speed-feed. Shakeri et al. (2017) found that Pegah, speed-feed and KFS2 produced higher shoot dry weight under saline and normal conditions when compared with more than 40 grain and forage bred lines released in Iran. In this study, the mean performance of forage genotypes was better than the grain genotypes and lower than the sweet genotypes for all the traits except juice yield.

By comparing the total average of sweet and grain sorghum genotypes, sweet differ phenotypically from grain sorghum genotypes by having a taller, sugar-rich juicy stem, and by producing higher fresh biomass. Interestingly, this performance was also found during evaluation trials at the ICRISAT, where those sweet sorghum genotypes generally had higher stem yield compared to grain yield, even in temperate regions (Kumar et al. 2010; Rao and Kumar 2013; Rao et al. 2009). These findings complement each other and show that sweet sorghum genotypes are not just genetically different from grain sorghum genotypes but also stable across different conditions in terms of yield production.

Correlations between agro-morphological traits

All calculated correlation coefficients are provided in Table 4. Significant positive associations with juice yield were recorded for the traits plant height ($r = 0.61^{*}$), fresh biomass yield ($r = 0.69^{*}$), cane yield ($r = 0.76^{**}$) and brix degree ($r = 0.57^{*}$). Different studies reported that cane yield positively correlates with higher biomass yield and cane-related traits (thickness and height) (Bakheit 1990; Donatelli et al. 1992; Almodares et al. 2007; Alhajturki et al. 2012). In addition, it was shown that sweet sorghum has developed different sugar yield-supporting mechanisms, by using the tall and thick stem as a major sink before grain filling stage (Bakheit 1990; Donatelli et al. 1992; Almodares et al. 2008).

The predicted ethanol yield showed significant positive correlation with biomass-related agro-morphological parameters viz., plant height ($r = 0.77^{**}$), leaf number ($r = 0.90^{**}$), leaf area ($r = 0.66^{*}$), fresh biomass yield ($r = 0.98^{**}$), bagasse yield ($r = 0.79^{**}$), brix degree ($r = 0.61^{*}$), juice ($r = 0.76^{**}$) and sugar yield ($r = 0.97^{**}$). This shows an expected positive correlation between source (leaf area) and sink tissues (stem biomass) since leaves are the primer source of sugar and stem tissues are the primary sink before they get translocated to the seeds (Ekefre et al. 2017).

Diversity of agro-morphological traits

Phenotypic traits are commonly used in assessment of genetic diversity since they provide a simple way of quantifying genetic variations that are of interest to plant breeding programs (Beuningen and Busch 1997). Data recorded on all 10 quantitative traits were used for clustering by Ward's minimum variance method. In the resulting dendrogram, the investigated sorghum genotypes are separated into two main clusters (Figure 2a).

Cluster I comprises three Iranian grain genotypes (Payam, Sepideh and Kimia) which recorded low in brix degree, fresh biomass and juice yield. The presence of these Iranian grain sorghum genotype in the same group indicates a possible common origin. Similar conclusions were drawn by Shakeri et al. (2017).

Cluster II is composed of all sweet and forage sorghum genotypes and the Syrian landrace Razinieh. Additionally,

Table 4. Phenotypic correlation coefficient of different agro-morphological traits obtained from sorghum genotypes.

Trait	PHT	LN	GLA	FBY	CY	BY	B°	JY	SY
PHT	1.00								
LN	0.90^{**}	1.00							
GLA	0.73^{**}	0.73^{**}	1.00						
FBY	0.79^{**}	0.92^{**}	0.73^{**}	1.00					
CY	0.77^{**}	0.90^{**}	0.66^{*}	0.98^{**}	1.00				
BY	0.56^{ns}	0.81^{**}	0.60^{*}	0.80^{**}	0.79^{**}	1.00			
B°	0.31^{ns}	0.37^{ns}	0.12^{ns}	0.56^{ns}	0.61^{*}	0.22^{ns}	1.00		
JY	0.61^{*}	0.57^{*}	0.41^{ns}	0.69^{*}	0.76^{**}	0.31^{ns}	0.57^{*}	1.00	
SY	0.50^{ns}	0.50^{ns}	0.26^{ns}	0.68^{*}	0.76^{**}	0.29^{ns}	0.86^{**}	0.89^{**}	1.00
EY	0.77^{**}	0.90^{**}	0.66^{*}	0.98^{**}	1.00^{**}	0.79^{**}	0.61^{*}	0.76^{**}	0.97^{**}

Note. Plant height (PHT), leaf number (LN), green leaf area (GLA), fresh biomass yield (FBY), cane yield (CY), bagasse yield (BY), brix degree (B°), juice yield (JY), sugar yield (SY) and theoretical ethanol yield (EY). $^{**}p < .01$, $^{*}p < .05$.

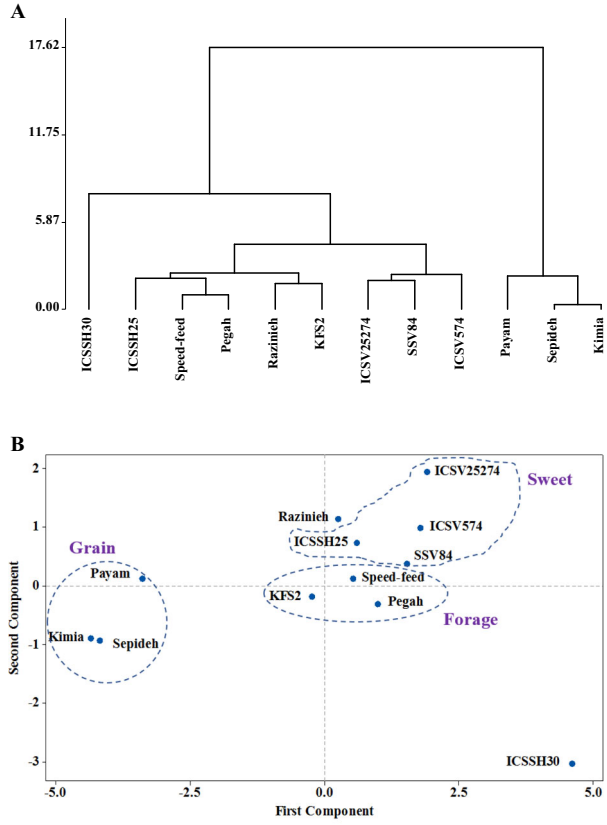

Figure 2. Clustering and principal component analysis based on 10 agro-morphological traits in 12 sorghum genotypes. (A) Dendrogram based on Euclidean distance and Ward's minimum variance method. (B) PCA plot showing scores for PC1 and PC2. Clusters have been highlighted corresponding to agronomic groups (sweet, grain and forage sorghum). ICSSH30 is the abbreviation of ICSSH30-11-ADP.

Table 5. Distance matrix of 12 sorghum genotypes based on 12 agro-morphological traits (below diagonal) and dominant markers (above diagonal) according to Ward's minimum variance method.

Genotypes	Razinieh	ICSSH30-11-ADP	ICSSH25	ICSV25274	ICSV574	SSV 84	Payam	Sepideh	Kimia	KFS2	Speed-feed	Pegah
Razinieh	**0.0**	8.7	8.2	9.2	8.8	8.5	9.2	9.2	9.5	9.2	9.2	9.1
ICSSH30-11-ADP	6.1	**0.0**	6.2	6.3	5.2	5.5	7.9	9.0	8.9	9.4	9.7	9.6
ICSSH25	1.9	5.8	**0.0**	7.7	6.5	6.1	8.3	8.9	8.8	9.2	9.3	9.4
ICSV25274	2.2	5.7	3.1	**0.0**	6.2	6.9	8.3	8.9	9.1	9.6	9.7	10.0
ICSV574	2.7	5.1	2.5	2.1	**0.0**	5.4	7.4	8.5	8.5	8.9	9.2	9.4
SSV84	2.1	4.7	2.8	2.0	2.4	**0.0**	7.3	8.3	8.3	8.9	9.4	8.9
Payam	3.9	8.6	4.7	5.6	5.6	5.1	**0.0**	7.1	7.1	7.7	8.3	7.5
Sepideh	5.0	9.0	5.3	6.8	6.4	6.0	1.8	**0.0**	6.5	7.8	8.4	8.7
Kimia	5.1	9.2	5.4	6.9	6.5	6.2	1.8	0.4	**0.0**	7.1	8.7	8.4
KFS2	1.8	5.7	2.3	3.2	3.1	2.1	3.4	4.1	4.3	**0.0**	8.0	7.6
Speed-feed	1.6	5.4	1.6	3.1	2.6	2.6	4.3	5.1	5.1	2.1	**0.0**	7.6
Pegah	2.0	4.7	2.0	3.0	2.2	2.1	4.6	5.3	5.5	2.1	1.0	**0.0**

cluster II can be subdivided into three groups. Group A, contains the Indian sweet sorghum genotypes (ICSV574, ICSV25274 and SSV84) with generally high fresh biomass and sugar yield, and group B the Iranian forage genotypes (Pegah, Speed-feed and KFS2) along with Razinieh and sweet sorghum genotype ICSSH25. Group C is made up by sweet sorghum genotype ICSSH30-11-ADP and is defined by its superior production of fresh biomass and sugar.

The mixture of genotypes from different agronomic groups within cluster II can be explained by three factors: First, the existence of significant phenotypic variability, secondly the heterogeneity of the agronomic group of forage sorghum, which also includes sweet sorghum genotypes, and thirdly the environmental constraints or management practices and their effect on the quantitative traits (Gepts 1993).

The majority of sweet sorghum genotypes were characterized by higher values in fresh biomass and sugar associated traits compared to the grain genotypes. These findings are in accordance with (Vietor and Miller 1990). Sinha and Kumaravadivel (2016) found morphological variation among sweet, grain and forage sorghum accessions collected from different parts of India using 10 morphological traits.

The broad trait diversity evident among and between the three agronomic groups of sorghum provides ample opportunities for genotype enhancement through breeding programs. Grouping accessions into similar agro-morphological, and most likely, genetically similar groups can help to select parents for crossing (Souza and Sorrells 1991). Estimates of genetic distance based on agro-morphological traits for 12 sorghum genotypes (Table 5) are within the range of 1.0 and 9.2. The minimum genetic distance was recorded between two forage sorghum genotypes (Pegah and Speed-feed). This means that they were almost uniform in terms of fresh biomass and sugar associated traits. The maximum genetic distance was estimated for the sweet sorghum genotype ICSSH30-11-ADP and the Iranian grain sorghum genotype Kimia as a result of the differences inherent to the particular agronomic group. While grain sorghum genotypes are bred to optimize grain yield, sweet sorghum has been selected for increased size and thickness of the stem.

PCA analysis provides a valuable tool to find traits that contribute most to the total amount of variation in a big data set and to assess the magnitude of variation (Tesfaye 2017). PCA results shows that 88.6% of total variation among genotypes is contained within the first two principal components, having an *eigenvalue* (1.6) greater than one (Table 6). The score plot of 12 genotypes based on the first two principal components is presented in Figure 2b. The first principal component (cane yield, fresh biomass yield, leaf number and plant height) explains 72.5% of the variation. The second principal component (brix degree, sugar yield, total leaf area and bagasse yield) contributes only 16.1% of the variation. With only four traits explaining most of the variation, strong correlations between agro-morphological traits are evident. To achieve better separation of different genotypes, other traits need to be considered.

Table 6. Eigenvectors, total variation, eigenvalue and cumulative variance derived from 10 agro-morphological traits of sweet, forage and grain sorghum genotypes.

Traits	PC1	PC2
Plant height	0.312	0.195
Leaf number	0.342	0.242
Green leaf area	0.264	0.393
Fresh biomass yield	0.363	0.075
Cane yield	0.368	−0.005
Bagasse yield	0.281	0.331
Brix °	0.232	−0.545
Juice yield	0.312	−0.308
Sugar yield	0.287	−0.492
Ethanol yield	0.368	−0.006
Eigenvalue	7.240	1.600
Total variance (%)	72.500	16.100
Cumulative variance (%)	72.500	88.600

Genetic diversity revealed by ISSR and RAPD markers

Dominant DNA markers provide a simple and fast way to assess genetic diversity between and among different sorghum genotypes and complements the agro-morphological diversity evaluation (Nkongola and Nsapato 2003). A total of 8 ISSR primers produced 96 bands, of which 57 were polymorphic, accounting for a polymorphism of 59.3%. The number of amplified bands varied between 8 (primer ISSR4) and 19 (primer ISSR6). The average number of polymorphic bands per primer was 7.1. In case of RAPD analysis, 24 RAPD random primers yielded clear and reproducible bands. A total of 212 RAPD bands were produced, of which 134 were polymorphic, accounting for a polymorphism of 63.2%. The number of amplified bands varied between 3 (primer OPK-7) and 15 (primer GLC-20) with an average of 9.6 (Table 2). Figure 3 shows two representative PCR amplification profiles, generated from genomic DNA of 12 sorghum genotypes

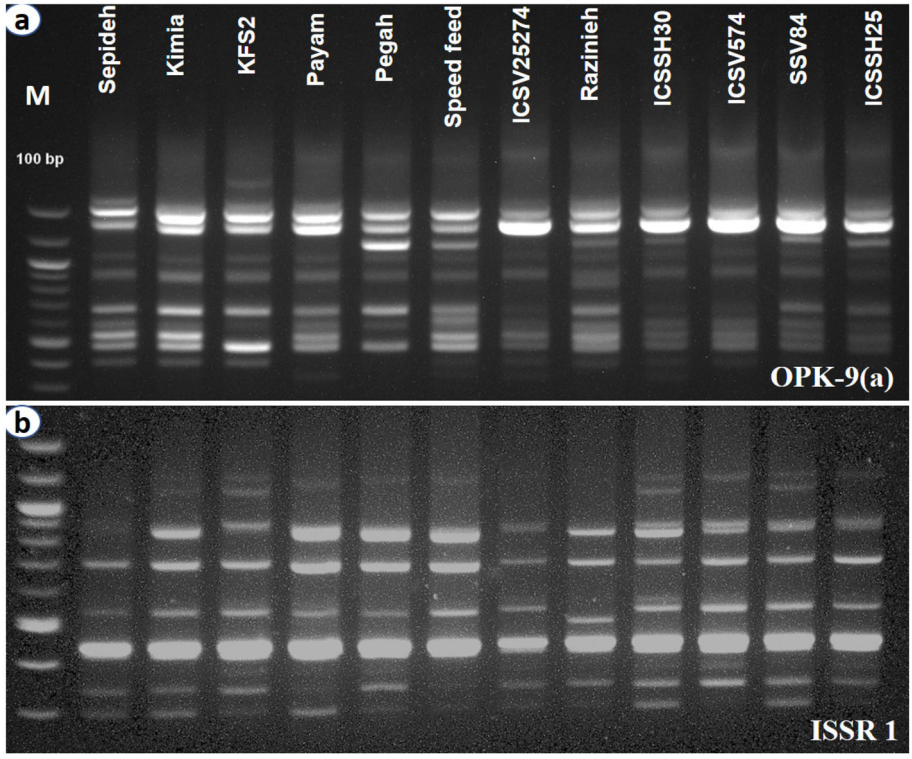

Figure 3. PCR amplification profile generated from genomic DNA of 12 sorghum genotypes with a: OPK-9(a) RAPD primer and b: ISSR 1 primer. M-marker = 100 bp. ICSSH30 is the abbreviation of ICSSH30-11-ADP.

with RAPD primer OPK-9(a) visualized on 1% agarose gel (Figure 3, top), and ISSR primer 1 visualized on 1.5% agarose gel (Figure 3, bottom). The degree of polymorphism observed in the present study was comparable with various reports of sorghum genetic diversity estimates using RAPD and ISSR (Tao et al. 1993; Nkongola and Nsapato 2003). While ISSR is known to be highly reproducible and stringent, RAPD is faster but with lower reproducibility, due to the lower annealing temperature, which is more prone to mismatching (Bornet and Branchard 2004).

The 12 genotypes were separated into two main clusters based on Euclidean distance created utilizing Ward's minimum variance method. The dendrogram was cut at a distance of 10 (Figure 4a). Cluster I included all sweet sorghum genotypes viz., ICSV25274, ICSSH30-11-ADP, ICSV574, SSV84 and ICSSH25, while cluster II included all grain and forage sorghum genotypes. Cluster II constituted three distinct groups. Group 1 includes the Syrian landrace Razinieh, group 2 is composed of three grain sorghum genotypes (Payam, Sepideh and Kimia) while group 3 consists of all forage sorghum genotypes (KFS2, Pegah and Speed-feed). According to the distance matrix, the genetic variation ranged from 5.2 to 10.0 (Table 5). The highest genetic variation was measured between the forage sorghum genotype Pegah and the sweet sorghum genotype ICSV25274. On the other hand, the least variation was observed between two sweet sorghum genotypes ICSSH30-11-ADP and ICSV574. Accordingly, these results demonstrate that ISSR and RAPD markers are useful and informative for evaluating genetic diversity. There was high genetic similarity among genotypes from each group that share a common origin. This can be explained by the existence of many alleles common to these genotypes (Creste et al. 2003). The PCA was performed with RAPD and ISSR data in order to establish the relationship between genotypes of different agronomic groups (Figure 4b). Distribution pattern of genotypes in this aspect was mainly similar to the result extracted from cluster analysis.

The present study indicates high similarity among Indian sweet, Iranian forage and grain sorghum genotypes based on molecular fingerprints. All sweet sorghum genotypes are adapted to the post-rainy season and were improved in India to increase sugar content of the stem, which could explain why, using PCR-based markers, they are placed distinctly separate from grain and forage genotypes. However, when agromorphological traits are considered, it is the Iranian grain sorghum genotypes that are placed distinctly separate as consequence of generally lower values in all traits compared to forage and sweet sorghum genotypes, which were grouped together in one main cluster. The genetics responsible for the increased capacity of stem tissue, which subsequently is used as a primary sink in sweet sorghum, is still not well understood (Irving 2015). A straightforward working hypothesis would propose that corresponding genotypes developed similar biomass partitioning mechanism. On the other hand, the Iranian grain and forage sorghum genotypes are contained within the same cluster at the molecular level indicative of a common ancestor (Shakeri et al. 2017). The two Iranian genotypes Kimia and Sepideh had one common grain parent FGS. Speed-feed is an Australian hybrid developed from crossings between a grain sorghum genotype and Sudan grass (*Sorghum* × *drummondii*). It is a very popular forage sorghum genotype with high productivity.

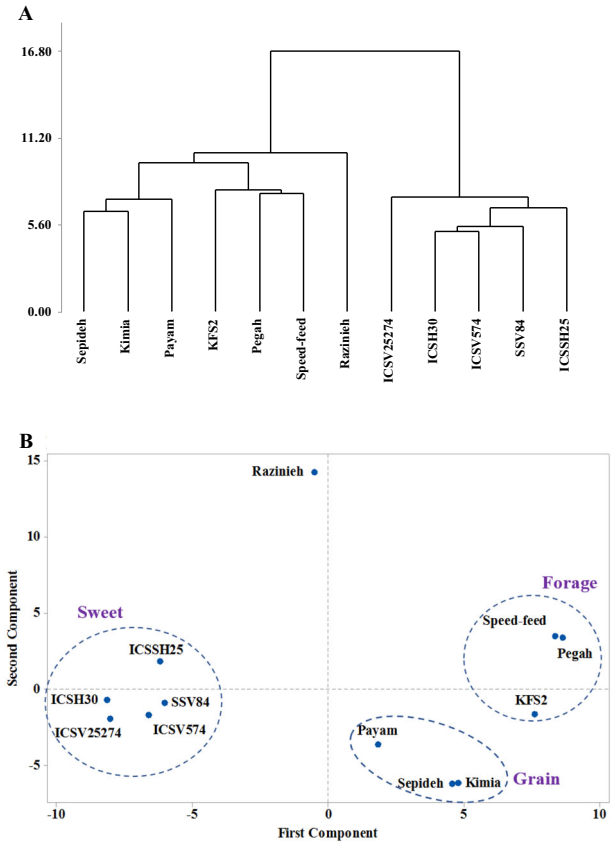

Figure 4. Clustering and principal component analysis based on RAPD and ISSR amplified fragments for 12 sorghum genotypes. (A) Dendrogram based on Euclidean distance and Ward's minimum variance method. (B) PCA plot showing scores for PC1 and PC2. Clusters have been highlighted corresponding to agronomic groups (sweet, grain and forage sorghum). ICSSH30 is the abbreviation of ICSSH30-11-ADP.

The Syrian landrace Razinieh holds a special position: within the corresponding main cluster it is separate from other grain sorghum genotypes, and all forage sorghum genotypes. Its position at the ancestral node suggests that some forage and grain sorghum genotypes may have been developed from a common ancestor, which is consistent with traditional knowledge tracing back the usage of Razinieh as a landrace to ancient times in Syria (Alhajturki et al. 2012). This landrace genotype has been subjected to a special plant breeding program using a bulk breeding strategy by the National Agricultural Institute in Syria to increase its grain yield, but it has remained morphologically and genetically more closely related to forage sorghum.

The phenotypic and the molecular diversity matrices exhibited no correlation ($r = 0.077$, p value $= .53$ at 5% significance level) obtained via the Mantel Test (Figure 5) which is in agreement with the inconsistencies observed between the clusters formed by the phenotypic and the molecular diversity analyses. The clusters obtained by the molecular diversity analysis were more consistent with the types and origin of the sorghum genotypes than the clusters obtained through the morphological diversity analysis. Based on morphological analysis, forage and sweet genotypes were close to each other but based on molecular analysis, forage and grain were more close to each other, therefore, the result of Mantel test is not significant.

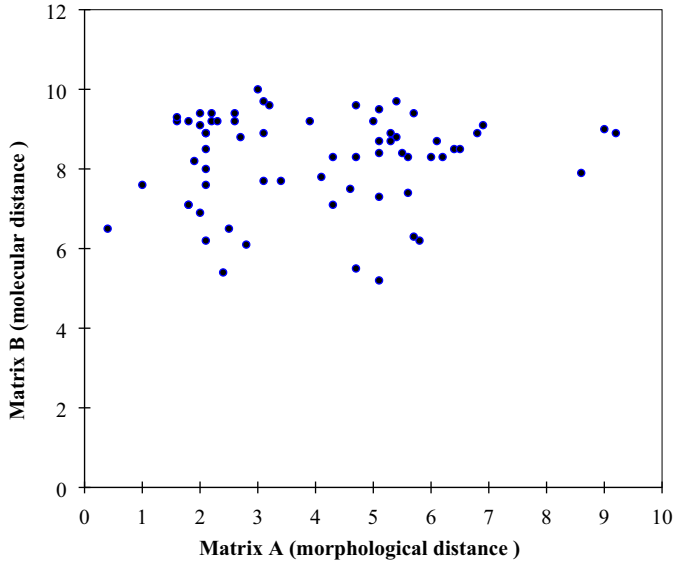

Figure 5. Mantel test for matrix correlation between morphological distance (Matrix A) and molecular distance (Matrix B). Pairwise morphological distance is plotted against molecular distance; $r = 0.077$; null hypothesis of r 0 (the matrices are not correlated.): one sided $p = .53$ from 10,000 randomizations.

Conclusions

Our results show that sorghum can be efficiently cultivated in temperate regions like Germany and has therefore great potential as renewable energy resources. This study showed that the studied genotypes have a wide range of variability in terms of sugar yield and related traits, which provide valuable resources for Sorghum improvement by breeding program in temperate zone. In general, sweet sorghum genotypes (especially ICSSH30-11-ADP) generated most fresh biomass and had the highest sugar yield, compared to grain and forage sorghum genotypes. The positive correlation between juice yield and some morphological traits could help indirect selection for higher ethanol and sugar lines. Classification based on molecular markers was more useful than that based on agronomic usage. Several genotypes, namely, ICSSH30-11-ADP, ICSV25274, ICSV574, SSV84 and Pegah were found to be excellent sugar and ethanol producers and superior for cane yield under one-season/location test. Therefore, there is a need for more trails across locations and years to validate these results and utilize these entries in further sorghum breeding programs to develop superior genotypes for biofuel production.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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