Breeding strategies for the adaptation of sorghum (Sorghum bicolor L. Moench) as a novel crop for temperate Europe

Inaugural Dissertation for a Doctorate Degree in Agricultural Sciences in the Faculty of Agricultural Sciences, Nutritional Sciences and Environmental Management

Examiners
1. Prof. Dr. Dr. h. c. Wolfgang Friedt
2. Prof. Dr. Rod Snowdon

Submitted by
M. Sc. Steffen Martin Windpassinger
from
Nidda, Germany

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‘The greatest service which can be rendered any country is to add a useful plant to its culture...One such service of this kind rendered to a nation is worth more to them than all the victories of the most splendid pages of their history, and becomes a source of exalted pleasure to those who have been instrumental in it.’

Thomas Jefferson (in a letter to Alexandre Giroud, Philadelphia, May 22, 1797)
Dedication

This work is dedicated to the persons whose love, care and support have enabled me to go so far: my wife Amelia and my parents Brigitte and Johann.
# Table of contents

<table>
<thead>
<tr>
<th>List of figures and tables</th>
<th>VI</th>
</tr>
</thead>
<tbody>
<tr>
<td>List of abbreviations</td>
<td>VII</td>
</tr>
</tbody>
</table>

## 1 General Introduction

1.1 Global importance of sorghum  
1.2 Domestication and biology of sorghum  
1.3 Abiotic and biotic stress tolerance of sorghum  
1.4 Crop types of sorghum  
1.5 Breeding of sorghum  
1.5.1 Historical aspects  
1.5.2 Hybrid breeding in sorghum  
1.5.3 Recent developments in sorghum breeding  
1.6 Importance and perspectives of sorghum in Europe  
1.7 Scope and aims

## 2 Publication I: Designing adapted sorghum silage types with an enhanced energy density for biogas use in temperate Europe

## 3 Publication II: Towards enhancement of early-stage chilling tolerance and root development in sorghum F₁ hybrids

## 4 General Discussion

4.1 Adaptation of maize in Central Europe- a blueprint for sorghum?  
4.2 Development of adapted lines as basis for hybrid breeding  
4.3 The potential of heterotic pattern in sorghum versus maize  
4.4 Seeking the adequate sorghum ideotype  
4.5 Conclusions

## 5 Summary

## 6 Zusammenfassung

## 7 References

## 8 Declaration of Honor

## 9 Acknowledgements

## Supplements
List of figures and tables

Fig. 1: Total production (average from 2009-2013) and grain yield per hectare (2009-2013 compared to 1979-1983) of the world’s top10 grain sorghum producing countries.

Fig. 2: Sorghum races *bicolor*, *durra*, *caudatum*, *kafir* and *guinea*

Fig. 3: (A) The sorghum morphotypes or races and their geographic distribution based on 469 representative germplasm accessions (B) The genetic relatedness among these accessions evaluated by neighbor-joining method, showing that clustering is firstly based on geographic origin and secondly on race (source: Morris et al. 2013).

Fig. 4: Different crop types (ideotypes) of sorghum. From left to right: sweet type with tall stems, leafy fodder type, short grain sorghum in front of tall fiber sorghum, early-maturing silage or dual-use type, broomcorn.

Fig. 5: Phylogram displaying the genetic relatedness in a sorghum diversity set (n=470) used in this study by neighbor-joining method, showing that clustering into five phylogenetic groups is predominantly based on geographic origin and race (visualization of the phylogram done by Dendroscope 3.4.4 (Huson et al. 2007)).

Fig. 6: Phylogram showing the distribution of established females (A-lines, dark red and bold), identified B-lines (possible new females, red) and restorers (blue) across the diversity set (visualization of the phylogram done by Dendroscope 3.4.4 (Huson et al. 2007)).

Fig. 7: Boxplots displaying the mid-parent heterosis (MPH) of factorial crosses between three females (all from group 1) and the four best-combining male parents of each phylogenetic group (see Fig. 5 and 6), respectively.

Table 1: World production and Top10 grain sorghum producing countries of Europe (source: FaoStat)

Table 2: World production and Top5 forage and silage sorghum producing countries of Europe (source: FaoStat)

Table 3: Correlation between Rogers’ genetic distance of hybrid parents (0= identical alleles at all marker loci, 1= different alleles at all marker loci) and hybrid biomass yield (dry matter) at Gross-Gerau and Moosburg 2013 (mean of both locations). Hybrids were assigned into six groups depending on their plant height.
## List of abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMS</td>
<td>cytoplasmic-male sterility</td>
</tr>
<tr>
<td>FM</td>
<td>biomass fresh matter</td>
</tr>
<tr>
<td>GCA</td>
<td>general combining ability</td>
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<tr>
<td>GS</td>
<td>genomic selection</td>
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<tr>
<td>LP</td>
<td>line <em>per se</em> performance</td>
</tr>
<tr>
<td>MeC</td>
<td>methane content (i.e., methane yield per dry matter unit)</td>
</tr>
<tr>
<td>MPH</td>
<td>mid-parent heterosis (= $F_1$-mid-parent value)</td>
</tr>
<tr>
<td>MPV</td>
<td>mid-parent value</td>
</tr>
<tr>
<td>QTL</td>
<td>quantitative trait locus/loci</td>
</tr>
<tr>
<td>SCA</td>
<td>specific combining ability</td>
</tr>
<tr>
<td>SNP</td>
<td>single nucleotide polymorphism</td>
</tr>
</tbody>
</table>
1 General Introduction

1.1 Global importance of sorghum

Sorghum (*Sorghum bicolor* L. Moench) is one of the world’s most important cereals, ranking 5th in terms of global production in the 2009-2013 period (http://faostat3.fao.org/browse/Q/QC/E). Thanks to its versatility and tolerance against most kind of abiotic stresses, it is of paramount importance for subsistence farming under low-input conditions in Africa and Asia (India). However, the major producer in the 2009-2013 period was the USA, and the worldwide Top 10 producers shown in Fig. 1 accounted for 76% of the global production (58.56 M metric tons per year). Generally, two groups of sorghum producing countries can be distinguished. On the one hand, countries as the USA, Mexico, Argentina, Australia and China produce sorghum on a commercial basis, principally for feed, export and recently also biofuel (ethanol). Due to the use of hybrid seed and modern farming practices, grain yields are relatively high (~ 4 t ha\(^{-1}\)) in these countries. However, it has to be taken into account that sorghum is principally cultivated on marginal sites which are too dry for other crops such as maize and wheat. Under optimal conditions, record yields of 20 t ha\(^{-1}\) for grain (Srinivasa et al. 2014, Boyer 1987) and 80 t dry matter ha\(^{-1}\) for biomass (Packer & Rooney 2014) have been reported in Texas. On the other hand, in countries like India, Nigeria, Sudan and other African countries, sorghum is mainly cultivated for human nutrition in traditional low-input farming systems, and yields are as low as 1 t ha\(^{-1}\) on average. The yield trend of the last 30 years is not concordant, and generally, yield improvements have lagged behind that of other major crops such as maize. While a substantial increase in yield per hectare was achieved e. g. in Australia, China, Argentina and Ethiopia, enhancements were comparatively low in the USA and Mexico, and even a decline was observed for Nigeria and Sudan (Fig. 1). Besides the use of grains as food or feed, sorghum has several other uses, which is amplified in chapter 1.4. Sorghum for forage and silage is grown on a large scale in Argentina, Mexico and the USA, which account for more than 80% of the world production for this item (http://faostat3.fao.org/browse/Q/QC/E). Cultivation of sweet sorghum allows for the production of sugar or ethanol in tropical and subtropical areas which are too dry or where winters are too cold for sugarcane. This is of growing importance especially in the USA and China, but also in other countries (Burks et al. 2015, Mocoeur et al. 2015, Regassa & Wortmann 2014). Further, biomass or fiber sorghum is used for cellulose production and bioenergy generation (Zegada-Lizarazu & Monti 2012).
1.2 Domestication and biology of sorghum

*Sorghum bicolor* L. Moench (2n = 20) belongs to the *Poaceae* family (tribe *Andropogoneae*, subtribe *Sorghinae*). The *Sorghum* genus consists of 22 species (Venkateswaran et al. 2014) and is separated into five taxonomic subgenera or sections: *Eu-Sorghum*, *Chaetosorghum*, *Heterosorghum*, *Para-Sorghum* and *Stiposorghum* (Dillon et al. 2007). *Sorghum bicolor* belongs to the section *Eu-Sorghum*, just as its progenitor *S. verticilliforum* (formerly *arundinaceum*, Kellog 2013) and *S. halapense* (Johnson grass, a very noxious weed). Phylogenetic studies demonstrate that sorghum is closely related to maize (*Zea mays*) and especially sugarcane (*Saccharum officinarum*). Evolutionary divergence of sorghum and sugarcane is estimated to have occurred only 5 M years ago, while maize separated 15-20 M years ago. Sorghum and sugarcane are each other’s closest relatives among cultivated crops (Dillon et al. 2007), and intergeneric crosses are possible (Bowers et al. 2003). Thanks to its relatively small (~ 730 Mbp), diploid and fully sequenced genome (Paterson et al. 2009), sorghum is an attractive model for functional genomics of the *Saccharinae* and other tropical C4-grasses.

The domestication of sorghum obviously began in Northeast-Africa (present Ethiopia and Sudan) around 4000-3000 BC (Dillon et al. 2007), although there is anthropological evidence suggesting that hunter-gatherers consumed sorghum already in 8000 BC (Smith and Frederiksen 2000). The practice of disruptive selection created numerous varieties of improved sorghum types, which were spread via the movement of people and trade routes into other regions of Africa, India (approx. 1500-1000 BC), the Middle East (approx. 900-700 BC), and is separated into five taxonomic subgenera or sections: *Eu-Sorghum*, *Chaetosorghum*, *Heterosorghum*, *Para-Sorghum* and *Stiposorghum* (Dillon et al. 2007). *Sorghum bicolor* belongs to the section *Eu-Sorghum*, just as its progenitor *S. verticilliforum* (formerly *arundinaceum*, Kellog 2013) and *S. halapense* (Johnson grass, a very noxious weed). Phylogenetic studies demonstrate that sorghum is closely related to maize (*Zea mays*) and especially sugarcane (*Saccharum officinarum*). Evolutionary divergence of sorghum and sugarcane is estimated to have occurred only 5 M years ago, while maize separated 15-20 M years ago. Sorghum and sugarcane are each other’s closest relatives among cultivated crops (Dillon et al. 2007), and intergeneric crosses are possible (Bowers et al. 2003). Thanks to its relatively small (~ 730 Mbp), diploid and fully sequenced genome (Paterson et al. 2009), sorghum is an attractive model for functional genomics of the *Saccharinae* and other tropical C4-grasses.

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BC) and China (approx. AD 400). From China, sorghum was introduced to Europe (Klein et al. 2015), where its first description dates back to 1204 (Piemont region, Italy) (Becker-Dillingen 1927), and from the 16th to 18th century, it was spread over Southern and Southeastern Europe, but its utilization was confined to broomcorn (a sorghum type used for making brooms and whiskbrooms) during this time (Dahlberg et al. 2011). The introduction of sorghum into America occurred as broomcorn during the 1750s (Berenji et al. 2011) and as grain- and sweet sorghum during the first half of the 19th century (Sleper & Poehlman 2006).

The adaptation to very different environments and the selection for different kinds of uses (grain, fodder, sugar, fiber) led to a huge morphological and genetic variety which is largely still untapped. The general appearance of sorghum during vegetative growth is similar to maize, but unlike modern maize varieties, sorghum shows tillering in a different extent depending on genotype, environment and their interaction (Alam et al. 2014). At the top of the tillers, perfect flowers develop. Sorghum is predominantly self-pollinating, but may show up to 50% cross pollination, depending on panicle structure and weather conditions during flowering (Osuna-Ortega et al. 2003). Five morphological subspecies or races (morphotypes) of *S. bicolor* and their intermediates can be distinguished based on panicle structure: *bicolor, caudatum, durra, guinea* and *kafir* (Fig. 2).

**Fig. 2:** Sorghum races *bicolor, durra, caudatum, kafir* and *guinea* (own records).
Bicolor and guinea have an open panicle, which is beneficial in humid climates to avoid fungal infections and explains the predominance of guinea in humid Western Africa. Bicolor is the primitive progenitor race from which all other races are derived (Brown et al. 2011). It is widely distributed, but nowhere dominant in Africa, and also present in Asia (Kimber et al. 2013). Caudatum and kafir have semi-compact panicles and are suitable for breeding of high-yielding grain types (Hariprasanna & Patil 2015). While caudatum types are primarily found in tropical savanna climates of Central Africa, kafir is predominant in Southern Africa and shows the strongest pattern of population subdivision relative to other races, indicating genetic bottlenecks resulting from a shift to a contrasting agroclimatic zone (Morris et al. 2013). Durra has a compact panicle with a characteristic ‘goose-neck’ and is mainly found in warm desert climates with a low risk of grain mold as the Nile valley of Egypt and Sudan, the Horn of Africa, Arabia and India (Kimber et al. 2013, Morris et al. 2013). In a phylogenetic study using a diversity panel, Morris et al. (2013) showed that accessions cluster preferentially into their geographic origin and secondly into races (Fig. 3).

**Fig. 3:** (A) The sorghum morphotypes or races and their geographic distribution based on 469 representative germplasm accessions (B) The genetic relatedness among these accessions evaluated by neighbor-joining method, showing that clustering is firstly based on geographic origin and secondly on race (source: Morris et al. 2013).
Hence, the panicle structure may be used as a simple morphological marker facilitating the development of genetically distinct heterotic groups (discussed in chapter 4.3).

Sorghum is originally a photosensitive short day plant. In the semiarid tropics of the Sahel, the rainy season ends quite reliably at a latitude-specific time in the fall at a day length below 12 hours, whereas its beginning is much more variable. Thus, local landraces are best adapted when flowering starts around 20 days before onset of the dry season regardless their sowing time, to allow for sufficient water supply during anthesis and grain filling period and dry conditions during ripening (Guitton et al. 2015). However, nowadays photo-insensitive varieties for temperate latitudes are available. Flowering time and photoperiodism of sorghum are controlled by four major maturity loci, designated $Ma_1$, $Ma_2$, $Ma_3$ and $Ma_4$, where dominant alleles cause late flowering (Quinby & Karper 1945, Quinby 1966). $Ma_1$ is a photoperiod sensitivity gene encoding PRR37, an inhibitor of flowering in long days (Murphy et al. 2011), and has the largest impact (Klein et al. 2008). A recessive mutation at this locus is sufficient to confer some temperate-adaptedness, and occurred independently in different parts of the world, allowing for establishment e. g. in temperate China (Klein et al. 2015). Most kafirs originating in extra-tropical Southern Africa already possessed the $ma_1$ allele when introduced into the US (Klein et al. 2015). $Ma_3$ encodes a phytochrome B (Childs et al. 1997), whereas the exact function of $Ma_2$ and $Ma_4$ is still unknown (Higgins 2013). More recently, two additional, epistatic maturity genes ($Ma_5$ and $Ma_6$, Rooney & Aydin 1999) and 40 quantitative trait loci (QTL) with predominantly small, additive effects on flowering time (Mace et al. 2013a) have been described.

Sorghum shows a remarkable variation for plant height. Dwarf types with a height of only 50 cm exist as well as tall biomass types with up to 5 m height. Similar to maturity, plant height is controlled by four unlinked major dwarfing loci ($Dw_1$, $Dw_2$, $Dw_3$, $Dw_4$) (Quinby & Karper 1954). Tallness is incompletely dominant, with heterozygotes being slightly shorter than tall-allele homozygotes (Brown & Paterson 2013). $Dw_3$ has been identified as an auxin transporter ($Pgp1/Pgp19$, Multani et al. 2003). Its recessive form is unstable, and reverts to the dominant form causing tallness, with one tall mutant plant occurring out of approx. 600-1200 plants in the field (Sleper & Poehlman 2006). In contrast to other cereals such as wheat and barley, in sorghum a short stature does not imply an effect on harvest index, and taller genotypes generally have a higher grain yield potential (George-Jaeggli et al. 2011), probably due to an increased availability of stem reserves which is especially beneficial under stress conditions (Blum et al. 1997). However, to facilitate combine-harvesting, modern grain types are usually recessive at three of the four $dw$ loci and do not exceed 170-180 cm plant height.
Extremely short 4-dwarfs are rather not used due to their very low yield potential (Sleper & Poehlman 2006).

1.3 Abiotic and biotic stress tolerance of sorghum

Sorghum has an outstanding tolerance against most kinds of abiotic stresses (Tari et al. 2013). Its photosynthetic pathway is C₄, conferring per se a high water use efficiency and adaptation to heat and intense irradiation. Per kg of accumulated dry matter, sorghum has been reported to need 332 L of water, whereas maize requires 368 L, barley 434 L and wheat 514 L (Reddy et al. 2011). Its exceptional drought tolerance is based on a low transpiration rate due to waxy leaves (Premachandra et al. 1994) and an extensive root system with a predominance of vertical growth already in juvenile stages (Singh et al. 2010) and final depths of up to 200 cm (Robertson et al. 1993). Recent studies highlight the importance of the nodal root angle for drought tolerance and stay green characteristics (Singh et al. 2012). Sorghum has a remarkable ability to acquire nutrients (Rehm & Espig 1996) and a high salinity tolerance (Almodares et al. 2008), allowing its growth also on poor soils within a pH range of 5-8.5 (Rehm & Espig 1996). Surprisingly, sorghum is also quite tolerant to water logging (Promkhambut et al. 2010). As an originally tropical plant, sorghum does not tolerate frost, and requires temperatures of more than 20 °C for optimal growth. Lower temperatures induce different grades of chilling stress and are especially problematic during emergence and seedling establishment (Pinthus & Rosenblum 1961, Peacock 1982), but also during flowering, when cold nights (<13 °C) can reduce pollen fertility (Downes & Marshall 1971, Osuna-Ortega 2003) and may cause a total loss of seed yield (Maulana & Tesso 2013). Similarly, heat stress during flowering also has detrimental effects on seed set and occurs at temperatures above 36-38 °C (Singh et al. 2015). Fortunately, there is a broad diversity for chilling tolerance in sorghum, facilitating breeding for higher latitudes and altitudes. Promising sources are kaoliangs from Northern China (Franks et al. 2006, Salas-Fernandez et al. 2014), but also landraces from highlands in Yemen, Burundi, Uganda and Ethiopia (Singh 1985). Varieties with a satisfying seed set even at night temperatures of 6 °C during flowering have been developed in Mexico (Osuna-Ortega et al. 2003).

In tropical and subtropical countries, sorghum is attacked by numerous diseases and pests. Sorghum downy mildew (Peronosclerospora sorghi) is probably the most destructive fungal disease, since it can cause stunting and death of plants already in juvenile stages. Leaf spots caused by fungi (e. g. Colletotrichum graminicola, Bipolaris sorghicola) or bacteria (e. g. Burkholderia andropogonis, Xanthomonas campestris) and fungal leaf blights (e. g. Exserohilum turcicum, Ramulispora sorghi) can also cause significant yield losses, depending
on the intensity of their infestation (Sleper & Poehlman 2006). Among insect pests, greenbug
(Schizaphis graminum) and sorghum midge (Contarinia sorghicola) are most widespread and
harmful. In semiarid regions of Africa and Asia, sorghum can be infested by the parasitic
weeds Striga hermonthica and S. asiatica. Striga parasites on roots and can kill sorghum due
to loss of water and nutrient supply. Resistance to Striga is based on impenetrable roots and
sorghum genotypes with a low production of sorgoleone, a stimulant released from sorghum
roots which is required for germination of Striga seeds (Sleper & Poehlman 2006).
In Europe, the disease and pest pressure on sorghum is much lower than in other parts of the
world. While the highly destructive sorghum downy mildew is still almost unknown, fungal
leaf spots and blights can be observed, but do not imply significant economic losses yet.
Greenbug and sorghum midge do not occur in Europe (Berenji & Dahlberg 2004), and
sorghum is proven to be a non-host for the Western Corn Rootworm (Diabrotica virgifera,
Oyediran et al. 2004), a devastating pest on maize.

1.4 Crop types of sorghum
Sorghum is an extremely versatile crop which is utilized for numerous purposes. The
selection for particular kinds of uses in distinct environments has created several crop types or
ideotypes according to Donald (1968), who defined an ideotype as an ideal plant form for a
particular environment or end-use. In traditional, non-mechanized subsistence farming, e.g. in
Africa and Asia, tall sorghum is preferred, since it offers multiple uses. While the grains are
for human nutrition, the shoot can be used for feed or construction material. However, in
commercial, mechanized farming, different crop types of sorghum can be distinguished depending on their end-use: grain, forage/silage, sugar, biomass/fiber and broomcorn (Fig. 4).

Fig. 4: Different crop types (ideotypes) of sorghum. From left to right: sweet type with tall
stems, leafy fodder type, short grain sorghum in front of tall fiber sorghum, early-maturing
silage or dual-use type, broomcorn (own records).
To prevent lodging and facilitate mechanical harvest, modern grain types usually do not exceed 170-180 cm plant height. In Africa and Asia, sorghum grains are used for foods like flat breads, fermented or unfermented porridges, couscous, and fried products (Leder 2004). In the USA, South America and Australia, the grains are predominantly utilized for animal feed and ethanol production, while a small percentage is used in the production of snacks and gluten-free foods (Taleon et al. 2012). The grain composition resembles other cereals, containing 60-75 % carbohydrates, 8-13 % protein and 4-6 % lipids (Franke 1989). The raw digestibility of sorghum starch is the lowest among cereals, since the starch granules are strongly associated with endosperm proteins (kafirins) restricting the accessibility of α-amylase to starch (Rooney & Pflugfelder 1986). This is considered as a negative aspect for animal feeding (Serna-Saldivar & Rooney 1995), but may be beneficial for human nutrition since it helps to lower the caloric intake (Barros et al. 2012). Depending on the pericarp color, sorghum grains can be white, yellow-lemon, red-brown and even black (Rooney et al. 2013, Dykes et al. 2014). Sorghum genotypes with dominant $B1_B2$ genes contain condensed tannins, which are stored in the testa layer. Hence, the pericarp color is not a reliable indicator for tannin content, even though it is widely erroneously believed that all sorghums with a red/brown pericarp contain tannins (Dykes & Rooney 2006). Tannin sorghums have a higher resistance to grain mold and a lower risk of bird damage, but the feed efficiency can be reduced by 10-30 % compared to non-tannin sorghums (Dykes & Rooney 2006). However, for human nutrition, tannins – as well as other phenolic compounds present in sorghum grains - are beneficial due to their antioxidant and anticarcinogenic effects (Yang et al. 2009).

Grain sorghum can also be used for whole-plant silage with a quality nearly equal to maize silage for cattle feeding when harvested at dough stage (Grant & Stock 1994). Especially convenient for this purpose are varieties with a plant height around the upper limit for grain sorghum (approx. 180 cm), which have an enhanced whole-plant dry matter yield at a still high grain: shoot ratio, which are referred to as silage types or dual-use types, since they can be harvested for both grain or silage use depending on the farmer’s preference (www.nt.gov.au/d/Content/File/p/Crop/514.pdf). Taller forage sorghums have lower energy values due to a reduced grain: shoot ratio, but higher biomass yields. Brown-midrib mutants ($bmr$), discernible by their midrib-color, have reduced lignin contents compared to their wild type due to mutations in the lignin- biosynthesis and hold the potential of an enhanced digestibility. Unfortunately, this mutation goes along with a lower yield potential, higher risk of lodging and delay in maturity compared to the wild type (Oliver et al. 2005, Pedersen et al. 2005). However, the extent of both agronomical drawbacks and enhanced digestibility depends strongly on the genetic background and not only on the mutation itself (Sattler et al. 2005).
S. bicolor x S. sudanense hybrids are suitable as ratoon crops, being valuable for hay production and pasture. When vegetative parts of sorghum are used for fodder, dhurrins, cyanogenic glucosides, are of concern. Their concentration is highest in young tissues and when plants are recovering from stress periods (Sher et al. 2012). Generally, mature plants are less critical, and ensilage greatly reduces the risk of cattle intoxication (Wheeler & Mulcahy 1989).

In contrast to typical fodder types, sweet sorghum types show much less tillering. They have tall, juicy stems which contain up to 25% sugar in the forms of sucrose, glucose and fructose (Ritter et al. 2008, Shiringani et al. 2010). Due to sink competition, sweet sorghum types have generally a low grain yield potential (Burks et al. 2015). Besides for sugar production as alternative to sugarcane, they are also traditionally used for syrup production in the US (Pfeiffer et al. 2010). Fiber sorghums are tall and late-flowering and look similar to sweet sorghum, but have dry stems rich in cellulose which is used for paper production (Zegada-Lizarazu & Monti 2012). Broomcorn can be of variable height and is primarily grown for its panicles consisting of long, fine, elastic branches which serve as raw material for manufacturing brooms, which is still of some economic importance e.g. in Hungary, Romania, Serbia and Mexico. Historically, broomcorn was the sorghum type that was first cultivated in Europe and the USA, previous to all other types introduced directly or indirectly from Africa (Berenji et al. 2011).

Sorghum is considered a promising bioenergy crop, due to its versatility and tolerance to drought and low-input conditions, which allows for its cultivation on marginal soils otherwise remaining uncultivated and hence mitigates the competition for land use between food and bioenergy crops. All of the sorghum crop types previously described can be used for bioenergy generation. Both grain and sweet types are used for ethanol production out of starch or sugar, which is conducted e.g. in the US and Australia. Forage and especially fiber types can be utilized for 2nd generation biofuels out of cellulose. The latter ones are also suitable for combustion (Zegada-Lizarazu & Monti 2012). In Europe, bioenergy generation is principally focused on biogas containing methane as energy carrier, which is subsequently converted into electricity. The optimal ideotype of sorghum for biogas/methane production remains to be defined. The methane yield per area is a product of dry matter yield per area and methane yield per dry matter unit (methane content) and can be enhanced via both factors. Presently, mostly forage and fiber types are grown for biogas use, with a high biomass dry matter yield potential, but a low energy density (Zeise & Fritz 2012). Silage types might be a smart alternative, offering a compromise between yield potential and energy density, which will be discussed in detail in chapter 2.
1.5 Breeding of sorghum

1.5.1 Historical aspects

Systematic sorghum breeding dates back to its domestication time, when seeds of non-shattering plants were selected by ancient farmers (Dillon et al. 2007). Subsequently, its spread to very different environments and both natural and human selection created an almost infinite number of locally adapted populations and landraces. However, modern breeding of sorghum began much more recently in the US by the end of the 19th century. In contrast to broomcorn, which was introduced from Europe already in the 1750s (Berenji et al. 2011), grain types came directly from Africa by slave ships in the first half of the 19th century. The number of founder cultivars in the US was limited and probably as narrow as about 20 sweet sorghum (so called 'sorgos') and 8 or 10 grain sorghum introductions (Sleper & Poehlman 2006). Among these grain sorghum founder cultivars, the varietal groups milo (race durra), kafir (race kafir), hegari and feterita (race caudatum) were most important. These first late-maturing and tall varieties were limited to subtropical areas in Texas and unsuitable for mechanical harvest. Farmers soon selected for short-stature, early maturing mutant plants, corresponding to mutations of the dwarf and maturity genes. Crosses between different desirable mutations facilitated the release of improved, combine-harvestable and early-maturing cultivars during the first half of the 20th century which allowed for cultivation as far north as Nebraska (Klein et al. 2008). The success achieved with hybrid maize also stimulated interest in developing hybrid sorghum varieties (Sleper & Poehlman 2006). Hybrid vigor in sorghum was already recognized in 1927 (Conner & Karper 1927), following varietal crosses in traditional breeding programs (Sleper & Poehlman 2006). The first generation of hybrids dates back to the late 1940s, when genetic male sterility in the variety Day Milo was utilized in crosses with Blackhull Kafir (Quinby 1974), realizing yield advantages of 40% compared to the cultivars in general use at that time (Klein et al. 2008). In 1952, both cytoplasmic-male sterility (CMS) and fertility restorers possessing Rf genes were discovered in different milo varieties (Stephens & Holland 1954), and the CMS system quickly replaced the use of the Day Milo genetic male sterile system (Klein et al. 2008). Commercial CMS-based hybrid seed production began in 1956, and only four years later, the area planted with hybrids reached 95%, and grain yields doubled compared to 1952 (Quinby 1974; Smith & Frederiksen 2000).

In spite of these impressive yield gains, the genetic base of the temperate-adapted breeding lines had remained narrow, and scientists recognized the limitations for further yield gains as well as biotic and abiotic stress tolerance. The main problem was that the huge diversity existing in the tropics was virtually locked-up due to photosensitivity. To overcome this
genetic bottleneck, in 1963 the Sorghum Conversion Program was established by the United States Department of Agriculture (USDA) to convert genetically diverse tropical accessions to temperate lines suitable for combine-harvesting (Stephens et al. 1967). Knowing about the inheritance of plant height and maturity in sorghum, the 4-\textit{dwarf} and photoperiod-insensitive cultivar \textit{BTx406} was used as a donor for temperate adaption in a backcrossing program. In tropical Puerto Rico, the exotic accession was crossed with \textit{BTx406}, resulting in a photoperiod-sensitive, tall \textit{F}_1 plant which was self-pollinated. The corresponding \textit{F}_2 seeds were then planted in Texas to select for short, early-maturing plants, whose seeds were sent back to Puerto Rico to repeat the backcrossing cycle up to five times (Higgins 2013). Through this program, about 850 converted and partially converted lines have been developed, providing novel sources for insect and disease resistance, drought tolerance, heterosis and grain quality for temperate areas (Rosenow & Dahlberg 2000) as well as adapted combine-harvestable genotypes for tropical environments (Sleper & Poehlman 2006). The impact of this program on sorghum improvement has been huge, and it is difficult to find present sorghum hybrids that do not have conversion lines in their pedigree (Gabriel 2005). Fully converted sorghum genotypes were expected to consist of 97\% recurrent, i.e. the respective tropical parent genome; however, recent studies show that the recovery of the exotic genome besides \textit{dwarf} and \textit{maturity} genes was not as complete as suggested. Especially on sorghum chromosome SB-06 which harbors the crucial \textit{ma}_1 and \textit{dw}_2 genes, extensive stretches of the donor genome survived, probably reflecting genetic hitchhiking and background selection caused by the elimination of deleterious alleles (Klein et al. 2008). As a result, little functional diversity in temperate sorghum genotypes has remained on SB-06, which contains roughly 10\% of all sorghum genes, limiting the adaptive potential especially for complex traits (Thurber et al. 2013). As in all other crops, the supply of genetic diversity remains a paramount goal in present sorghum breeding to ensure genetic gains and resistance sources. Recent studies underline the importance of a continuous integration of diverse, exotic material into modern breeding programs (Jordan et al. 2011a) and the possibilities of the secondary gene pool in the wild species \textit{S. bicolor} subsp. \textit{verticilliflorum} and \textit{S. propinquum} (Dillon et al. 2007, Mace et al. 2013b, Muraya 2014, Venkateswaran et al. 2014).

1.5.2 Hybrid breeding in sorghum

In developed countries, almost all grain sorghums are hybrid varieties. Heterosis for grain yield is principally due to an increased number of grains per branch, mostly at the lower branches of the panicle (Blum 1970), and a larger panicle itself (Miller & Kebede 1984). The average high-parent heterosis for grain yield is estimated at 20-60\% (Axtell et al. 1999).
Further, heterosis is also expressed for plant height, maturity (Kirby & Atkins 1968) and abiotic stress tolerance, e. g. chilling stress (Pinthus & Rosenblum 1961, Yu & Tuinstra 2001, Windpassinger et al. 2016). Unfortunately, heterotic groups in sorghum are not as clearly defined as in temperate maize breeding (Jordan et al. 2003, Gabriel 2005). However, the advent of inexpensive marker technologies facilitates the analysis of genetic diversity in breeders’ materials and the work towards the establishment of such groups in the near future (Monk et al. 2014), which will be amplified in chapter 4.3. Despite the availability of several different CMS-systems (Schertz 1983), commercial hybrid seed production relies worldwide almost exclusively on the *milo* A1 CMS-system discovered in 1952 (cf. Jordan et al. 2011b). This cytoplasmic genetic uniformity of commercial sorghum is risky, since a disease might be associated with a particular cytoplasm, as occurred for maize in the outbreak of southern corn leaf blight (*Bipolaris maydis*) in 1969/70 (Reddy et al. 2007, Jordan et al. 2011b). The A2 CMS-system seems a promising alternative, since hybrids created using this cytoplasm yielded comparably to A1 cytoplasm-based hybrids (Moran & Rooney 2003, Reddy et al. 2007), whereas the A3 cytoplasm had negative effects on hybrid yield (Moran & Rooney 2003). One drawback of the A2 cytoplasm is that lines restoring the A1 cytoplasm are often not restorers in the A2 cytoplasm. In this regard, the mapping and characterization of *Rf3*, a gene restoring fertility in both cytoplasms, may be a basis to select for universal restorers (Jordan et al. 2011b).

Hybrid breeding is also applied in forage (Ross et al. 1979) and fiber sorghums (Murphy et al. 2014, Packer & Rooney 2014). The pronounced heterosis for plant height allows for the production of tall biomass hybrids on short, combine-harvestable A-lines, provided that the restorer is taller or the complementary action of *dwarf* genes is used (Ross et al. 1979, Packer & Rooney 2014). By exploiting the complementary action of the maturity loci *Ma1*, *Ma5* and *Ma6*, photoperiod-sensitive, high-biomass hybrids can be produced on photoperiod-insensitive parental lines, permitting seed production of these hybrids also in temperate areas (Rooney & Aydin 1999, Mullet et al. 2010). In contrast, present sweet sorghum varieties are still mostly line cultivars (Pfeiffer et al. 2010), since the sugar content (brix) is an additive, non-heterotic trait (Makanda et al. 2009, Pfeiffer et al. 2010) and there is a scarcity of sweet short-stature A-lines (Pedersen et al. 2013). However, the expression of heterosis for stem biomass yield may imply an enhancement of total sugar yield per area unit (Makanda et al. 2009, Pfeiffer et al. 2010). Hence, first sweet sorghum hybrids have been released (Pfeiffer et al. 2010) and research is going on, also on the background to provide improvements on seed supply which can rather be accomplished on short A-lines than on traditional, tall sweet sorghum line cultivars (Pedersen et al. 2013). Since sugar yield is higher in sterile hybrids due to avoidance
of sink competition, the preferential use of the $A_3$ cytoplasm in which most male lines do not effect fertility restoration has been suggested (Pfeiffer et al. 2010).

1.5.3 Recent developments in sorghum breeding

Regardless of the crop type, both maximum exploitation of heterosis and improvements of line per se performance are considered components of the best breeding strategy (Packer & Rooney 2014), which is also based on the experiences in maize hybrid breeding (Duvick 1999). A recently discovered, multi-seeded ($msd1$) mutant might boost the sorghum grain yield potential, since in this mutant in contrast to the wild-type also the pedicellate spikelets are fertile, increasing the total seed weight per panicle by 30 to 40% (Burow et al. 2014). However, the usefulness of this mutation under field conditions remains to be demonstrated.

To ensure yield stability, a sufficient level of tolerance to abiotic and biotic stress is crucial. Sorghum has a high tolerance against most kinds of abiotic stress per se (Tari et al. 2013), but further improvements are definitely necessary to meet the requirements of its harsh production environments. The availability of a sorghum standard genome sequence (Paterson et al. 2009) and the dramatically decreasing costs for molecular markers have led to the discovery of numerous quantitative trait loci (QTL) and candidate genes for stress tolerance and quality traits (e.g. Madhusudhana 2014, Anami et al. 2015), allowing the development of molecular breeding approaches. Especially useful in this regard are single nucleotide polymorphism (SNP) markers, which occur at a high frequency in the genome and can be easily detected by automated, so called Next Generation Sequencing methods as Illumina™. Drought tolerance has been shown to be influenced by QTL for nodal root angle (Mace et al. 2012). For early-stage chilling tolerance of sorghum, several QTL have been identified and partially confirmed in different studies (Knoll et al. 2008, Burow et al. 2011, Fiedler et al. 2012 & 2014, Bekele et al. 2014), the feasibility of marker-assisted selection for this trait has been shown (Knoll & Ejeta 2008) and first potential candidate genes have been found (Bekele et al. 2014, Upadhyaya et al. 2015). However, besides a general heterosis (Yu & Tuinstra 2001), little is known about the inheritance and expression in F$_1$ hybrids, which will be further discussed in chapter 3 (Windpassinger et al. 2016). Regarding sugar and biofuel traits, several studies have identified significant QTL in biparental populations (Ritter et al. 2008, Murray et al. 2008a, Murray et al. 2008b, Shiringani et al. 2010, Shiringani & Friedt 2011, Mocoeur et al. 2015) and genome-wide diversity panels (Murray et al. 2009, Burks et al. 2015). Genomic selection (GS) is considered a promising alternative to QTL mapping for traits with several small-effect QTL and lower heritability. Besides selection for yield, GS has also been successfully tested for abiotic stress tolerance like drought tolerance in maize (Ziyomo &
Bernardo 2013) and frost tolerance in winter wheat (Zhao et al. 2013). For sorghum chilling
tolerance, Bekele (2014) showed the effectiveness of GS in a recombinant inbred line (RIL)
population, obtaining a cross-validation accuracy of 0.30-0.55.

Besides yield and stress tolerance, the enhancement of grain quality is a global breeding goal.
Sorghum is the staple food for the majority of the population in Africa and Central India, who
mainly do not have access to a balanced and healthy diet and thus depend on sorghum for
their micronutrient requirements (Parthasarathy Rao et al. 2006). Biofortification of sorghum
is considered the most cost-effective and sustainable solution for tackling micronutrient
deficiencies in developing countries of arid-tropical and subtropical regions. Large genetic
variability for Iron (Fe) and Zinc (Zn) concentrations in grains underlines the possibility of
genetic enhancement for these traits, and the development of high yielding hybrids with high
grain Fe and Zn concentration is considered feasible (Kumar et al. 2013a). Sorghum lines
combining enhanced protein and starch digestibility have been developed through
conventional breeding by Texas A & M University (e.g. Jampala et al. 2012). To alleviate
vitamin A deficiencies in semiarid Africa, β-carotene biofortified transgenic sorghum is being
developed (Lipkie et al. 2013, You et al. 2015) with support from the Bill & Melinda Gates
has been regarded a recalcitrant crop for genetic transformation due to the rapid production of
phenolic compounds hampering tissue culture (Liu et al. 2014), and even though the first
transgenic sorghum plants were obtained by microprojectile bombardment already by Casas
et al. in 1993, transformation efficiency has stagnated at less than 1% for a long time (Liu et
al. 2014). However, this has greatly been improved in recent years, and transformation
efficiencies of between 10 and 25% are now routinely achieved by both microprojectile (Liu
& Godwin 2012) and Agrobacterium (Gurel et al. 2009) based methods. Further applications
of transgenic sorghum are comparable to those in other major crops and include herbivore
(Visarada et al. 2014) and herbicide tolerance. Up to date, no transgenic sorghum has been
commercially released yet (Visarada & Sai Kishore 2015), but it is expected in the near future
(Liu et al. 2014). One major concern is the gene flow to wild and weedy sorghums, e. g.
outcrossing with the notorious rhizomatous weed Sorghum halapense (Kumar et al. 2013b,
Liu et al. 2014). However, this risk is rather defined by the characteristic of the trait than by
the method it was achieved. Herbicide tolerance in sorghum has already been developed by
conventional breeding, introgressing tolerance to acetolactate synthase (ALS) herbicides
identified in naturally occurring shattercane (S. bicolor ssp. bicolor) (Tuinstra & Al-Khatib
2006). The release of ALS-tolerant sorghum varieties had been expected for 2016 (Monk et
al. 2014), but right now it seems that it will take longer, since also the supposedly resistant
variety is significantly stressed by herbicide application and there are also issues regarding yield performance (http://agfax.com/2015/07/24/sorghum-herbicide-tolerance-needed-but-still-several-years-off-dtn/). Against the background of the mentioned risk of gene flow, it remains to be seen if herbicide-tolerant sorghum will have more than short-term success.

1.6 Importance and perspectives of sorghum in Europe

Compared to other continents, sorghum is only of minor importance in Europe up to now, and its grain sorghum only accounts for 2% of global production (http://faostat3.fao.org/browse/Q/QC/E). France, Italy, Russia and Ukraine account for approx. 90% of both grain sorghum area and production in Europe (Table 1). Comparing the pentade 2009-2013 with the period 1999-2003, a dramatic increase of sorghum production can be observed for Romania and Ukraine. The same applies for Austria, which is listed by the FAO as sorghum producer since 2010. More modest growths took place in all other countries, while there was a decline in Bulgaria and France. Average yields in Austria, France, Italy and Spain are notably higher than in the world main producers using hybrid seed (see 1.1), while in the Eastern European countries yields are low, suggesting a much more extensive production.

Table 1: World production and Top10 grain sorghum producing countries of Europe (source: FaoStat)

<table>
<thead>
<tr>
<th>Country</th>
<th>Production in 2013 (t)</th>
<th>Production area in 2013 (ha)</th>
<th>Mean Production 2009-2013</th>
<th>Production 2009-2013 relative to 1999-2003 (100)</th>
<th>Yield 2009-2013 (t ha⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>World total</td>
<td>62,295,137</td>
<td>42,228,265</td>
<td>58,562,447</td>
<td>102</td>
<td>1.4</td>
</tr>
<tr>
<td>Europe total</td>
<td>1,251,870</td>
<td>393,027</td>
<td>869,506</td>
<td>128</td>
<td>4.0</td>
</tr>
<tr>
<td>Ukraine</td>
<td>354,400</td>
<td>129,343</td>
<td>168,380</td>
<td>1156</td>
<td>2.4</td>
</tr>
<tr>
<td>Italy</td>
<td>316,919</td>
<td>51,034</td>
<td>257,805</td>
<td>128</td>
<td>6.1</td>
</tr>
<tr>
<td>France</td>
<td>278,800</td>
<td>51,344</td>
<td>279,080</td>
<td>78</td>
<td>5.7</td>
</tr>
<tr>
<td>Russia</td>
<td>172,044</td>
<td>119,475</td>
<td>59,981</td>
<td>143</td>
<td>1.1</td>
</tr>
<tr>
<td>Romania</td>
<td>49,829</td>
<td>21,025</td>
<td>32,025</td>
<td>934</td>
<td>2.4</td>
</tr>
<tr>
<td>Spain</td>
<td>45,300</td>
<td>8207</td>
<td>40,037</td>
<td>117</td>
<td>5.0</td>
</tr>
<tr>
<td>Austria</td>
<td>10,200</td>
<td>1899</td>
<td>8425</td>
<td>na</td>
<td>6.4</td>
</tr>
<tr>
<td>Serbia</td>
<td>10,000</td>
<td>3003</td>
<td>7622</td>
<td>na</td>
<td>2.9</td>
</tr>
<tr>
<td>Bulgaria</td>
<td>5000</td>
<td>2994</td>
<td>6353</td>
<td>80</td>
<td>2.1</td>
</tr>
<tr>
<td>Hungary</td>
<td>5000</td>
<td>1901</td>
<td>8083</td>
<td>110</td>
<td>2.1</td>
</tr>
</tbody>
</table>
The production of forage and silage sorghum is listed by FaoStat for Portugal, France, Italy, Hungary, Spain and Greece (Table 2). It is unclear which proportion of this production is dedicated for bioenergy generation, which is focused on biogas rather than bioethanol in Europe. In Germany, forage sorghum is grown almost exclusively for biogas production on an estimated area of up to 25000 ha which is not listed by the FAO yet.

Table 2: World production and Top5 forage and silage sorghum producing countries of Europe (source: FaoStat)

<table>
<thead>
<tr>
<th>Country</th>
<th>Production in 2013 (t FM)</th>
<th>Production area in 2013 (ha)</th>
<th>Mean Production (t FM) 2009-2013</th>
<th>Production 2009-2013 relative to 1999-2003 (=100)</th>
<th>Yield (t FM ha⁻¹) 2009-2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>World total</td>
<td>25,231,288</td>
<td>1,213,924</td>
<td>23,734,070</td>
<td>103</td>
<td>20.8</td>
</tr>
<tr>
<td>Europe total</td>
<td>1,381,000</td>
<td>48,850</td>
<td>1,320,060</td>
<td>109</td>
<td>28.3</td>
</tr>
<tr>
<td>Portugal</td>
<td>460,000</td>
<td>21,005</td>
<td>430,000</td>
<td>119</td>
<td>22.1</td>
</tr>
<tr>
<td>France</td>
<td>370,000</td>
<td>10,511</td>
<td>368,000</td>
<td>96</td>
<td>35.4</td>
</tr>
<tr>
<td>Italy</td>
<td>352,000</td>
<td>7652</td>
<td>347,800</td>
<td>117</td>
<td>46.0</td>
</tr>
<tr>
<td>Hungary</td>
<td>162,000</td>
<td>6694</td>
<td>126,600</td>
<td>422</td>
<td>25.0</td>
</tr>
<tr>
<td>Spain</td>
<td>37,000</td>
<td>1800</td>
<td>40,840</td>
<td>31</td>
<td>21.0</td>
</tr>
</tbody>
</table>

Several factors suggest that sorghum will continue to gain importance in Europe, for both fodder and bioenergy use. Having a similar production technique and diversity of end uses, it can serve as a substitute for maize. Regardless its crop type and end use, sorghum has the advantages of a higher tolerance against both drought (Schittenhelm & Schroetter 2014) and temporary flooding (Promkhambut et al. 2010), events predicted to increase due to climate change, and lower production costs due to reduced fertilization and crop protection requirements. However, probably the most important benefit, especially on the background of the European policy towards transgenic Bt-maize, is its resistance against the Western Corn Rootworm (Diabrotica virgifera) (Oyediran et al. 2004), which is regionally causing devastating damages in European maize production. Being an annual crop and providing high biomass yields along with flexibility in crop rotations, sorghum is more attractive as a bioenergy crop for farmers than perennial crops such as Miscanthus. Presently, its chilling sensitivity is a major constraint for a successful cultivation outside areas with warm summers,
such as the Mediterranean and Southeastern parts of Europe. In Central Europe, the vegetation period is too short and not warm enough to allow for maturity of grain sorghum, except for favored regions as the Rhine valley and lowland areas of Southeastern Austria. Also for silage and biomass type sorghum, the short growing season notably limits the biomass yield potential compared to maize, which is sown around three weeks earlier than sorghum.

Maize is the most important crop for biogas use in Germany by far, since it has the highest potential of dry matter and methane yield at most sites (Herrmann 2013) and hence the highest marginal returns for farmers (Theiß et al. 2013, Kornatz et al. 2014). The biogas boom has thus led to a substantial increase of maize acreage in Germany, causing concerns about biodiversity and increase of maize pests. The ratio of maize silage in biogas plants was limited to 60% in 2012 (German law ‘EEG 12’), giving opportunities for sorghum as a substitute. In spite of its shorter growing season, sorghum can already outperform maize in terms of biomass dry matter yield in drought-prone environments, e.g. diluvial soils in Eastern Germany. However, even at similar biomass dry matter yields, the marginal returns of sorghum are substantially lower than maize. Reasons therefore are lower dry matter contents, implying higher transportation costs (Theiß 2013 et al., Kornatz et al. 2014) and inferior energy densities (Zeise & Fritz 2012), leading to lower methane yields per area unit (Theiß et al. 2013). These inconveniences are mainly caused by the sorghum crop types grown for biogas use, even though an enhancement of chilling tolerance would allow for higher dry matter content (and yield) thanks to a longer vegetation period.

In 2015, seven sorghum varieties for silage use (i.e. almost exclusively biogas use in Germany) were listed by the Federal Plant Variety Office (Bundessortenamt, www.bundessortenamt.de/internet30/fileadmin/Files/PDF/bsl_getreide_2015.pdf; p. 190). Six of them are tall, late-flowering biomass types, whose lower energy density compared to maize can be explained by the lack of starch containing grains. Additionally, more cultivars of this variety type are merchandised without registration in Germany. One of the seven listed varieties (Farmsugro180) represents a grain type, which has a substantially reduced biomass yield potential, but a higher energy density (Zeise & Fritz 2012). Further, several Sorghum bicolor x S. sudanense hybrids (e.g. Gardavan, KWS Sole) are merchandised without registration in Germany. They are earlier and somewhat shorter than the previously mentioned biomass types. While they do not differ in their energy density, their yield potential is considerably lower than that of biomass types (Zeise & Fritz 2012). A new ideotype in form of an early-maturing silage type might combine the advantages of both biomass- and grain types and overcome some of the present drawbacks of sorghum
cultivation for biogas use in Central Europe, which will be discussed in detail in chapter 2 (Windpassinger et al. 2015).

1.7 Scope and Aims

This thesis aims to contribute to the adaptation and establishment of sorghum as a novel crop in temperate Europe. Even though sorghum is an extremely versatile crop with numerous uses (chapter 1.4), presently its utilization as an alternative bioenergy crop is the main focus for Central Europe. Unlike maize, current sorghum varieties grown for biogas use fail to combine high biomass yields with adequate dry matter content for silage and satisfying energy density (chapter 1.6). To make sorghum competitive, the design of a novel ideotype with enhanced energy density and dry matter content may represent a decisive advancement (chapter 2, Windpassinger et al. 2015). Regardless its end-use, improvements in early-stage chilling tolerance allowing for earlier sowing are of paramount importance for adaptation in Central and Northern Europe and escape of summer drought in Southern and Southeastern Europe. An efficient breeding strategy for the development of chilling-tolerant hybrid cultivars requires a profound understanding of heterosis, combining ability and relation between line and hybrid performance for this trait (chapter 3, Windpassinger et al. 2016). Further, breeding strategies for enhancement of adaptation, quality and yield traits of sorghum will be discussed, considering which lessons can be learnt from temperate maize breeding in the last century. The establishment of heterotic groups may be fruitful for an efficient hybrid breeding in sorghum, and examples for the usefulness of SNP-markers in this regard will be presented (chapter 4.3).
Chapter 2:

Publication I

‘Designing adapted sorghum silage types with an enhanced energy density for biogas generation in temperate Europe’


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Designing adapted sorghum silage types with an enhanced energy density for biogas generation in temperate Europe

Steffen Windpassinger a, Wolfgang Friedt a,*, Martin Frauen b, Rod Snowdon a, Benjamin Wittkop b

a Department of Plant Breeding, Research Center for BioSystems, Land Use and Nutrition (IFZ), Heinrich-Buff-Ring 26-32, 35392 Gießen, Germany
b Norddeutsche Pflanzenzucht Hans-Georg Lembke KG, Hohenlieth, 24363 Holtsee, Germany

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Abstract

Sorghum is regarded a promising bioenergy crop and may be an alternative to maize for biogas use in temperate Europe in the near future. Presently, the principal shortcoming compared to maize besides chilling sensitivity during juvenile development is that current sorghum varieties fail to combine a high dry matter yield with adequate dry matter content for silage and satisfying methane yield. Our major goal was to evaluate whether early-maturing silage type Sorghum bicolor hybrids with a higher contribution of panicles and grains to total dry matter may allow an improvement of methane yield, representing a novel, alternative variety type for biogas use in temperate Europe. Our results indicate that under adequate conditions, they reach higher dry matter contents. Due to enhanced methane yields per dry matter unit which offset slightly lower dry matter yields, their methane yield per area unit is similar or superior to existing biomass type varieties. Since panicles account for up to 50% of total dry matter yield, seed set and maturity in cool environments represent critical factors for yield stability. Breeding efforts focus on chilling tolerance during flowering to avoid male sterility and on the development of appropriate parental lines combining earliness and adequate height. While selection for maturity can be reliably conducted on per se performance of parental lines, hybrid yield is predominantly determined by general combining ability.

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1. Introduction

Sorghum (Sorghum bicolor L. Moench), an extremely versatile and resilient crop, represents an essential staple food in Africa and India, and an important commodity in countries like the USA, Mexico, Argentina and Australia. Especially in the US, sorghum is also used for ethanol production out of grain or sugar. In Europe, notably Germany, Austria and Italy, where bioenergy is focused on biogas rather than ethanol, sorghum recently has drawn attention as a novel bioenergy crop.

Biogas, obtained by anaerobic digestion of organic matter, contains methane as energy carrier, which is subsequently converted into electricity. Besides slurry, maize silage is the principal substrate used in biogas plants. As a bioenergy crop, maize presently has the highest potential of dry matter and methane yield at most sites throughout Germany [1]. The policy towards renewable energies has thus gone along with a substantial increase of maize growing area in Germany. Due to concerns about biodiversity in crop rotations and soil conservation together with the increase of maize pests, in 2012 the maximum input of maize silage as substrate in biogas plants was limited to 60% dry mass fraction in Germany [2]. As a substitute for maize, sorghum is already grown on approx. 25000 ha here. Principal advantages of sorghum as a bioenergy crop include resistance against the Western Corn Rootworm (Diabrotica virgifera) [3] and a high tolerance to drought [4] and low-input conditions. In contrast to other novel bioenergy crops such as miscanthus, sorghum is an annual crop and allows
the maintenance of flexibility in crop rotations, and its production technique is very similar to maize, making it amenable for farmers and not implying the need of new investment.

However, since breeding of sorghum as a bioenergy crop for temperate Europe is still in its infancy, several drawbacks must be overcome to make it a viable alternative to maize. Its sensitivity to cool temperatures, especially during juvenile stage, represents a major constraint for a successful adaptation to temperate climates, since it delays sowing compared to maize by around 3–4 weeks and thus notably limits the potential growth period. Unless maize, present commercial sorghum variety types used for biogas production fail to combine a high dry matter yield (DMY) with essential quality requirements as a satisfying energy density, described as methane content (MeC, methane yield per dry matter unit), and an adequate dry matter content (DMC, mass fraction of dry matter) of at least 28% for silage. In consequence, the average methane yield per area unit (MeY) and thus the marginal return of existing biogas sorghum varieties is usually lower than maize [5], making it only second choice for biogas production until now. Presently, three different sorghum variety types are grown for biogas production in Central Europe: 1) Tall biomass type hybrids (up to 4 m) not flowering before September, too late for grain development and principally prone to lodging, with a high DMY, but suboptimal DMC and MeC [6,7]; 2) Earlier S. bicolor x sudanense hybrids with an adequate DMC, but a substantially lower DMY and suboptimal MeC; 3) early and short grain sorghum hybrids with satisfying DMC and MeC, thanks to the notable proportion of starch containing grains in the overall biomass, but a low biomass DMY due to the short stature.

The question is whether these three variety types will remain the sole options for biogas sorghum, or if a shift in plant architecture might allow the combination of satisfying DMC and MeC with high biomass yield. Variety types with a plant height of 200–300 cm, intermediate between grain and current biomass types, and a sufficiently early anthesis allowing appropriate panicle and grain development, hereafter referred to as silage types, might be a smart alternative, taking up the advantages of grain types in regard to DMC and MeC, and complementing them with a higher biomass yield potential due to a taller stature. Their plant architecture and height would somehow resemble silage maize, where the cob accounts for at least 50% of total DMY [8] even in high-biomass varieties which are used for biogas generation. The experience that tall, late-maturing maize varieties with a reduced cob proportion, obtained by the exploitation of late-maturing material and the introgression of short-day genes from tropical populations [9], have not become widely accepted by farmers in temperate Europe supports the idea that silage type sorghum might be a better choice for biogas production than current biomass types. However, up to now, adapted sorghum silage type varieties are not available in Central Europe.

The principal aim of this study is to assess whether these silage types, represented by pre-selected experimental hybrids, may be a viable alternative to currently grown sorghum varieties for biogas use. In this regard, we have analyzed the performance of silage type hybrids regarding the key traits DMY, DMC, MeC and MeY and compared them with representative checks from the existing standard variety types. Further, to facilitate selection in future breeding programs, we have investigated how different agronomical and morphological traits are correlated to these key traits in hybrids, and to which extent the per se performance of inbred lines can be used for hybrid prediction.

2. Material and methods

In 2013, 489 experimental hybrids were tested along with their parents, incl. 4 cytoplasmic male sterile (CMS) female lines and 195 males (not all cross combinations included), in trials at two locations in Germany with one replication per site: Moosburg/Isar (MB) represents a typical maize growing area in Bavaria (Southern Germany), whereas Gross-Gerau (GG) near Frankfurt/Main stands for favorable sorghum growing conditions, thanks to high average temperature and light soil (Table 1). Plot size was 9 m² at MB and 3.75 m² at GG, with row-spacing of 75 cm and a seed rate of 25 m⁻² in both environments. Experimental hybrids were grouped as families according to their paternal lines and grown adjacent to the respective male line in randomized blocks together with several replications of female lines and checks across the blocks. Sowing date was June 7, 2013 at both locations (around two weeks delayed compared to the recommended sowing date due to cool and rainy weather before). Fertilizer and herbicide applications were executed following good agronomical practice. Genotypes showing lodging at one or both locations were excluded from further analyses, so that a total number of 201 experimental hybrids could be tested along with their parental lines in both environments. Parental lines of the experimental hybrids were early maturing grain or dual-use types mostly originating from the USDA Sorghum Conversion Program [10] and also some biomass and sugar types, mainly derived from a cross between the sweet sorghum line S579 and the grain sorghum line M71 [11]. Fresh matter yield (FMY), dry matter content (DMC) and plant height (PH) were scored in parents and hybrids as well. Dry matter yield (DMY) was calculated as product of FMY and DMC. Principal aims of these trials were 1) to select appropriate silage type hybrids for more detailed analyses in the following year, and 2) to learn about the correlation between mid-parent value and hybrid performance, the magnitude of midparent-heterosis, and the accuracy of general combining ability (GCA) for hybrid prediction. For statistical analyses, the two locations were considered as replications.

The 39 best performing experimental hybrids - principally silage types – were selected and re-tested in 2014 at the experimental locations of GG and Rauschholzhäusern (RH) along with 5 check varieties (biomaiss hybrids Biomass150, Herkules, KWS Tarzan and KWS Thor and S. bicolor x sudanense hybrid Gardavan). RH represents an adequate location to select for adaptation to cool climate, with temperatures around 2 °C lower than GG and heavy loamy soil showing retarded warming in spring (Table 1). Entries were planted at both locations in 9 m² plots with 75 cm row spacing and a seed rate of 25 m⁻². The experimental design was a randomized split-plot design consisting of four different subgroups depending on plant height to minimize neighbor effects, and complete randomization within the respective subgroups, with two replications at GG and three at RH. Fresh matter yield (FMY), dry matter content (DMC), plant height (PH), days to anthesis (DTA), stem diameter (SD), seed set and grain maturity were scored. Moreover, shortly before harvest 10 representative plants per entry were stripped into their key components stem, leaves and panicle. DMC of each sample was measured and the respective proportion of these components on total dry matter yield (DMY) was calculated.

Methane content (MeC, i.e. methane yield per dry matter unit) was measured on all 5 check varieties and 8 representative silage type experimental hybrids. For this purpose, at harvest a sample of milled fresh matter (particle size 2–5 mm) was ensiled in jars. Measurements of biomaiss volume per dry matter unit and methane concentration were conducted in three technical replications as described in detail by Mahmood & Honermeier [12]. A maize silage mixture consisting of five commercial varieties grown in RH was used as a reference biomaiss sample. Methane yield per area unit (MeY) was calculated as a product of mean MeC and DMY (all volumes reported are at standard temperature and pressure conditions of 298 K and 100.3 kPa). Starch content of these entries was...
Table 1
Characteristics of the different field trial environments.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Coordinates</td>
<td>49° 55' N, 8° 29' E</td>
<td>49° 55' N, 8° 29' E</td>
<td>48° 28' N, 11° 56' E</td>
<td>50° 44' N, 8° 50' E</td>
</tr>
<tr>
<td>Altitude</td>
<td>90 m</td>
<td>90 m</td>
<td>421 m</td>
<td>270 m</td>
</tr>
<tr>
<td>Soil type</td>
<td>Sandy loam</td>
<td>Sandy loam</td>
<td>Loam</td>
<td>Loam</td>
</tr>
<tr>
<td>Date of sowing</td>
<td>07/06</td>
<td>15/05</td>
<td>07/06</td>
<td>22/05</td>
</tr>
<tr>
<td>Date of harvest</td>
<td>16/10</td>
<td>14/10</td>
<td>23/10</td>
<td>02/10</td>
</tr>
<tr>
<td>Mean daily maximum temperature from sowing to harvest (°C)</td>
<td>23.9</td>
<td>24.8</td>
<td>22.3</td>
<td>22.3</td>
</tr>
<tr>
<td>Precipitations from sowing to harvest (mm)</td>
<td>345</td>
<td>347</td>
<td>361</td>
<td>340</td>
</tr>
</tbody>
</table>

measured by a polarimetric method as described by Naumann & Bassler [13].

Statistical analyses were conducted using IBM SPSS Statistics Version 22 (IBM Software, Armonk, NY, USA).

3. Results and discussion

3.1. Prediction of hybrid performance based on parental lines

The correlation between mid-parent value and hybrid performance observed in the two-location trial of 2013 differed among the traits. It was relatively high for DMC (r = 0.73**) and PH (r = 0.67**), implying that selection of suitable parental lines in a breeding program can be reliably conducted for these agronomical traits. Breeders can assign their lines into different maturity groups analogue to maize, where strong correlations for DMC have also been described [14]. In contrast, the correlation between mid-parent value and hybrid performance was only medium for DMY (r = 0.40**, Fig. 1A). As expected, hybrids as a group attained a significantly higher DMY and PH than their parental lines, and mid-parent heterosis (F1 - mid-parent value) was on average 64% for DMY and 46% for PH. The strong mid-parent heterosis for PH shows that silage type hybrids with a PH above 200 cm can be produced on shorter hybrid parents convenient for mechanical harvest, facilitating hybrid seed production. In contrast, there was no mid-parent heterosis for DMC.

The general combining ability (GCA) is a major criterion to identify and select superior parents. The GCA for DMY of three female and 45 male lines was calculated based on the performance of their factorial crosses. The coefficient of determination (R²) between the sum of parental GCA effects and the performance of their respective hybrids shows the accuracy of GCA for hybrid prediction [15]. In this case, an R² value of 0.68 (Fig. 1B) for DMY suggests that the GCA for parental lines is an adequate predictor for hybrid performance, and that specific combining ability (SCA) effects play only a minor role. These results confirm earlier studies with forage sorghum hybrids [16–19].

Further, the correlation between per se performance of the tested male lines and their respective GCA for DMY was calculated, resulting in a medium correlation of r = 0.44**, comparable to the correlation between mid-parent value and hybrid performance observed for this trait. Similar results were reported for sorghum grain yield [20]. Results from ICRISAT [21] suggest that it might be sufficient to select for high green fodder productivity in male lines, which would give high-yielding hybrids even if the biomass productivity of the female lines was low. For biogas maize, a higher correlation (r = 0.65**) between per se performance for DMY and GCA was found [14]. In consequence, the improvement of parental lines for yield and adaptation traits can also be seen as an indirect selection for a higher GCA and may subsequently lead to hybrids with enhanced biomass yield, as has been shown for sorghum grain yield, where according to Doggett [22] about one half of the yield increase until the 1980ies could be attributed to better parents. However, GCA eventually remains the crucial trait for hybrid performance in sorghum. Unfortunately, heterotic groups are not as clearly defined in sorghum as in maize, and also the correlation between genetic distance of the parents and heterosis is too weak to be of a practical value for breeders [23,24]. Hence, if new, diverse lines are tested for GCA, it seems recommendable to employ various, genetically diverse or F1 testers [25].

3.2. Optimal variety type and methane yield – insights from field trials in 2014

For the agronomical performance trials at GG and RH in 2014, significant differences were observed among entries (n = 44) for all considered traits (Table 2). Due to the higher temperature at GG, flowering began on average 15 days earlier than at RH, and DMC at harvest was 7% higher (Table 3). DMY was superior at GG by 43% on average. This was due to the substantially higher DMC and not to FMY, which was even slightly higher at RH. While entries differed in their MeY at both sites, significant differences in MeC were only found in the trial at GG (Table 2).

3.2.1. Potential of silage type hybrids regarding yield and methane content

Performance of sorghum silage types differed between the experimental sites, and variation for MeC and MeY was higher than for check varieties (Table 4). This can mainly be explained by differences in seed set, which is crucial for yield and quality parameters of silage types since panicles account for 40–50% of total DMY under optimal conditions (see 3.2.2). Flowering at RH was retarded and coincided for most genotypes with several chilly nights of around 5°C, resulting in poor seed set due to male sterility. While at GG all entries except the four biomass hybrid checks and one experimental hybrid had complete seed set ranging from dough stage to maturity at harvest in most cases, at RH only 12 hybrids achieved a satisfying seed set, with maturity not beyond milk-ripe stage. This phenomenon of male sterility caused by low temperature in sorghum has already been described e.g. [26,27] and complicates the development of silage types for areas prone to cold nights during flowering. Though, fortunately genetic variation for this trait is available and varieties adapted to cold nights have already been developed in Mexico [27] and India [28]. An escape strategy by choosing hybrids with an earlier anthesis, reducing the incidence of cold nights, would be an alternative for cropping areas in Central-Northern Europe, but implies an even stronger limitation for biomass yield potential.

To determine the number of subsets being significantly different from one another, a Student–Newman–Keuls test was conducted for DMY and DMC. Analyzing both locations together, 5 homogeneous subsets for DMY were identified, the best one only consisting of the biomass-type check varieties Biomass150, Herkules, KWS Thor and KWS Tarzan. Considering this trait for both locations separately,
four subsets were found for GG, with the best subset not only comprising the four mentioned check varieties, but also 15 experimental hybrids. This demonstrates that under adequate conditions silage type hybrids can basically be competitive for yield potential. In contrast, for RH the best of the 8 subsets consisted of the four check varieties only. Analyzing DMC in the same way for both sites together, 11 subsets were identified, the best one comprising 11 experimental hybrids and the check variety Gardavan, whereas the other check varieties had a significantly lower DMC.

**Fig. 2A** depicting the mean values of both sites, underlines the high DMY of the four biomass check varieties compared to the experimental hybrids, but also shows that they did not achieve a

<table>
<thead>
<tr>
<th>Trait</th>
<th>df</th>
<th>Genotypic variance (both locations)</th>
<th>Genotypic variance (GG)</th>
<th>Genotypic variance (RH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMY</td>
<td>43</td>
<td>68 804.31**</td>
<td>33 080.40**</td>
<td>43 064.05**</td>
</tr>
<tr>
<td>DMC</td>
<td>43</td>
<td>46.94***</td>
<td>31.58**</td>
<td>19.41**</td>
</tr>
<tr>
<td>DMY</td>
<td>43</td>
<td>2485.07**</td>
<td>1035.69**</td>
<td>2039.66**</td>
</tr>
<tr>
<td>MeC</td>
<td>12</td>
<td>6985.78*</td>
<td>10 307.93**</td>
<td>2720.28</td>
</tr>
<tr>
<td>MeY</td>
<td>12</td>
<td>3 288 559.58**</td>
<td>3 401 480.10**</td>
<td>3 254 849.83**</td>
</tr>
<tr>
<td>DTA</td>
<td>40</td>
<td>333.91**</td>
<td>64.96**</td>
<td>411.29**</td>
</tr>
<tr>
<td>PH</td>
<td>43</td>
<td>16 271.89**</td>
<td>7914.34**</td>
<td>9054.31**</td>
</tr>
<tr>
<td>SD</td>
<td>43</td>
<td>12.68**</td>
<td>5.27**</td>
<td>12.65**</td>
</tr>
<tr>
<td>Stem DMY</td>
<td>43</td>
<td>4173.56**</td>
<td>2984.71**</td>
<td>1774.82**</td>
</tr>
<tr>
<td>Panicle DMY</td>
<td>43</td>
<td>1467.23**</td>
<td>1666.93**</td>
<td>260.06**</td>
</tr>
<tr>
<td>Leaf DMY</td>
<td>43</td>
<td>172.10**</td>
<td>76.39**</td>
<td>176.35**</td>
</tr>
</tbody>
</table>

*Significant at 0.05 level, **Significant at 0.01 level.

Fig. 1. Correlation between mid-parent value and hybrid performance (A, n = 201) and sum of parental GCA effects and hybrid performance (B, n = 135) for DMY (means of Gross-Gerau and Moosburg 2013).
necessary DMC of at least 28%. In contrast, the early check cv. Gardavan was outperformed regarding DMY and DMC by several of the new experimental hybrids. Fig. 2B and C show both sites separately and illustrate the overall better performance of silage types at RH compared to GG as compared to RH. The higher MeC of checks at RH was significantly higher than that of checks at GG and also the negligible difference in mean MeC of the digestibility of shoot and leaves. The higher MeC of checks at RH compared to GG and also the negligible difference in mean MeC of these compared to GG and also the negligible difference in mean MeC of the digestibility of shoot and leaves. The higher MeC of checks at RH compared to GG and also the negligible difference in mean MeC of the digestibility of shoot and leaves.

### Table 3
Overview of descriptive statistics for observed traits at Gross-Gerau and Rauischholzhausen (2014), comprising 39 experimental hybrids and five check varieties (Biomass150, Gordovan, Herkules, KWS Tarzan and KWS Thor).

<table>
<thead>
<tr>
<th>Trait</th>
<th>n</th>
<th>GG (Min)</th>
<th>GG (Max)</th>
<th>GG (Mean)</th>
<th>RH (Min)</th>
<th>RH (Max)</th>
<th>RH (Mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMY (Mg ha⁻¹)</td>
<td>44</td>
<td>43.0</td>
<td>86.9</td>
<td>58.6</td>
<td>50.0</td>
<td>101.9</td>
<td>65.0</td>
</tr>
<tr>
<td>DMC (%)</td>
<td></td>
<td>24.9</td>
<td>39.9</td>
<td>31.4</td>
<td>19.1</td>
<td>29.0</td>
<td>24.2</td>
</tr>
<tr>
<td>DMY (Mg ha⁻¹)</td>
<td>44</td>
<td>18.1</td>
<td>29.2</td>
<td>22.3</td>
<td>11.5</td>
<td>24.2</td>
<td>15.6</td>
</tr>
<tr>
<td>MeC (L kg⁻¹)</td>
<td>13</td>
<td>236.3</td>
<td>427.8</td>
<td>310.3</td>
<td>253.9</td>
<td>377.3</td>
<td>320.9</td>
</tr>
<tr>
<td>MeY (m₃ ha⁻¹)</td>
<td>13</td>
<td>5445</td>
<td>9925</td>
<td>7430</td>
<td>3685</td>
<td>7250</td>
<td>5713</td>
</tr>
<tr>
<td>DTA (d)</td>
<td>44</td>
<td>75</td>
<td>120</td>
<td>81.8</td>
<td>75</td>
<td>no anthesis</td>
<td>97.3</td>
</tr>
<tr>
<td>PH (cm)</td>
<td>44</td>
<td>168</td>
<td>450</td>
<td>273.4</td>
<td>162</td>
<td>397</td>
<td>267.2</td>
</tr>
<tr>
<td>SD (mm)</td>
<td>44</td>
<td>12.8</td>
<td>19.8</td>
<td>15.6</td>
<td>12.7</td>
<td>23.5</td>
<td>18.0</td>
</tr>
<tr>
<td>Stem DMY (Mg ha⁻¹)</td>
<td>44</td>
<td>6.7</td>
<td>22.1</td>
<td>11.3</td>
<td>6.7</td>
<td>17.0</td>
<td>10.3</td>
</tr>
<tr>
<td>Stem DMC (%)</td>
<td>44</td>
<td>18.7</td>
<td>34.5</td>
<td>25.8</td>
<td>18.2</td>
<td>31.8</td>
<td>24.2</td>
</tr>
<tr>
<td>Stem proportion total DMY (%)</td>
<td>44</td>
<td>33.6</td>
<td>78.8</td>
<td>50.1</td>
<td>51.5</td>
<td>75.3</td>
<td>65.1</td>
</tr>
<tr>
<td>Panicle DMY (Mg ha⁻¹)</td>
<td>44</td>
<td>2.0</td>
<td>11.8</td>
<td>7.8</td>
<td>0.7</td>
<td>5.3</td>
<td>2.1</td>
</tr>
<tr>
<td>Panicle DMC (%)</td>
<td>44</td>
<td>38.1</td>
<td>66.3</td>
<td>56.4</td>
<td>22.6</td>
<td>40.8</td>
<td>34.4</td>
</tr>
<tr>
<td>Panicle proportion total DMY (%)</td>
<td>44</td>
<td>7.3</td>
<td>52.6</td>
<td>35.4</td>
<td>3.7</td>
<td>32.2</td>
<td>13.7</td>
</tr>
<tr>
<td>Leaf DMY (Mg ha⁻¹)</td>
<td>44</td>
<td>2.1</td>
<td>4.8</td>
<td>3.1</td>
<td>1.9</td>
<td>5.5</td>
<td>3.4</td>
</tr>
<tr>
<td>Leaf DMC (%)</td>
<td>44</td>
<td>22.1</td>
<td>31.4</td>
<td>27.1</td>
<td>17.6</td>
<td>31.0</td>
<td>25.3</td>
</tr>
<tr>
<td>Leaf proportion total DMY (%)</td>
<td>44</td>
<td>7.9</td>
<td>19.0</td>
<td>14.1</td>
<td>11.3</td>
<td>35.0</td>
<td>21.6</td>
</tr>
</tbody>
</table>

*Volumes are at standard temperature and pressure conditions of 298 K and 100.3 kPa.*

### Table 4
Detailed comparison of genotypic variance (mean squares) for methane content (MeC) and methane yield (MeY) of different variety types at Gross-Gerau (GG) and Rauischholzhausen (RH) in 2014.

<table>
<thead>
<tr>
<th>Trait</th>
<th>df</th>
<th>Genotypic variance (both locations)</th>
<th>Genotypic variance (GG)</th>
<th>Genotypic variance (RH)</th>
<th>Location variance</th>
<th>Genotype* location variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeC (entries)</td>
<td>12</td>
<td>6985.78*</td>
<td>10 307.93**</td>
<td>2720.28</td>
<td>2141.29</td>
<td>7708.86**</td>
</tr>
<tr>
<td>MeC (checks)</td>
<td>4</td>
<td>501.55</td>
<td>1073.14</td>
<td>1066.91</td>
<td>19 807.34*</td>
<td>1638.50</td>
</tr>
<tr>
<td>MeC (silage types)</td>
<td>7</td>
<td>6567.47</td>
<td>12 090.13**</td>
<td>4033.78</td>
<td>26020.17</td>
<td>9485.87**</td>
</tr>
<tr>
<td>MeC (checks vs. silage types)</td>
<td>1</td>
<td>26 703.38**</td>
<td>48 068.76**</td>
<td>139.26</td>
<td>2894.01</td>
<td>1398.55</td>
</tr>
<tr>
<td>MeY (entries)</td>
<td>12</td>
<td>3 288 559.58**</td>
<td>3 401 480.10**</td>
<td>3 254 849.83**</td>
<td>45 364 895.91**</td>
<td>3 540 422.84**</td>
</tr>
<tr>
<td>MeY (checks)</td>
<td>4</td>
<td>2 788 682.60**</td>
<td>1 646 002.29*</td>
<td>1 525 152.21</td>
<td>187 384.92</td>
<td>406 614.66</td>
</tr>
<tr>
<td>MeY (silage types)</td>
<td>7</td>
<td>3 746 239.52**</td>
<td>4 245 712.18**</td>
<td>4 138 216.25**</td>
<td>67 363 448.00**</td>
<td>2 610 147.28**</td>
</tr>
<tr>
<td>MeY (checks vs. silage types)</td>
<td>1</td>
<td>6 423 473.63</td>
<td>4 513 766.80</td>
<td>25 086 729.13**</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

*a significant at 0.05 level, **significant at 0.01 level.*

### 3.2. Trait correlations and their implications for breeding of silage type sorghum hybrids

Among the plant components influencing DMY, the stem was the most important and also most stable one, contributing to 50% of total DMY at GG and 65% at RH. In comparison, the relative importance of leaves was low. The panicle yield differed strongly between both locations due to the different seed set. While the mean panicle contribution to DMY was only 14% at RH, being less important than leaves, it was 35% at GG, with a genotypic maximum beyond 50%, resembling the plant architecture of silage maize [8], and an absolute panicle yield of more than 10 Mg ha⁻¹ (Table 3). Since DMC between both sites differed more for panicles than for stems and leaves, the 7% higher DMC at GG can be...
explained rather by a higher panicle to stem ratio than by differences in DMC of stems and leaves. Except for panicle DMC, the measurements for all traits correlated between the two sites, with high correlations for PH (r = 0.95**), FMY (0.88**), DTA (0.83**), Stem DMY (0.76**) and total DMY (0.75**), and medium correlations for SD, Panicle DMY, Leaf DMY, Stem DMC and Leaf DMC (r = 0.56**, 0.56**, 0.50**, 0.45** and 0.40**, respectively).

Regarding correlations among traits, 49 of 66 trait combinations showed significant correlations at GG, whereas only 31 were significant at RH (Table 6). Correlations were concordant for both sites in 27 cases. Total DMY was strongly related to PH (r = 0.71** and 0.80**) and stem DMY (r = 0.73** and 0.95**) and moderately correlated with leaf DMY (r = 0.40** and 0.54**) in both environments. However, these high correlations can be mainly attributed to the tall, high-yielding check varieties. Excluding these varieties, the correlation between DMY and PH dropped to r = 0.39* (GG) and r = 0.56** (RH). Further, the impact of stem DMY for total DMY decreased strongly at GG (r = 0.39*), whereas it remained extremely high at RH (r = 0.92**). At GG, the DMC showed a medium positive correlation with panicle DMY and a medium negative correlation with stem DMY, while in RH, panicle DMY was not related to DMC at all. Subject to the condition that seed set and maturity are sufficient, a satisfying DMC can thus primarily be achieved by a substantial contribution of panicle yield. Panicle DMC in GG showed an extremely high negative correlation with DTA, while in both environments the DMC of leaves and stems, being correlated with each other, did not depend on flowering time. Hence, the higher DMC of early-flowering hybrids is due to a higher panicle DMC and panicle proportion to total biomass, and not caused by changes in stem or leaf DMC.

Interestingly, PH did not show any correlation with DTA or DMC. On the other hand, PH was negatively correlated with panicle DMY at both locations. As expected, stem DMY depended strongly on PH, but surprisingly, its correlation with SD was only medium at GG and inexistent for RH. Interestingly, stem DMY showed a strong negative correlation with panicle DMY in GG. At first sight, this finding...
seems to underline the difficulty to combine a high DMY with an optimal DMC, but it should not be taken as a general result. In contrast to other cereals such as wheat and barley, in sorghum a short stature does not imply an effect on harvest index, and taller varieties (biomass types: triangles; bicolor x sudanense cv. Gardavan: circle), Gross- Gerau 2014.

Table 6
Pearson’s correlation coefficient (r) between the observed traits for Gross-Gerau (below the diagonal) and Rauscholzhausen (above the diagonal) in 2014 (n = 41 for DTA and n = 44 for all other traits).

<table>
<thead>
<tr>
<th></th>
<th>FMY</th>
<th>DMY</th>
<th>DMC</th>
<th>DTA</th>
<th>PH</th>
<th>SD</th>
<th>Stem DMY</th>
<th>Stem DMC</th>
<th>Panicle DMY</th>
<th>Panicle DMC</th>
<th>Leaf DMY</th>
<th>Leaf DMC</th>
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<tbody>
<tr>
<td>FMY</td>
<td>1.00*</td>
<td>0.77**</td>
<td>0.59**</td>
<td>0.39**</td>
<td>0.37*</td>
<td>0.26</td>
<td>0.65**</td>
<td>0.72**</td>
<td>0.58**</td>
<td>0.53</td>
<td>0.51**</td>
<td>0.54**</td>
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<tr>
<td>DMY</td>
<td></td>
<td>1.00**</td>
<td>0.67**</td>
<td>0.42**</td>
<td>0.32**</td>
<td>0.27</td>
<td>0.68**</td>
<td>0.66**</td>
<td>0.55**</td>
<td>0.51</td>
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</tr>
<tr>
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<td></td>
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<td>0.48**</td>
<td>0.36</td>
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<tr>
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<td></td>
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<td>1.00**</td>
<td>0.66**</td>
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<td>0.72**</td>
<td>0.77**</td>
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<tr>
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<td></td>
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<td>1.00**</td>
<td>0.66**</td>
<td>0.72**</td>
<td>0.77**</td>
<td>0.67**</td>
<td>0.60</td>
<td>0.63**</td>
<td>0.64**</td>
</tr>
<tr>
<td>SD</td>
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<td></td>
<td></td>
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<td></td>
<td>1.00**</td>
<td>0.64**</td>
<td>0.72**</td>
<td>0.67**</td>
<td>0.60</td>
<td>0.63**</td>
<td>0.64**</td>
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<tr>
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<td></td>
<td></td>
<td>1.00**</td>
<td>0.64**</td>
<td>0.72**</td>
<td>0.67**</td>
<td>0.60</td>
<td>0.63**</td>
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<td>Stem DMC</td>
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<td>0.64**</td>
<td>0.72**</td>
<td>0.67**</td>
<td>0.60**</td>
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<tr>
<td>Panicle DMY</td>
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<td></td>
<td></td>
<td>1.00**</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>1.00**</td>
<td>0.64**</td>
<td>0.72**</td>
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<tr>
<td>Leaf DMC</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.00**</td>
<td>0.64**</td>
</tr>
<tr>
<td>Leaf DMY</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.00**</td>
</tr>
</tbody>
</table>

*Significant at 0.05 level, **Significant at 0.01 level.

4. Conclusions
Although originally a tropical plant, due to its diverse benefits, sorghum may also gain importance as a biogas crop under temperate conditions. We consider the silage types described in this work as a superior alternative to the currently existing variety types used for this purpose. With a panicle proportion of 40–50% of total DMY and a height suitable for chopper-harvesting without difficulties, they offer the advantages of enhanced DMC and MeC similar or even superior to silage maize. Thanks to their significantly higher MeC, silage types may outperform biomass varieties in terms of MeY, in spite of hitherto slightly lower DMY. However, the difference in DMY between the best performing biomass check and the highest yielding silage type experimental hybrid (Fig. 4) at GG (2014) was 15%, but the DMC of this silage type was 10% higher, thanks to a panicle: stem ratio of 1:1 instead of 1:10. Hence, silage types are expected to match biomass types regarding DMY in the near future, and a goal of 30 Mg ha⁻¹ and, assuming a MeC of 400 l kg⁻¹, a total MeY of 12 dam³ ha⁻¹ seems to be realistic.

Fig. 3. Scatter-plot showing the performance in methane yield (MeY) and methane content (MeC) of 8 representative silage type experimental hybrids (squares) and 5 check varieties (biomass types: triangles; bicolor x sudanense cv. Gardavan: circle), Gross- Gerau 2014.
fully exploit these benefits, seed set and maturity at dough stage for silage harvest are considered mandatory and represent critical factors of sorghum production in cooler environments. Therefore, principle breeding goals are chilling tolerance during juvenile development to permit earlier sowing and enhance yield potential as well as chilling tolerance during flowering to guarantee high seed set and grain yield. The development of adapted parental lines combining adequate grain and biomass yield using an efficient approach, e.g. GCA tests, are considered the best breeding strategy. The adaptation of sorghum to cooler climates faces similar challenges than that of maize in the last century, however, thanks to novel molecular breeding tools, this is expected to be achievable in a significantly shorter time.

Acknowledgments

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References

Chapter 3:

Publication II

‘Towards enhancement of early-stage chilling tolerance and root development in sorghum $F_1$ hybrids’


CHILLING/FREEZING STRESS

Towards Enhancement of Early-Stage Chilling Tolerance and Root Development in Sorghum F1 Hybrids

S. Windpassinger, W. Friedt, I. Deppé, C. Werner, R. Snowdon & B. Wittkop

Department of Plant Breeding, Research Center for BioSystems, Land Use and Nutrition (IFZ), Giessen, Germany

Keywords
chilling tolerance; combining ability; heterosis; hybrid performance; root development; sorghum breeding

Abstract
Sorghum (*Sorghum bicolor* L. Moench) is regarded a drought-tolerant alternative to maize as a bioenergy and fodder crop, but its early-stage chilling sensitivity is obstructing a successful implementation in temperate areas. While several studies have identified quantitative trait loci (QTL) underlying chilling tolerance-related traits in sorghum lines, little is known about the inheritance of these traits in F1 hybrids. We have conducted a comprehensive approach to analyse heterosis, combining ability and the relation between line *per se* and hybrid performance for emergence and early shoot and root development comprising both field trials and controlled environment experiments including chilling tests. To our best knowledge, this is the first study analysing heterosis for sorghum root parameters under chilling. Our results show that most traits are heterotic and that the mid-parent values are rather poor predictors of hybrid performance. Hybrid breeding programmes should focus on efficient GCA tests and the establishment of genetically diverse pools to maximise heterosis rather than on a too strict selection among lines based on their *per se* performance. The medium-to-high heritabilities estimated for seedling emergence and juvenile biomass suggest that a robust breeding progress for these complex traits is feasible.

Introduction

Sorghum (*Sorghum bicolor* L. Moench) is regarded a promising alternative to maize as a bioenergy and fodder crop in temperate climates such as Central and Western Europe, thanks to its tolerance to abiotic stresses (e.g. Berenji and Dahlberg 2004, Promkhambut et al. 2010, Tari et al. 2013, Schittenhelm and Schroetter 2014), nutrient efficiency (Subbarao et al. 2009) and pest resistance (e.g. to *Diabrotica*, Oyediran et al. 2004). Being a C4 plant of tropical origin, sorghum is sensitive to temperatures below 15 °C, especially during germination and juvenile stages (Yu and Tuinstra 2001). An improved chilling tolerance is thus mandatory for a successful adaptation to higher latitudes, but would also be beneficial for subtropical regions where sorghum is already well established, since earlier sowings in spring would allow a better utilisation of winter moisture (Patane et al. 2006). Chilling adversely affects nearly all physiological and developmental processes in plants, but the photosynthetic apparatus is considered most sensitive, especially when chilling, as it frequently occurs under field conditions in late spring, coincides with high light intensity (Wise 1995, Fracheboud et al. 2004). Root development and structure have also been highlighted as crucial for chilling tolerance in maize (Hund et al. 2008, 2012) and sorghum (Bekele et al. 2014), which differ notably in their juvenile root development. In contrast to maize, nodal roots develop quite late in sorghum, and until 4- to 5-leaf stage, water and nutrient uptake relies only on the primary root and its respective lateral roots (Singh et al. 2010). Fortunately, there is a broad variation for chilling tolerance among different sorghum accessions, and tolerant sources have been identified (Salas-Fernandez et al. 2014). Several recent studies have gained first insights into the genetic architecture of this trait, finding several quantitative trait loci (QTL) of interest (Knoll et al. 2008, Burow et al. 2011, Fiedler et al. 2012, Bekele et al. 2014), which may be useful for marker-assisted selection in the future. However, because most commercial varieties are hybrids, for breeders it is essential to learn more about the inheritance of chilling tolerance traits to select the most appropriate...
parental lines. The superiority of hybrids in this regard was shown shortly after the advent of the first commercial sorghum hybrids in the 1950s (Pinthus and Rosenblum 1961, Blum 1969). More recently, Yu and Tuijnstra (2001) and Tiryaki and Andrews (2001) confirmed heterosis for chilling tolerance traits and provided first valuable insights regarding their inheritance. However, the results of these studies were not concordant, and either only field experiments (Yu and Tuijnstra 2001) or controlled environments (Tiryaki and Andrews 2001) were used for scoring. We have analysed a broad set of parental lines along with their factorial crosses under field trials with different sowing dates as well as under contrasting temperature regimes in controlled environments, aiming at a more comprehensive understanding of combining ability and the correlation between line per se and hybrid performance for emergence, shoot and root development.

Besides its importance for designing breeding strategies, the correlation between hybrid and mid-parent performance is crucial for deciding whether QTL mapping studies for chilling tolerance should focus on inbred lines or rather on hybrids (Bhosale et al. 2007).

Materials and Methods

Germplasm

Four cytoplasmic male sterile (CMS) seed parent lines (females), 16 diverse pollinator lines (males) and their factorial crosses (n = 64) were analysed (Table 1). Due to limited seed availability of a few crosses, the factorial was not complete for all trials. While the females are breeding lines from the USA and Mexico, the males are of very different origin. Two males are recombinant inbred lines (RILs) derived from a cross between the sweet sorghum line SS79 and the grain sorghum line M71, developed by Dr. Willy Wenzel, Potchefstroom (South Africa), and described previously by Shiringani et al. (2010), whereas the remaining ones originate from the USDA Sorghum Conversion Program (Stephens et al. 1967) and are of diverse origin (see Table 1). To exclude the influence of different environments on seed traits, all seeds were taken from one seed lot, which was produced in Puerto Vallarta, Mexico, under optimal conditions and harvested in April of 2013. Further, seeds were treated with MaximXL™ (Syngenta, agent fludioxonil) at the label rate to avoid fungal infections distorting the results.

Field trials

Field trials were conducted at two sites in Germany during the years 2013 and 2014: Poel (PL, 53°9’9”N, 11°4’7”E, 19 m a. s. l.), a small island near Wismar in the Baltic Sea, is characterised by a maritime climate with retarded warming in spring and light soils (loamy sand); Giessen (GI, 50°6’0”N, 8°6’5”E, 158 m a. s. l.), located in the Lahn River valley in Western Germany, has heavy clay soils and higher daily temperature amplitudes (Table 2). In 2013, the trials were sown at the recommended (‘normal’) planting dates for sorghum at these locations and chilling stress was only light, while in 2014, sowing was approx. 1 month earlier and, in consequence, growth conditions were harsh. At both sites, a randomised complete block design (RCBD) with two replications was used. Entries were grown in single-row plots (2.5 × 0.5 m) at GI and

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Overview of the parental lines used in the study (origin and subspecies of conversion lines according to Dr. William L. Rooney, personal communication)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Line</td>
<td>Sex</td>
</tr>
<tr>
<td>A1102</td>
<td>Female</td>
</tr>
<tr>
<td>A1104</td>
<td>Female</td>
</tr>
<tr>
<td>AK011</td>
<td>Female</td>
</tr>
<tr>
<td>ATX623</td>
<td>Female</td>
</tr>
<tr>
<td>SC 136</td>
<td>Male</td>
</tr>
<tr>
<td>SC 207</td>
<td>Male</td>
</tr>
<tr>
<td>SC 299</td>
<td>Male</td>
</tr>
<tr>
<td>SC 525</td>
<td>Male</td>
</tr>
<tr>
<td>SC 538</td>
<td>Male</td>
</tr>
<tr>
<td>SC 569</td>
<td>Male</td>
</tr>
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<td>SC 689</td>
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<tr>
<td>SC 695</td>
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<tr>
<td>SC 721</td>
<td>Male</td>
</tr>
<tr>
<td>SC 733</td>
<td>Male</td>
</tr>
<tr>
<td>SC 1104</td>
<td>Male</td>
</tr>
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<td>SC 1160</td>
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<td>SC 1271</td>
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<td>SC 1307</td>
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</tr>
<tr>
<td>SMRL-096</td>
<td>Male</td>
</tr>
<tr>
<td>SMRL-221</td>
<td>Male</td>
</tr>
</tbody>
</table>
double-row plots (2.5 × 1.0 m) at PL, with 0.5 m row spacing, 50 seeds per row and 2 cm sowing depth at both sites. Fertiliser and herbicide applications were executed following good agronomical practice. Approx. 4 weeks after sowing, final emergence was counted, vigour was scored on a scale from 1 (poor) to 9 (excellent) and 10 representative plants per plot were harvested by hand (approx. 1 cm above ground) and dried overnight at 105 °C to determine the shoot dry weight per plant (SDW). At PL, also the days from sowing to the 3- and 5-leaf stage (D3L and D5L) were scored to record the time of juvenile development.

Controlled environment experiments

Five controlled environment experiments were conducted under sterile or semi-sterile conditions (see Table 3). Gel-based root assays were carried out in incubators, while the experiments analysing whole-seedling development under chilling stress were conducted in a climate chamber. Seedling development under warm conditions (control) was assessed in a greenhouse chamber in the summer to receive natural light. The day/night period was set at 14/10 h in all experiments to resemble actual Central European conditions in late spring. Because sterilised sand was used as substrate to facilitate the scoring of root traits, adequate plant nutrition was provided by a repeated application of Murashige & Skoog Basal Salt Mixture™ (Duchefa Biochemie B. V., Haarlem, The Netherlands). For the climate chamber and greenhouse experiments, sowing depth was 2 cm and a RCBD with 4 replications was used.

The two climate chamber chilling stress experiments differed in their setting regarding the onset of cool temperature. During the first one (chilling during emergence, CDE), temperatures were kept constant at 13/10 °C, so that emergence and subsequent seedling development took place under cool conditions. The number of emerged seedlings was counted each day, and 28 days after sowing, the final emergence was scored. The Emergence Index (EI) was calculated as described by Smith & Millet (1964) using the formula:

\[
\text{EI} = \frac{\text{actual emergence}}{\text{potential emergence}} \times 100
\]

### Table 2 Climate data of the field trial sites during the duration of the respective experiments

<table>
<thead>
<tr>
<th>Site</th>
<th>Year</th>
<th>Trial dates (sowing-harvest)</th>
<th>Mean temp. [°C]</th>
<th>Mean max. temp. [°C]</th>
<th>Mean min. temp. [°C]</th>
<th>Absolute max. and min. temp. [°C]</th>
<th>Mean soil temp. [°C]</th>
<th>Min. soil temp. [°C]</th>
<th>Precipitations [mm]</th>
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<tr>
<td>Giessen (GI)</td>
<td>2013</td>
<td>June 5–July 5</td>
<td>17.4</td>
<td>23.3</td>
<td>11.5</td>
<td>34.5/5.1</td>
<td>20.5</td>
<td>17.7</td>
<td>44</td>
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<tr>
<td></td>
<td>2014</td>
<td>May 6–June 5</td>
<td>13.8</td>
<td>19.8</td>
<td>7.8</td>
<td>28.5/2.3</td>
<td>17.3</td>
<td>14.2</td>
<td>68</td>
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<tr>
<td>Poel (PL)</td>
<td>2013</td>
<td>June 5–July 8</td>
<td>16.5</td>
<td>20.7</td>
<td>12.3</td>
<td>31.7/8.3</td>
<td>18.8</td>
<td>15.6</td>
<td>79</td>
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<td></td>
<td>2014</td>
<td>May 6–June 10</td>
<td>15.1</td>
<td>19.4</td>
<td>10.8</td>
<td>30.3/6.7</td>
<td>16.1</td>
<td>10.4</td>
<td>93</td>
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### Table 3 Overview of the controlled environment experiments and gel-based assays

<table>
<thead>
<tr>
<th>Experiment/ Treatment</th>
<th>Temperature conditions</th>
<th>Light</th>
<th>Duration</th>
<th>Pots, plant density and substrate</th>
<th>Traits scored</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chilling during emergence (CDE)</td>
<td>13/10 °C</td>
<td>14 h, 60 W m⁻²</td>
<td>28 days</td>
<td>12 × 12 × 12 cm, 32 seeds/pot, sterilised sand, 4 × fertilised with 100 ml 0.25 × MS¹</td>
<td>Emergence index (EI), final emergence, leaf greenness, root dry weight (RDW), primary root length (PRL), shoot length (SL), shoot dry weight (SDW), vigour</td>
</tr>
<tr>
<td>Chilling after emergence (CAE)</td>
<td>First 10 days after sowing 25/18 °C, then 13/10 °C</td>
<td>14 h, 60 W m⁻²</td>
<td>96 days</td>
<td>12 × 12 × 12 cm, 16 seeds/pot, sterilised sand, 4 × fertilised with 100 ml 0.25 × MS¹</td>
<td>Seedling survival under chilling, root dry weight (RDW), shoot dry weight (SDW)</td>
</tr>
<tr>
<td>Control</td>
<td>25/18 °C</td>
<td>14 h, natural light</td>
<td>14 days</td>
<td>Cylindrical 16.8 × 19.2 cm pots, 1 plant/pot, 4 × fertilised with 100 ml 0.25 × MS¹</td>
<td>Root dry weight (RDW), primary root length (PRL), shoot length (SL), shoot dry weight (SDW)</td>
</tr>
<tr>
<td>Cold Gel Assay (CGA)</td>
<td>13/10 °C</td>
<td>14 h, 105 W m⁻²</td>
<td>28 days</td>
<td>10 plants per gel chamber (23.5 × 23.5 × 1 cm), gels consisting of 6 g agarose and 4.4 g MS¹ per l H₂O</td>
<td>Primary root length (PRL)</td>
</tr>
<tr>
<td>Warm Gel Assay (WGA)</td>
<td>25/18 °C</td>
<td>14 h, 105 W m⁻²</td>
<td>14 days</td>
<td>10 plants per gel chamber (23.5 × 23.5 × 1 cm), gels consisting of 6 g agarose and 4.4 g MS¹ per l H₂O</td>
<td>Primary root length (PRL), lateral root length (LRL), lateral root number (LRN)</td>
</tr>
</tbody>
</table>

¹Murashige & Skoog Basal Salt Mixture™ (Duchefa Biochemie B. V., Haarlem, The Netherlands).
were in the same physiological state (214 days after sowing, because at that time, the seedlings ing conditions for sorghum. Harvest was carried out experiment. SDW and RDW were measured at the end of the onset date of lower temperature, the days of survival to chilling (trait ‘seedling survival’) were calculated for each entry. Based on the symptoms of irreversible chilling injury, such as complete chlorophyll degradation, necrosis or wilting. Based on the onset date of lower temperature, the days of survival to chilling (trait ‘seedling survival’) were calculated for each entry. SDW and RDW were measured at the end of the experiment. In contrast, the goal of the second climate chamber experiment (chilling after emergence, CAE) was to evaluate the reaction of sorghum seedlings to the same temperatures (13/10 °C) after emergence under warm conditions (25/18 °C). The temperature regime was changed 7 days after emergence (10 days after sowing) when the seedlings were in the 2-leaf stage, and stress conditions were maintained until all entries had succumbed to chilling. An entry was scored ‘dead’ when 50 % of the plants showed symptoms of irreversible chilling injury, such as complete chlorophyll degradation, necrosis or wilting. Based on the onset date of lower temperature, the days of survival to chilling (trait ‘seedling survival’) were calculated for each entry. SDW and RDW were measured at the end of the experiment. The control experiment aimed to provide optimal growing conditions for sorghum. Harvest was carried out 14 days after sowing, because at that time, the seedlings were in the same physiological state (2–3 leaves) as in the two previously described chilling stress experiments (CAE and CDE) at harvest. Root structure was analysed in gel-based assays. Gel chambers (23.5 × 23.5 × 1 cm) were γ-rayed and filled with 0.5 l gel, which was prepared under sterile conditions and consisted of 6 g agarose and 4.4 g Murashige & Skoog Basal Salt Mixture™ (Duchefa Biochemie B. V., Haarlem, The Netherlands) per l demineralized H₂O. A vertical space of approx. 5 cm in the gel chamber was not filled to facilitate shoot development. Five seeds per entry (spacing approx. 2 cm) were placed approx. 0.5 cm into the gels. The seedling which showed the slowest germination was excluded from further analyses, so that four plants per entry were scored and considered as replications. Gel chambers were completely wrapped with aluminium foil to avoid any light exposure of the seeds during germination. Germination and subsequent development occurred under controlled conditions in incubators (see Table 3). After germination, the gel-free space provided for shoot development was removed from the foil, so that only the roots remained in the dark. At the end of the experiments, the gels were scanned and root structure was analysed with ImageJ version 1.45 (Rasband 1997-2014). Primary root length (PRL) was measured, and the number of lateral roots (LRN) was counted. The mean of the three longest lateral roots per plant was taken for lateral root length (LRL).

Statistical analyses

A general linear model was used for statistical analyses in which genotypes, females, males, location, year and treatment were considered as fixed and replicates as random effects. Female × male interaction was utilised to compute the specific combining ability (SCA) variance (Yu and Tuinstra 2001).

Broad-sense heritability for emergence, SDW and vigour under field conditions was calculated using the formula:

\[
h^2 = \frac{\sigma_G^2}{\sigma_G^2 + \frac{\sigma_{G \times L}^2}{n_L} + \frac{\sigma_{G \times Y}^2}{n_Y} + \frac{\sigma_{G \times L \times Y}^2}{n_{LYR}}}\]

where \(\sigma_G^2\) is the genetic variance, \(\sigma_{G \times L}^2\) the genotype × location variance, \(\sigma_{G \times Y}^2\) the genotype × year variance, \(\sigma_{G \times L \times Y}^2\) the genotype × location × year variance, \(\sigma_{\text{Error}}^2\) the error variance and \(n_{LYR}\) the number of locations, years and replications.

The heritability of RDW and SDW in the controlled environment experiments was calculated using the formula:

\[
h^2 = \frac{\sigma_G^2}{\sigma_G^2 + \frac{\sigma_{G \times T}^2}{n_T} + \frac{\sigma_{G \times E}^2}{n_{TR}}}\]

where \(\sigma_G^2\) is the genetic variance, \(\sigma_{G \times T}^2\) the genotype x treatment variance, \(\sigma_{G \times E}^2\) the error variance and \(n_{TR}\) the number of treatments and replications.
Results

Reaction to chilling stress

Highly significant differences among entries were observed under both field trials and controlled environment experiments (Tables 4 and 5, Table S1). Only for seedling emergence at GI 2013, no differences were found (Table S1), but this environment was characterised by an overall relatively poor emergence which was caused rather by dry conditions (Table 2) than by chilling. Considering lines and hybrids separately, differences remained significant for most traits. Variation was generally higher among males than females, and for most traits in controlled environment chilling stress experiments (CAE and CDE), performance of males was better (Table 5, Table S3). As expected, SDW was reduced under cold conditions. For the field trials, year effects on SDW were highly significant due to early sowings in 2014.

Table 4 (a) Genotypic variances (mean squares) of field trials and their interactions across all locations and years; (b) Genotypic variances (mean squares) of field trials and their interactions, partitioned into years (2013 and 2014), that is normal and early sowing dates

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>Emergence</th>
<th>Shoot dry weight (SDW)</th>
<th>Vigour</th>
<th>2013 (normal sowing)</th>
<th>2014 (early sowing)</th>
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<td></td>
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<td>48060.90***</td>
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<td>33262.47***</td>
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<td>1.03**</td>
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<td></td>
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<tr>
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<td>44032.87***</td>
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<tr>
<td>SCA (F x M)</td>
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<td>17656.51</td>
<td>0.77</td>
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<td>38.94***</td>
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<td>Loc x GCA (M)</td>
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<td>16639.14</td>
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<td>11052.65</td>
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</table>

*Significant at 0.05 level; **Significant at 0.01 level; ***Significant at 0.001 level.

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Table 5 Genotypic variances (mean squares) from controlled environment experiments

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>Emergence EI1</th>
<th>El1</th>
<th>Vigour Leaf Gr2</th>
<th>Leaf Gr3</th>
<th>Shoot dry weight (SDW)</th>
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<td>CDE3</td>
<td>CDE3</td>
<td>CDE3</td>
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<td>3.93***</td>
<td>6.36***</td>
<td>11.54***</td>
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<tr>
<td></td>
<td>CAE, Ctr: 69</td>
<td>396.13***</td>
<td>23.25***</td>
<td>3.29***</td>
<td>8.14***</td>
<td>7.55***</td>
</tr>
<tr>
<td></td>
<td>CDE: 71</td>
<td>397.36***</td>
<td>21.52***</td>
<td>3.93***</td>
<td>6.36***</td>
<td>11.54***</td>
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<td>8.14***</td>
<td>7.55***</td>
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<td>23.25***</td>
<td>3.29***</td>
<td>8.14***</td>
<td>7.55***</td>
</tr>
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<td>0.60</td>
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<td>7.75***</td>
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<td></td>
<td>CAE, Ctr, CGA, WGA: 14</td>
<td>765.53***</td>
<td>11.56*</td>
<td>2.98**</td>
<td>7.38***</td>
<td>7.75***</td>
</tr>
<tr>
<td>Hybrids (H)</td>
<td>CDE: 63</td>
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<td>6.96***</td>
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<td>5.06***</td>
<td>5.60***</td>
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<tr>
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<td>CGA, WGA: 52</td>
<td>154.06**</td>
<td>6.96***</td>
<td>2.33***</td>
<td>5.06***</td>
<td>5.60***</td>
</tr>
<tr>
<td>M vs. F</td>
<td>1</td>
<td>956.09</td>
<td>246.56***</td>
<td>15.98***</td>
<td>35.78***</td>
<td>19.77***</td>
</tr>
<tr>
<td>L vs. H</td>
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<td>906.10***</td>
<td>119.21***</td>
<td>53.76***</td>
<td>461.82***</td>
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<tr>
<td>F (GCA)</td>
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<td>665.83***</td>
<td>29.14***</td>
<td>10.22***</td>
<td>49.03***</td>
<td>29.83***</td>
</tr>
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<td>M (GCA)</td>
<td>CDE: 15</td>
<td>143.11</td>
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<td>7.80**</td>
<td>3.08**</td>
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<td>6.26***</td>
</tr>
<tr>
<td>F × M (SCA)</td>
<td>CDE: 45</td>
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<td>1.58</td>
<td>1.74*</td>
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<tr>
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<td>CAE, Ctr. 33</td>
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<td>5.29*</td>
<td>1.58</td>
<td>1.74*</td>
<td>3.80***</td>
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<td>CGA, WGA: 34</td>
<td>6.174</td>
<td>5.29*</td>
<td>1.58</td>
<td>1.74*</td>
<td>3.80***</td>
</tr>
<tr>
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<td>3.90</td>
<td>1.18</td>
<td>0.92</td>
<td>1.32</td>
</tr>
</tbody>
</table>

1Emergence index.  
2Leaf greenness.  
3Chilling during emergence.  
4Chilling after emergence.  
5Control.  
6Cold gel assay.  
7Warm gel assay.  
8Lateral root length.  
9Lateral root number.  

*Significant at 0.05 level; **Significant at 0.01 level; ***Significant at 0.001 level.

At GI, the relative SDW in 2014 was only 16.5 % as compared to 2013 implying a sixfold decrease (Table S4), while at PL, the difference was much smaller (77.6 % SDW 2014 relative to 2013), probably due to a higher temperature difference between both years at GI (3.6 °C) than at PL (1.4 °C) and cold night temperatures at GI 2014 (Table 2). Under controlled environments, relative SDW at CDE compared to the control (15.3 %) showed a very similar extent of reduction as between early and later sowing in GI. Year effects were also significant for emergence, but not concordant. At GI, emergence was better in 2014, due to dry conditions in 2013, while at PL, emergence was reduced in 2014 vs. 2013 as expected due to lower soil temperature. Genotype × Location × Year interaction was only significant for SDW of lines (Table 6). In field trials, heritability was medium for emergence and medium to high for SDW and vigour (Table 7). Due to the higher genotypic variance among lines, heritability was higher for lines than for hybrids. For the controlled environments, a higher heritability for lines was observed as well, and heritability for SDW was slightly higher than for RDW. RDW under chilling stress (CDE: control) was reduced to a similar extent as SDW, and the development of lateral roots was completely repressed in the cold gel assay (CGA).

Heterosis

Hybrids performed better than inbred lines in most cases, as indicated by the significant line vs. hybrid effects (i.e. average MPH). However, relative values for SDW under chilling stress (ratio CDE: control, GI 2014: GI 2013) were similar for
lines and hybrids, while relative RDW and PRL were slightly higher in hybrids than in lines (Table S4). For field emergence, average MPH was significant only at early sowing (Table 4). MPH for SDW under field conditions was stronger expressed at PL than GI (Table 8), whereas its magnitude under controlled environment conditions did not depend on temperature and was approx. 60% in all three treatments (Table 9). In contrast, MPH for RDW, even though significant in all three treatments, was higher under cold, and MPH for PRL was manifested only under cold (CDE and CGA). Regarding root structure, significant MPH was found for the length (LRL) of lateral roots, but not for their number (LRN) (Table 5). The finding that LRN is clearly an additive trait is also supported by the high correlation between line per se performance and male GCA (Table 9).

HPH for field traits was only found in one case, and also under controlled environment experiments, HPH was not commonly expressed (Table 9). Most cases of HPH were observed for SDW at CAE and CDE treatments, while for SDW at control and RDW HPH only occurred exceptionally.

Combining ability

Considering all environments, field emergence and SDW were principally affected by GCA effects, even though SCA effects were also significant (Table 4a). Partitioning these traits into normal (2013) and early (2014) sowing (Table 4b), GCA effects remained highly significant in both cases, while SCA effects were only significant for emergence
after early sowing. Under controlled chilling stress (CAE and CDE), female GCA effects were highly significant for all traits (Table 5). Although male GCA effects were also significant except for emergence, the female impact was generally stronger. Only for the trait ‘seedling survival’ (CAE treatment), a higher influence of male GCA effects was observed. In contrast, SDW and RDW in the control treatment were only influenced by male GCA effects, just like root structure characteristics (LRL and LRN) in the gel-based assay (Table 5). While SCA effects were not significant under control conditions, most traits under chilling stress (CAE and CDE) were also influenced by SCA.

The accuracy of the sum of parental GCA for prediction of hybrid performance differed among the traits (Tables 8 and 9). It was high for RDW, especially under permanent chilling (CDE), leaf greenness, seedling survival and root structure traits (LRL and LRN), while it was relatively low for emergence (both field trials and CDE treatment) and EI (CDE treatment). Also SDW was only determined by parental GCA effects to around 50% (except at CAE).

Relationship between line per se and hybrid performance

Under controlled environment conditions, the correlations between mid-parent value (MPV) and hybrid performance were significant for most traits, but only weak to medium. In contrast, under field conditions MPV and hybrid performance for SDW were only correlated at the early sowings of 2014, and no correlation was found for field emergence.

Line per se performance (LP) and GCA under controlled environment conditions were strongly related for males for seedling survival and some root traits, and for females for leaf greenness, RDW under permanent cold (CDE) and SDW at control conditions. However, for the last trait female GCA effects were not significant, so that selection for SDW based on line performance alone

### Table 6 Mean squares of entry, location and year effects and their interactions from field trials at Giessen (GI) and Poel (PL) 2013 and 2014

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Type of entry</th>
<th>d.f.</th>
<th>Emergence</th>
<th>Shoot dry weight</th>
<th>Vigour</th>
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<td>336.93***</td>
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<td>29651.98***</td>
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<tr>
<td>Year H</td>
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<td>82.79</td>
<td>3757198.96***</td>
<td>38.94***</td>
</tr>
<tr>
<td>Entry * Location H</td>
<td>52</td>
<td></td>
<td>149.61*</td>
<td>24586.49***</td>
<td>0.55</td>
</tr>
<tr>
<td>Entry * Year H</td>
<td>52</td>
<td></td>
<td>244.92***</td>
<td>12637.34</td>
<td>0.80</td>
</tr>
<tr>
<td>Location * Year H</td>
<td>1</td>
<td></td>
<td>232352.33***</td>
<td>299721.42***</td>
<td>0.05</td>
</tr>
<tr>
<td>Entry * Location * Year</td>
<td>52</td>
<td></td>
<td>115.46</td>
<td>12847.07</td>
<td>0.67</td>
</tr>
<tr>
<td>Error H</td>
<td>212</td>
<td></td>
<td>97.40</td>
<td>11052.65</td>
<td>0.57</td>
</tr>
</tbody>
</table>

*Significant at 0.05 level; **Significant at 0.01 level; ***Significant at 0.001 level.

### Table 7 Broad-sense heritability estimates for major traits estimated in field trials and controlled environment experiments

<table>
<thead>
<tr>
<th>Type of entry</th>
<th>Field trials</th>
<th>Controlled environments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Emergence</td>
<td>Shoot dry weight</td>
</tr>
<tr>
<td>Lines and Hybrids</td>
<td>0.55</td>
<td>0.66</td>
</tr>
<tr>
<td>Lines</td>
<td>0.60</td>
<td>0.70</td>
</tr>
<tr>
<td>Hybrids</td>
<td>0.53</td>
<td>0.57</td>
</tr>
</tbody>
</table>
would be ineffective. In contrast, under field conditions LP and GCA were not related except for emergence at GI 2014.

Correlations between controlled environments and field trials

To avoid distortions by heterosis, correlations between controlled environments and field trials [means of both locations for early (2014) and normal (2013) sowing dates] were calculated for lines and hybrids separately (Fig. 1). Due to the higher genotypic variance, there were more and stronger correlations for lines than for hybrids. For lines, EI at CDE treatment and SDW in all three controlled environment experiments showed medium-to-strong correlations to field SDW at both sowing dates. Interestingly, PRL was related to field SDW, while there was no correlation for RDW. For hybrids, most correlations between field SDW and traits scored under controlled environment conditions were found for the early sowings of 2014. Surprisingly, final emergence at CDE was closer related to field emergence at regular (2013) than early sowing dates for both lines and hybrids. Further, hybrids with a higher number of lateral roots were slower in their juvenile development (D5L) and less vigorous at normal sowing dates.

Discussion

Heterosis and combining ability for chilling tolerance and juvenile development in sorghum

Our results confirm the heterotic character of sorghum juvenile biomass and seedling emergence under chilling as also described in previous studies (Tiryaki and Andrews 2001, Yu and Tuinstra 2001). To our best knowledge, this is the first study analysing heterosis for sorghum root traits under different temperature treatments, showing that MPH for root growth is stronger expressed under cold. However, HPH was not predominantly observed in our study, contrasting to Yu and Tuinstra (2001), who found, for example, HPH for SDW at early sowing in 75 % of the hybrids. This may be explained by the equal yielding of female and male parents in their study, while in contrast, the females used in our study showed a significantly inferior performance than males for many traits (see Tables 4 and 5, Table S2 and S3), and differences between males with good LP and females were probably too large to allow for HPH.

A detailed discussion on the basic causes of heterosis for chilling tolerance is beyond the scope of this article. However, the similar relative values of parental lines and hybrids suggest that the superiority of hybrids was rather due to heterosis – regardless the environment – than to specific physiological settings. Hence, a strong expression of heterosis is desirable not only for yield traits per se, but also for chilling tolerance and juvenile development. However, it is unclear to which extent a superior early vigour is relevant for final yield. In earlier studies, a significant delay of flowering and maturity was observed when sorghum was subjected to chilling after emergence (Kapangowda et al. 2013, Maulana and Tesso 2013), but this did finally not cause a significant reduction of grain yield (Maulana and Tesso 2013). For maize hybrids, only weak associations between early growth traits and final dry matter yield were found under Central European conditions (Strigens et al. 2012). Nonetheless, sorghum substantially lags behind maize regarding chilling tolerance, and improvements are essential to make it ‘fail-safe’ and

Table 8 Expression of heterosis, GCA prediction accuracy and Pearson’s correlation (r) between line per se performance (LP) and GCA and between mid-parent values (MPV) and hybrid performance for traits evaluated in field trials

<table>
<thead>
<tr>
<th>Item</th>
<th>Giessen (GI)</th>
<th>Poel (PL)</th>
<th>Giessen (GI)</th>
<th>Poel (PL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(%) of hybrids with sign. HPH 1</td>
<td>53</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Max. HPH 2 [%]</td>
<td>53</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Average MPH 3 [%] (all MPV 3-hybrid comparisons)</td>
<td>53</td>
<td>–2.1</td>
<td>10.6***</td>
<td>1.1</td>
</tr>
<tr>
<td>GCA 4 prediction accuracy</td>
<td>53</td>
<td>0.19</td>
<td>0.38</td>
<td>0.56</td>
</tr>
<tr>
<td>Correlation GCA 5 M: LP 6 M</td>
<td>15</td>
<td>0.01</td>
<td>–0.02</td>
<td>0.08</td>
</tr>
<tr>
<td>Correlation GCA 5 F: LP 6 F</td>
<td>4</td>
<td>0.09</td>
<td>0.90*</td>
<td>0.45</td>
</tr>
<tr>
<td>Correlation MPV 3: hybrid performance</td>
<td>53</td>
<td>0.02</td>
<td>0.15</td>
<td>0.06</td>
</tr>
</tbody>
</table>

1High-parent heterosis.
2Mid-parent heterosis.
3Mid-parent value.
4General combining ability.
5Line per se performance.
enhance the overall yield potential thanks to earlier sow-
ing, even though direct associations between juvenile
chilling tolerance and final yield may not be traceable
under all conditions.

The differences in the relative importance of female
and male GCA effects for the same traits under different
temperature conditions may be due to different ratios
of heterotrophic and autotrophic growth. Heterotrophic
growth is determined by a prevalence of seed traits
(Bhosale et al. 2007), which are determined to a higher
extent by the female parent (Yu and Tuinstra 2001),
while at autotrophic growth, the male parent is theoreti-
cally of equal importance. Under controlled chilling stress
(CAE and CDE), the impact of female GCA on RDW
and SDW was stronger, suggesting a principally hetero-
trrophic growth due to the inhibition of photosynthesis.
In contrast, under warm conditions (control) these traits
were influenced by male GCA effects only, probably due
to an earlier change to autotrophic growth and hence a
reduced importance of the female seed parent. Male
effects were also stronger for seedling survival, which is
an indicator of chilling tolerance at autotrophic growth.
However, the lack of female GCA effects on RDW and
SDW at autotrophic growth (control) and root structure
(LRL an LRN) should not be generalised, as it probably
just reflects the low variation among the females used in
this study. Final emergence and EI were principally deter-
mined by female GCA effects, as expected due to high

<table>
<thead>
<tr>
<th>Item</th>
<th>n</th>
<th>Em. 1 CDE 4</th>
<th>El 2 CDE 3</th>
<th>Vig-our CDE 4</th>
<th>Leaf Gr 3 CDE 4</th>
<th>Shoot dry weight (SDW) CDE 4</th>
<th>CAE 5</th>
<th>Ctr 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hybrids with sign. HPH 11</td>
<td>CDE: 64 CAE, Ctr.: 51</td>
<td>1.6%</td>
<td>4.7%</td>
<td>1.6%</td>
<td>1.6%</td>
<td>29.7%</td>
<td>41.2%</td>
<td>3.9%</td>
</tr>
<tr>
<td>max. HPH 11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>average MPH 12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GCA prediction accuracy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Correlation GCA M: LP M</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Correlation GCA F: LP F</td>
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<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Correlation MPV hybrid performance</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Emergence.
2 Emergence index.
3 Leaf greening.
4 Chilling during emergence.
5 Chilling after emergence.
6 Control.
7 Cold gel assay.
8 Warm gel assay.
9 Lateral root length.
10 Lateral root number.
11 High-parent heterosis (=F1- better performing parent).
12 Mid-parent heterosis (F1- mid-parent value).
13 General combining ability.
14 Line per se performance.
15 Mid-parent value.
importance of seed traits. Due to the lack or the low extent of male GCA contribution and SCA effects, the sum of parental GCA is not an adequate predictor for these traits. Also SDW (except at CAE treatment) was only determined by the sum of parental GCA effects to approx. 50 %, which is unexpected, as GCA is considered a good predictor for biomass yield of forage sorghum hybrids (Blum 1968, Tarumoto 1969, Grewal and Paroda 1974, Ross et al. 1979, Windpassinger et al. 2015). However, the deviation during juvenile development can be explained by a higher female impact and SCA effects especially under chilling. Studies on maize (Hodges et al. 1997, Strigens et al. 2012) also showed a high influence of SCA on emergence and early biomass. In contrast, root traits, leaf greenness and seedling survival of hybrids can be reliably predicted by the sum of parental GCA effects, facilitating the identification of superior combiners among the breeding lines.

Controlled chilling stress experiments as predictors for field performance

The medium-to-strong correlations for line SDW among and between all controlled environments and field trials suggest that the variation among our lines for shoot development at juvenile stages is maintained independently from temperature conditions, supported by the high heritability for this trait. Hence, selection for vigorous lines could be
theoretically conducted in any environment, although variation is higher under cold. Because also for hybrids SDW at CDE was related to field performance, scoring of this trait under controlled permanent chilling stress may be useful for pre-selection prior to field testing. Final emergence at CDE showed a stronger correlation to field emergence at normal than early sowing. Probably, conditions of CDE (13/10 °C) were not harsh enough to simulate field emergence in cold and heavy soils after early sowing. For comparison, Yu et al. (2004) observed the highest correlation between field emergence and growth chamber emergence at 11/8 °C, while Salas-Fernandez et al. (2014) reported the result of a 7-day cold test at 10 °C in non-sterilised soil as the best predictor for early field emergence.

The finding that hybrids with a higher number of lateral roots (LRN) were slower in their juvenile development (DSL) under field conditions suggests trade-off effects between root and shoot development. However, particularly under drought stress, a higher number of lateral roots may be beneficial for seedling establishment, as indicated by the specific correlation of LRN to field emergence in GI 2013 (r = 0.35*). Seedling survival was suggested as the best surrogate trait for selection due to its correlation between both emergence and biomass under chilling stress in a sorghum RIL-population (Bekele et al. 2014). In contrast, in our study seedling survival showed basically an intrinsic correlation to SDW and RDW in the same experiment (CAE) and was not related to field performance. As outlined above, survival can be explained as the ability to maintain metabolism at autotrophic growth under cold, underlying a different genetic control than emergence and heterotrophic growth. Temperature conditions at CAE were chosen to be consistent with CDE and to allow a maximal variation, based on the results of Bekele et al. (2014). Under field conditions, maintenance of autotrophic growth under chilling stress is a crucial factor, as a fast emergence is fruitless if the seedling subsequently succumbs to chilling. However, even at earlier sowing dates, temperature will not be constantly low but rather tends to rise with days after sowing since spring advances. Hence, to simulate field conditions, a short but stronger chilling especially at night would be more realistic.

Implications for developing appropriate breeding strategies
Mid-parent value (MPV) is a poor predictor of hybrid performance for chilling tolerance traits in sorghum, which is
not surprising, as very similar results were found in maize (Hodges et al. 1997, Presterl et al. 2007, Strigens et al. 2012, Revilla et al. 2014). At first sight, these findings suggest influences of both additive and dominance genetic effects (Smith 1986). However, due to the stronger influence of female parents at emergence and heterotrophic growth and the theoretically equal influence of females and males at autotrophic growth, MPV does not seem the best criterion to predict hybrid performance, anyway. For a successful establishment of sorghum in temperate Central Europe, a satisfying and fast emergence in cold and heavy soils, a rapid juvenile growth at variable conditions, both warm and suboptimal, and the ability to withstand short early-summer periods of severe chilling with night temperatures close to freezing are crucial requirements. For the grower, earlier sowings make no sense if the seedlings just emerge and survive but then stagnate in growth, as the field would rapidly be overgrown by weeds better adapted to low temperatures. Hence, hybrids must be superior at both the heterotrophic and autotrophic stages to achieve maximal growth during juvenile development.

Hybrid emergence is mainly determined by female GCA effects, but deviations due to SCA effects are to be expected under chilling at early sowings. Unfortunately, variation for emergence was low among the sorghum females used in our experiments, and a correlation between female LP and GCA was only found in one field trial. Testing more females comprising a higher variation, possibly a more stable correlation between female LP and GCA might be detected. Emergence of male parents is less important for their hybrids than that of females; however, it should not be completely neglected.

Prediction of hybrid shoot biomass at juvenile stage, being an indicator of fast and robust development, can rather be based on GCA than on LP (Fig. 2). In consequence, future studies on juvenile biomass should focus on testcross rather than on line performance, and genomic selection approaches may be more efficient than QTL studies. However, sorghum breeders need to continuously improve their line material regarding early vigour, as this will also result in better performing hybrids. In our study, the poor performance of females probably was a limiting factor of hybrid performance and HPH expression. Hence, both pools should be improved, as a good chilling tolerance of males only would not be sufficient.

The root weight of hybrids is predominantly determined by GCA effects, and the correlation between LP and GCA for this trait was high for females at heterotrophic growth (constant chilling) and for males at autotrophic growth (control), clearly facilitating future breeding. Further, the number of lateral roots (LRN) in hybrids, being a non-heterotic trait, depends strongly on LP. Unfortunately, the scoring of root traits is too laborious to be implemented in a breeding programme, so that marker-assisted selection, in this case based on LP, would be extremely helpful. Nevertheless, as the number of female (CMS) lines in a commercial sorghum breeding programme is usually limited anyway, an analysis of their root traits might be worthwhile. Leaf greenness is an indicator of photosynthetic activity, representing a key trait for chilling tolerance. In our study, a simple visual scoring was utilised to grade variation for this trait. Thanks to the clear predominance of GCA effects, a high correlation between female LP and GCA and the easy scoring, selection for this trait on female lines is considered recommendable. Selection for seedling survival under chilling at autotrophic growth can be conducted on LP per se. Due to the specific temperature conditions, no correlation of seedling survival under controlled conditions to field performance was observed in our study. For breeding programmes, application of short, but intense chilling is probably more promising. The possibility of marker-assisted selection based on QTL studies for LP would also be advantageous to enhance this trait.

In addition to an enhanced LP, a strong heterosis is the second key for early-stage chilling tolerance. Because hybrid breeding programmes generally aim at a maximum
exploitation of hybrid vigour, this precept should not imply difficulties, even though heterosis for early (seedling) vigour, yield components and yield itself may not be directly associated. Unfortunately, heterotic groups are not as clearly defined in sorghum as in maize (Jordan et al. 2003), but new sequencing technologies may assist breeders to establish such groups in the future.

Conclusions
An enhancement of early-stage chilling tolerance of sorghum is crucial for its successful adaptation as an alternative crop in temperate climates. Our results show that chilling tolerance and juvenile development of sorghum hybrids are heterotic traits for which the mid-parent values are poor predictors. Thus, hybrid breeding programmes should focus on efficient GCA testing and the development of genetically distinct pools to maximise heterosis. A too strict selection among parental lines based on their per se performance is not considered recommendable, even though a certain level of chilling tolerance on the female side should be desirable for seed production.

Hence, whole genome association studies and genomic selection for GCA seem to be a more efficient approach for future breeding research than QTL studies based on line per se performance. The medium-to-high heritabilities for seedling emergence and juvenile biomass estimated in field trials suggest that a significant breeding progress for these traits should be feasible in the near future.

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We express our gratitude to Dr. William L. Rooney (Texas A & M University) for providing USDA sorghum conversion lines. Further, we thank Norddeutsche Pflanzenzucht Hans-Georg Lemke KG (NPZ) for conducting field trials at Poel and Bärbel Frenz and Mona Nehl for excellent technical assistance. This research was funded by FNR (Fachagentur Nachwachsende Rohstoffe e. V., Germany) grants 22006911 and 22007011.

References


Tarumoto, I., 1969: Studies on forage sorghum breeding. V. Heterosis and combining ability in F1 hybrids between male-sterile lines and other morphological groups. JPN J. Breed. 19, 94–99.


Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1 Genotypic variances (mean squares) from field trials in Giessen and Poel for all observed traits and years.

Table S2 Descriptive statistical traits from field trials in Giessen and Poel for all observed traits and years.

Table S3 Descriptive statistical traits from controlled environment experiments.

Table S4 Relative values [%] of different traits, comparing chilling during emergence and control experiment and early and normal sown field trials, respectively.
4 General Discussion

Thanks to its resilience, versatility and potential for genetic enhancement, sorghum is expected to be of increasing importance for global food and energy security. There are all prerequisites for a robust breeding progress, sufficient investments provided: (i) a huge genetic diversity in the primary and secondary gene pool (Mace et al. 2013b, Muraya 2014, Venkateswaran et al. 2014), (ii) a diploid, fully sequenced genome (Paterson et al. 2009), (iii) a relatively simple inheritance of plant height (Quinby & Karper 1954) and maturity (Quinby & Karper 1945, Quinby 1966) which allows for access to tropical diversity also for temperate breeding (Stephens et al. 1967), (iv) and established hybrid breeding systems comprising several CMS sources for exploitation of heterosis (Stephens & Holland 1954, Schertz 1983).

Experiences in maize breeding show that both systematic exploitation of heterosis by using genetically distinct hybrid parents and improvements of line per se performance are crucial for an optimal breeding progress in yield and adaptation (Duvick 1999), which is considered transferable to sorghum (Packer & Rooney 2014). The following chapters will discuss breeding strategies for temperate adaptation of sorghum on the background of these dogmata, the results of chapter 2 and 3 (Windpassinger et al. 2015 & 2016) and describe which lessons can be learnt from maize breeding.

4.1 Adaptation of maize in Central Europe- a blueprint for sorghum?

‘Few agronomic improvements during the 20th century rival the development of hybrid maize’ (Duvick 2001)

Maize shows that the successful adaptation of an originally tropical C4 plant into temperate environments, presently as far north as Southern Scandinavia, is feasible, suggesting similar possibilities for sorghum. Nowadays, most of the global maize production is yielded in temperate areas of the US, China and Europe, far outside of its origin in tropical Mexico. However, it should be considered that the history of temperate-adaptedness in maize is far longer than in sorghum. Already in pre-Columbian times, maize was spread in temperate North America up to present Southern Canada (Matsuoka et al. 2002), and recent results suggest that divergence between tropical and temperate maize occurred at least 3,400 years ago (Liu et al. 2015). The first maize was probably brought to Europe by Columbus himself after having been collected in the Caribbean, but these landraces remained confined to Southern Spain due to their tropical background (Rebourg et al. 2003). North American early flint types were introduced only shortly afterwards, being recorded in Germany already in
A second wave of introductions of North American *flints* into Central Europe is assumed for the 17th century (Brandolini 1969). Originating from these introductions, local landraces (e.g. *Gelber Badischer*, *Rheintaler*) developed under a strong selection pressure for early maturity and tolerance to cool spring temperatures. However, maize cultivation remained restricted to certain favored regions, as the Upper Rhine Valley in Germany or Alpine valleys in Switzerland, where grain ripening was possible due to frequent warm Foehn winds in fall (Peter et al. 2009). At that time, sorghum was also present in Europe, but remained confined to its southeastern parts with warm summers, and was grown for the particular use of broomcorn (Dahlberg et al. 2011), meaning that there was no direct selection for grain or biomass productivity. Temperate sorghum adapted to cool spring temperatures and short growing seasons developed in Northern China from ~ 800 AD onwards (Klein et al. 2015), and in the 13th century, grain type *kaoliangs* and broomcorn diverged in this area (Kimber 2001). The temperate adaptation of sorghum for modern breeding in the US by the end of the 19th century is much more recent and based on early-maturity due to mutations of the *ma* genes rather than improvements in chilling tolerance, as shown by the superior chilling tolerance of Chinese *kaoliang* compared to US breeding lines (Franks et al. 2006).

The fast expansion of maize acreage in Central Europe after the Second World War can be attributed to: (i) the existence of adapted *flints* and (ii) their heterotic pattern with North American *dents* as a result of geographic isolation (Schnell 1992), which allowed an optimal exploitation of heterosis in well-adapted and high-yielding hybrids. Unfortunately, both prerequisites do not exist yet for sorghum. However, sorghum also has a crucial advantage for temperate adaptation: its flowering time is principally controlled by a few major genes with large impact (see 1.2), which allows for a fast access to extremely diverse, tropical material for temperate breeding, as was successfully proven by the Sorghum Conversion Program (Stephens et al. 1967). In contrast, the genetic architecture of maize flowering time is extremely quantitative, and there is no evidence for any single-large-effect QTL (Buckler et al. 2009), impairing a fast use of germplasm from different latitudes. Enhancements in the quantitative and epistatic trait chilling tolerance (Bekele et al. 2014) are therefore more challenging than breeding for adequate maturity in sorghum.
4.2 Development of adapted lines as basis for hybrid breeding

‘Good hybrids are not found; they are made from good inbreds’ (Troyer & Wellin 2009)

In spite of the exploitation of heterosis for yield and also stress tolerance, the development of well-adapted inbred lines is crucial for hybrid breeding. For grain sorghum, about one half of the yield increase until the 1980s is ascribed to better parents (Doggett 1988), and for maize, adapted open-pollinated (OP) cultivars preceded the success of hybrids in Central Europe. For breeders, it is essential to know to what extent line performance can be used to predict hybrid performance, which is quantified by the correlation between line per se performance (LP) and general combining ability (GCA), or alternatively, mid-parent value (MPV) and hybrid performance. According to quantitative-genetic theory (Smith 1986), the correlation coefficient $r$ (LP, GCA) is expected to be higher for traits with a predominantly additive gene action than for traits with dominant gene effects. The $ma$ genes controlling photoperiodism and flowering time in sorghum are dominant for photosensitivity and late flowering, but among genotypes with the same configuration of $ma$ alleles, flowering time in sorghum is considered largely additive, with a small degree of dominance for early flowering (Kirby & Atkins 1968). Results from the breeding station of Gross-Gerau (GG, 49° 55’ N, 8° 29’ E) obtained in 2013 and 2014 with a set of 4 females, 17 males and their factorial crosses confirm these assumptions. The observed correlation between LP and GCA was $r= 0.87^{**}$ for males and $r= 0.98^{*}$ for females, with a mid-parent heterosis (MPH) of around 2 days or 3% for early flowering, and a similar correlation between MPV and hybrid performance ($r= 0.81^{**}$). Dry matter content is an important trait for silage quality and also of quantitative inheritance, with a correlation between MPV and hybrid performance of $r= 0.73^{**}$ [average of Gross-Gerau and Moosburg (48° 28’ N, 11°56’ E) 2013, see Windpassinger et al. 2015]. The results regarding MPH were not concordant for this trait; while a small degree of heterosis was observed at GG, as expected due to the earlier flowering of hybrids, there was no MPH at Moosburg. Since flowering time is strongly correlated to the dry matter content of panicles, but only weakly related to the dry matter content of shoot and leaves (Windpassinger et al. 2015), this discrepancy may be due to differences in the panicle : shoot ratio between the two locations. Nevertheless, inbred lines can be clustered into different maturity groups analogue to maize, where very high correlations between LP and GCA were observed for flowering time and dry matter content ($r= 0.95^{**}$ and $0.92^{**}$, respectively, Grieder et al. 2012b).
Plant height also showed a high correlation between MPV and hybrid performance ($r=0.67^{**}$), suggesting a predominantly quantitative inheritance if no complementary action of $dw$ genes is involved. The observed MPH of 46% for this trait permits the production of silage types with a plant height between 200 and 300 cm, defined as a novel ideotype for biogas generation (Windpassinger et al. 2015), using hybrid parents which are still suitable for mechanical harvest. Biomass productivity and increased height need to come from the restorer side, since A-lines should be short grain types suitable for combine-harvesting which are not taller than the male lines to ensure a proper pollination in hybrid seed production. Hence, breeders must know to what extent the height of males is reflected in their hybrids. In this case, the height of males (LP) was strongly related to their GCA ($r=0.74^{**}$, calculated for the factorial crosses of 45 males on 3 females, means of Gross-Gerau and Moosburg 2013) as expected, due to the high correlation between MPV and hybrid performance. For maize, similar results were reported, with a correlation between LP and GCA of 0.77** (Grieder et al. 2012b).

While selection for maturity and plant height can reliably be conducted on lines, it is uncertain if a selection on methane content (MeC) of lines is efficient. The crucial trait is methane yield per area, which depends on both dry matter yield and MeC, and while silage types had a significantly higher MeC than biomass types, variation within one group of ideotypes was low (Windpassinger et al. 2015). The execution of batch trials to assess MeC is laborious and time consuming, and would only be worthwhile on parental inbred lines if there was a high relation to hybrid performance. High-throughput near-infrared spectroscopy (NIRS) based measurements which permit the calculation of theoretical methane contents are a more efficient approach for practical purposes. For biogas maize, a medium correlation ($r=0.59^{**}$) between LP and GCA for MeC was found (Grieder et al. 2012b). For sorghum, the corresponding correlation for this trait in our experiments was lower ($r=0.43$), probably due to the small number of lines evaluated here (8 restorer lines tested on 4 females in a factorial design, means of Gross-Gerau 2013 and 2014). There was no mid-parent heterosis for MeC; in contrast, the MeC of hybrids was slightly lower (-8% on average), which might be due to the taller stature of hybrids and a reduced panicle: shoot ratio.

The enhancement of early-stage chilling tolerance is of utmost importance for a successful establishment of sorghum in Central Europe. In this work, a comprehensive approach to analyze the relation between line per se and hybrid performance was conducted, comprising both field trials and controlled environment experiments (Windpassinger et al. 2016). The results show that most traits are heterotic with a low or medium correlation between MPV and
hybrid performance, indicating non-additive gene action. These findings are in concordance with studies on maize (Hodges et al. 1997, Presterl et al. 2007, Strigens et al. 2012, Revilla et al. 2014). On the one hand, the observed heterosis for emergence under cold and juvenile development at both warm and suboptimal temperatures facilitates a robust breeding progress for adaptation. On the other hand, the poor prediction accuracy of line per se for hybrid performance suggests that a testing only among lines is not sufficient, requiring higher capacities to evaluate also the chilling tolerance of hybrids. Traits which depend highly on seed characteristics, as emergence and heterotrophic growth, are stronger influenced by the female than by the male parent. Hence, a special focus should be laid on the development of cold tolerant A-lines (sterile females). In this study, the poor performance of the used females was probably a limiting factor for expression of high-parent heterosis and hybrid performance.

Yield remains the decisive trait, as a function of all events and plant responses during the whole vegetation period, including stress tolerance, adaptation and adequate flowering time, all based on the genetic potential. Correlations between mid-parent value and hybrid performance for biomass dry matter yield ($r= 0.40^{**}$) and male per se performance and GCA ($r= 0.44^{**}$), respectively, were medium, suggesting that GCA is a far better predictor of hybrid yield than line per se performance. The same is true for chilling tolerance traits; however, there are several points in favor of a rather strong pre-selection based on LP for both yield and stress tolerance. According to Falconer & Mackay (1996), the effectiveness of a pre-selection can be quantified by the relative efficiency (RE), expressed as the ratio of indirect response to selection based on line performance, over the response to direct selection on GCA:

$$RE = \frac{i(LP) \ h(LP) \ r(LP, GCA)}{i(GCA) \ h(GCA)}$$

where $i$ is the selection intensity, $h$ the square root of heritability and $r(LP, GCA)$ the correlation between line per se performance and GCA. Hence, besides $r(LP, GCA)$, the relative efficiency of selection based on LP depends on the ratios $i(LP):i(GCA)$ and $h(LP):h(GCA)$. The heritability greatly depends on the number of environments in which the genotypes are tested. In current breeding programs, test crosses are usually evaluated at more locations than lines, implying a more robust $h(GCA)$. Though, due to a higher genotypic variance among lines and no masking effects of the tester, a higher $h(LP)$ can be expected if lines and test crosses are tested in an equal number of locations or environments, as shown for
seedling emergence and juvenile biomass (Windpassinger et al. 2016). Since the number of lines evaluated in a breeding program tends to be (much) higher than the number of test crosses, $i(LP)$ is usually substantially higher than $i(GCA)$, suggesting a satisfying efficiency of selection on LP. Studies on maize hybrid breeding show that the yield of inbred lines has increased almost twice as fast as heterosis yields since the 1930s, implying a decrease in the relative importance of heterosis (Duvick 1999). Consequently, it seems recommendable for maize breeders to replace preliminary testcross trials with finished-inbred yield trials, saving money and time of testcross production. A stronger focus on line development also leads to a higher genetic diversity and stress tolerance, since the latter one differentiates more among lines than hybrids (Troyer & Wellin 2009). One explanation for heterosis is the dominance theory, which states that deleterious, recessive alleles which tend to accumulate in inbred lines are offset in $F_1$ hybrids due to the effect of the dominant alleles in the other hybrid parent, pointing at a higher heterosis in crosses of genetically distant inbred lines which probably have a more complementary configuration of these alleles (Falkoner & Mackay 1996). The continuous improvement of inbred lines has reduced the number of deleterious alleles, thus lowering the relative magnitude of heterosis and increasing the association between line and hybrid performance (Troyer & Wellin 2009). Recent studies show a medium to high correlation between LP and GCA for maize (e.g. $r= 0.65**$, Grieder et al. 2012b), supporting these assumptions. However, since sorghum breeding for Central Europe is in its infancy, the adaptation of lines is suboptimal, suggesting a higher importance of heterosis and lower correlations of line per se and hybrid performance. This could be shown in this work with $r(LP, GCA)=0.44**$ and a strong average mid-parent heterosis of 64% for biomass dry matter yield, which is higher than the expected average mid-parent heterosis for sorghum grain yield assumed at 30-40% (Duvick 1999). On the other hand, sorghum as a naturally predominantly autogamous crop is far more tolerant to inbreeding than maize, where even the survival of inbreds was critical at the beginning of line breeding a century ago. Hence, gains in yield and adaptation of sorghum lines might be achieved within a significantly shorter time, especially taking into account the support of modern, molecular breeding tools.

While maturity and plant height of inbred lines can be assessed even in small plots at one location, the evaluation of traits with a lower heritability as yield and chilling tolerance is more expensive and requires several locations. Field trials for scoring of chilling tolerance traits may not always work well, since environmental conditions may be too harsh or too mild for an optimal differentiation. Controlled-environment chilling tests can be conducted as a pre-selection of new lines during winter, thus reducing the amount of lines to be tested under
field conditions in spring. In this study, the emergence index (an index describing speed and completeness of emergence) and seedling shoot dry weight of lines at 13/10 °C in a climate chamber showed a relatively strong relation to their juvenile shoot dry weight in early sown field trials (r= -0.68** and r= 0.69**, respectively). In contrast, correlations between emergence at 13/10 °C in sterilized sand and field emergence were low, suggesting that the controlled conditions were not harsh enough to screen efficiently for this trait. Recently, a 7-day cold test at 10 °C in non-sterilized soil was reported as the best predictor for early field emergence (Salas-Fernandez et al. 2014). Marker-assisted selection would be extremely helpful for chilling tolerance. For a pre-selection of hybrid parents, QTL studies should be rather based on tester cross (GCA) than line performance for most traits, due to their heterotic character and low correlations between LP and GCA (Windpassinger et al. 2016). However, for a sustainable breeding progress towards chilling tolerance, enhancements in LP are essential, requiring QTL studies on lines which might also enable the introgression of desirable genes from sources of chilling tolerance such as *kaoliang* accessions into elite lines (Knoll et al. 2008). Several QTL for germination, emergence and early growth traits under chilling stress have been identified in biparental populations (Knoll et al. 2008, Burow et al. 2011, Bekele et al. 2014) and on a diversity panel comprising 194 biomass breeding lines from KWS company (Fiedler et al. 2012 & 2014). The feasibility of marker-assisted selection in half-sibling populations has been shown (Knoll & Ejeta 2008) and candidate genes playing a role for cell division and growth under chilling stress have been identified (Bekele et al. 2014). Nevertheless, in spite of regions on chromosome 1 (SB-01) which were found in all of these studies, most of the detected QTL are not transferable, due to environmental effects and the specific genetic background of biparental populations. Most recently, in a genome-wide association study on a diverse sorghum mini core collection (242 lines) from ICRISAT, only one marker locus was significantly associated with low temperature germination and none with vigor (Upadhyaya et al. 2015). The lack of stable marker-trait associations in genetically diverse accessions may also be due to different physiological mechanisms to cope with stress which depend on distinct alleles, but nevertheless result in similar phenotypes (Maccaferri et al. 2011). Genetic studies on the quantitative traits of sorghum sugar content (brix) and juice yield gave similar results as for chilling tolerance. While numerous significant QTL for brix were identified in biparental populations (Ritter et al. 2008, Murray et al. 2008a, Murray et al. 2008b, Shiringani et al. 2010, Mocoeur et al. 2015), only one significant QTL for this trait was found using a genome-wide association study (Murray et al. 2009), and Burks et al. (2015) found midrib color, a well-known morphological marker for juicy stems, to be more predictive for sugar yield than any SNP marker. For such quantitative traits, Genomic
Selection (GS) may be a more efficient approach. The feasibility of GS for sorghum chilling tolerance has been shown by Bekele (2014) in a proof-of-concept study on a biparental recombinant inbred line (RIL) population, obtaining a cross-validation accuracy of 0.30-0.55. Unfortunately, the prediction accuracy of GS has been shown to drop dramatically if applied on genetically diverse material instead RIL-populations (Schön et al. 2015), representing a principal limitation in practical breeding programs. Solutions to overcome this inconvenience may be found in the near future, as current research on the optimization of GS for sorghum biomass traits on a diversity panel demonstrates (Yu et al. 2015).

4.3 The potential of heterotic pattern in sorghum versus maize

The single most important element of a breeding program is the recognition and utilization of heterotic pattern. This recognition both simplifies and increases the efficiency of all subsequent operations (Sprague, 1984)

Even though in the previous chapter a decline in the relative importance of heterosis in maize breeding has been described, all hybrid breeding programs are and will be based on it. For an efficient and directed exploitation of heterosis, the discovery or development of heterotic pattern is a prerequisite. Once established, breeding progress can principally be achieved by continuous improvements of lines within the heterotic groups as outlined in 4.2. The concept of heterotic patterns and groups has first been developed in maize breeding. According to Melchinger & Gumber (1998), a heterotic group is ‘a group of related or unrelated genotypes from the same or different populations, which display similar combining ability and heterotic response when crossed with genotypes from other genetically distinct germplasm groups’, and the term heterotic pattern refers to ‘a specific pair of two heterotic groups, which express high heterosis and consequently high hybrid performance in their cross’. Heterotic patterns in maize were discovered - or rather developed - during the introduction of commercial single cross hybrids in the US in the mid-1960s, which implied the need for female parents with a satisfying per se performance to make hybrid seed production economically viable. At that time, there were only few maize inbred lines meeting this requirement, and the inbreds B14, B37, B73 and their respective combinations, developed from the Iowa Stiff stalk synthetic (BSSS), became the preferred female parents (Tracy & Chandler 2004, Reif et al. 2005). Inbreds that combined well with BSSS were assigned into the male pool, forming a pre-heterotic pattern germplasm which was not clearly structured at the beginning, but diverged with ongoing inter-population improvement (Duvick et al. 2004). Today, maize breeding for Central Europe is based on the heterotic pattern between well-adapted European flints and
high-yielding dents from the US (see 4.1). In this case, the genetic distance between these two heterotic groups which is a prerequisite of heterosis is the result of a century-long geographic isolation and not of directed breeding as for the heterotic groups used in US maize breeding. The establishment of heterotic groups is sought for in all hybrid crops including sorghum, since it facilitates a reliable, high mean heterosis and an increasing importance of general (GCA) over specific combining ability (SCA) (Reif et al. 2005), thus reducing the number of testcrosses necessary to find the best hybrid combinations.

In sorghum, heterotic groups are not clearly defined yet (Jordan et al. 2003, Menz et al. 2004, Gabriel 2005, Monk et al. 2014), and the number of scientific publications on this issue is surprisingly limited. Early breeding methods and the subsequent use of the CMS system for hybrid production are reasons for their absence. This situation is similar to other hybrid crops relying on CMS systems, such as sunflower (Reif et al. 2013). Prior to the discovery of the CMS system in sorghum in the 1950s, classical line breeding methods were applied in the US, including the practice of intermating cultivars from different origins and working groups which compromised the potential heterotic patterns. In consequence, female A-lines developed from the 1950s onwards had a shared ancestry with the existing male R-lines (Klein et al. 2008). The access to a genetically diverse germplasm provided by the Sorghum Conversion Program in the 1960s would have been a chance to develop heterotic groups based on geographic origin and race, but for practical reasons, breeders preferred to group their material rather based on fertility reaction. Therefore, B- and R-lines have become surrogate groups, and since the development of new A-lines out of B-lines is labor-intensive and time consuming, breeders have focused on R-line development, resulting in a notably higher diversity among elite R- than elite B-lines (Menz et al. 2004). The selection of R-lines based on their combining ability with established A-lines has led to a pre-heterotic pattern comparable to US dent maize in the 1960s (see above). Hybrids made among elite R-lines and among elite B-lines were shown to be significantly lower yielding compared to A x R hybrids, even though these groups did not show a consistent genetic dissimilarity characteristic of actual heterotic groups (Gabriel 2005). Thus, decades of breeding efforts in the US have resulted in the selection of good combiners to the complementary group, but have not entailed phylogenetic divergence as in maize breeding. In contrast, a study on the genetic diversity of breeding lines from India revealed clustering according to fertility reaction (Ganapathy et al. 2012), showing that the concept of heterotic groups applies for that material. Nowadays, the availability of inexpensive molecular markers provides new opportunities for a systematic development of actual, genetically diverse heterotic groups on a global scale also for sorghum. However, a shift from the simple B- and R grouping towards phylogenetic grouping
would require considerable efforts by breeders. It would imply more crosses between related B- and R-lines which are traditionally rather avoided, and additionally a stronger focus on A/B-line development, since a higher diversity on the female side may unlock more heterotic patterns.

The establishment of a new breeding program for adapted sorghum silage type hybrids for Central Europe holds the opportunity for the development of heterotic groups from the very beginning, avoiding the inconveniences of a later shift in grouping as would be the case for existing breeding programs e. g. in the US. In this regard, knowledge about the phylogenetic relationships among the breeding lines is essential. In the present study, a broad diversity set (n=470) comprising representative accessions from the Sorghum Conversion Program (so called ‘conversion lines’), diverse sweet sorghum lines and US breeding lines was genotyped with a 3K-Illumina SNP Chip developed by Bekele et al. (2013). Neighbor-joining method (Saitou & Nei 1987) shows (Fig. 5) that this diversity set clusters into five phylogenetic groups and that genetic relatedness is principally based on geographic origin and morphotype, confirming the results of Brown et al. (2011) and Morris et al. (2013) (see Fig. 3).

**Fig. 5:** Phylogram displaying the genetic relatedness in a sorghum diversity set (n=470) used in this study by neighbor-joining method, showing that clustering into five phylogenetic groups is predominantly based on geographic origin and race (visualization of the phylogram done by Dendroscope 3.4.4 (Huson et al. 2007)).
Hence, this information can help to group new germplasm even if no marker data are available. However, in the present study the phylogenetic group 2, consisting mainly (but not exclusively) of biomass- and sweet sorghum types, does not fit into this scheme, since it is based on the ideotype (crop type) and not on the morphotype (race). In contrast, Ritter et al. (2007) reported that sweet sorghum lines cluster with grain sorghums of similar racial origin, suggesting a polyphyletic origin of sweet sorghum which is also supported by the high diversity found among sweet sorghum accessions (Murray et al. 2009). Also in the present study, sugar and grain types of the race *durra* originating in India cluster together within group 4. Hence, the clustering of sweet- and biomass-types in group 2 may be rather explained by separate breeding programs and their geographic origin in Southern Africa, which was one of the main sources for sorghum germplasm introductions into the USA (Sleper & Poehlman 2006).

The present sorghum diversity set comprises valuable base-material for breeding for Central Europe, since most of the genotypes are photoinsensitive and flower early enough to allow for seed production even at the Gross-Gerau field station (49° 55’ N, 8° 29’ E). Additionally, a high variation for chilling tolerance can be observed (unpublished data). The four CMS-lines used in this study originate in the US and Mexico and cluster in the same group (Fig. 6), which consists principally of *kafirs*. This may suggest that their genetic background traces back to the first introductions into the US where *kafirs* played an important role, underlining the lack of genetic diversity among elite female lines as reported by Menz et al. (2004). Most of the inbred lines in the diversity set were scored for their fertility reaction in A1 cytoplasm. The results show that B- and R-lines can be found in all phylogenetic groups, but with different ratios (Fig. 6).
**Fig. 6:** Phylogram showing the distribution of established females (A-lines, dark red and bold), identified B-lines (possible new females, red) and restorers (blue) across the diversity set (visualization of the phylogram done by Dendroscope 3.4.4 (Huson et al. 2007)).

While R-lines predominate in group 3 consisting mainly of *caudatums*, B-lines occur more frequently in group 1 (*kafirs*) which also harbors the established A-lines, suggesting the potential of a heterotic pattern between these two genetically distant groups. However, the frequency of B-lines also among conversion lines from Ethiopia within group 4 points at the possibility of more than one heterotic pattern.

Heterosis is considered to increase with genetic distance (Falkoner & Mackay 1996), but this theorem is not always empirically traceable, as it has been shown in studies on rice (Yan et al. 2010), rapeseed (Girke et al. 2012) and alfalfa (Riday et al. 2003) which found no significant correlation between genetic distance and heterosis. Other factors such as the adaptation of hybrid parents may have a higher impact on hybrid performance, and important QTL influencing heterosis are rather located in particular chromosome regions and not distributed evenly over the genome as the molecular markers used for calculation of genetic distance (Jordan et al. 2003). For grain sorghum hybrids, studies from Australia (Jordan et al. 2003) and the US (Gabriel 2005) observed relatively low, but significant correlations between the genetic distance of the parental lines and hybrid yield ($r= 0.42^{**}$, Jordan et al. 2003; $r=...
0.32**, Gabriel 2005) and mid-parent heterosis (r= 0.37**, Gabriel 2005). In the present study, the correlation between Rogers’ genetic distance (Rogers 1972) and hybrid performance including mid-parent heterosis (MPH) was analyzed for biomass dry matter yield on 203 experimental hybrids (representing an incomplete factorial of 4 females and 99 males) which were tested at two field sites (Gross-Gerau and Moosburg 2013, the mean of both locations was used for statistical analyses). Hybrid yield was stronger related to MPH (r= 0.69**) than to the mid-parent value (r= 0.33**), but both hybrid yield and MPH were not related to genetic distance (r= 0.11 and r= -0.03, respectively) if the complete set was analyzed. Due to the high variation for plant height among the hybrids, ranging from 120 cm to 304 cm, and the correlation between plant height and biomass yield (r= 0.62** in this case), the hybrids were grouped to minimize the masking effect of plant height (Table 3).

Table 3: Correlation between Rogers’ genetic distance of hybrid parents (0= identical alleles at all marker loci, 1= different alleles at all marker loci) and hybrid biomass yield (dry matter) at Gross-Gerau and Moosburg 2013 (mean of both locations). Hybrids were assigned into six groups depending on their plant height.

<table>
<thead>
<tr>
<th>Plant height of hybrids (cm)</th>
<th>n</th>
<th>Range of Rogers’ genetic distance</th>
<th>Range of hybrid biomass yield (dry matter) (t)</th>
<th>Pearson’s correlation (r) between Rogers’ genetic distance and hybrid yield</th>
<th>Pearson’s correlation (r) between Rogers’ genetic distance and mid-parent heterosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>120-150</td>
<td>33</td>
<td>0.19-0.54</td>
<td>12.0-19.6</td>
<td>-0.18</td>
<td>-0.21</td>
</tr>
<tr>
<td>150-175</td>
<td>45</td>
<td>0.26-0.59</td>
<td>10.6-21.9</td>
<td>0.35*</td>
<td>0.05</td>
</tr>
<tr>
<td>175-200</td>
<td>53</td>
<td>0.25-0.58</td>
<td>12.3-23.1</td>
<td>0.21</td>
<td>0.09</td>
</tr>
<tr>
<td>200-225</td>
<td>35</td>
<td>0.33-0.55</td>
<td>14.2-24.4</td>
<td>-0.02</td>
<td>-0.28</td>
</tr>
<tr>
<td>225-250</td>
<td>28</td>
<td>0.34-0.56</td>
<td>15.3-26.9</td>
<td>0.01</td>
<td>0.04</td>
</tr>
<tr>
<td>&gt;250</td>
<td>9</td>
<td>0.29-0.51</td>
<td>18.4-23.5</td>
<td>-0.52</td>
<td>-0.31</td>
</tr>
</tbody>
</table>

*: significant at 0.05 level

However, genetic distance was not related to mid-parent heterosis in any case, and a significant positive correlation between genetic distance and hybrid yield was only found for the group of 150-175 cm plant height. The reports on the relation of genetic distance to grain yield of sorghum cited above were on entries of a similar plant height and gave a comparable result. In the present study, biomass yield was scored, but the grain yield has a strong impact on biomass yield in this range of plant height (Windpassinger et al. 2015). In contrast, the stem is the most important component of biomass yield in taller hybrids, and its height is principally determined by the configuration of the \( dw \) alleles. Hence, the importance of genetic distance might decrease for taller hybrids, since the most decisive factor influencing
yield is less quantitative. While Jordan et al. (2003) and Gabriel (2005) used elite lines which were well-adapted to the agroclimatic conditions of their studies, in the present case the parental lines differed in their level of adaptation to Central Europe, which may imply a further masking effect on the importance of genetic distance. Moreover, the genetic distance was possibly high enough to allow for satisfying levels of heterosis in most of the crosses, as shown by the high average MPH of 64% in this study which is superior to the average MPH for US sorghum grain yield assumed at 30-40% (Duvick 1999). If more crosses between closely related parents were involved, perhaps a higher correlation between genetic distance and heterosis might be found.

Fig. 6 shows that five phylogenetic groups can be distinguished, and that all females cluster in group 1. In order to assess if these groups differ in their suitability as complementary heterotic groups to the existing females, the four male lines of each group whose factorial crosses on three females had the highest average MPH were compared (Fig. 7).

![Boxplots displaying the mid-parent heterosis (MPH) of factorial crosses between three females (all from group 1) and the four best-combining male parents of each phylogenetic group (see Fig. 5 and 6), respectively.](image)

**Fig. 7:** Boxplots displaying the mid-parent heterosis (MPH) of factorial crosses between three females (all from group 1) and the four best-combining male parents of each phylogenetic group (see Fig. 5 and 6), respectively.

The factorial crosses of the best-combining males of group 3 had a significantly higher MPH than factorial crosses including the best-combining males of group 1 and 5, suggesting a heterotic pattern to females in group 1 which may be useful for further breeding especially due to the predominance of restorers in group 3. The low MPH attained in crosses where males and females belonged to the same group (group 1) underlines the necessity of heterotic groups built on the basis of phylogenetic studies. The observed predominance of GCA over SCA effects for sorghum biomass yield (Windpassinger et al. 2015) facilitates the
identification of superior combiners among the groups by test crosses, which can be used for
the development of new breeding lines in genetically diverse pools which should be
developed and maintained separately from each other. Unfortunately, no Chinese *kaoliang*
were included in the present diversity set. Due to their geographic isolation, they are
considered to form an at least partially separate group (Morris et al. 2013, see Fig. 1), which
might serve as a heterotic group resembling the role of *flints* thanks to their temperate-
adaptedness.

Altogether, these results confirm the usefulness of heterotic groups also in sorghum, even
though genetic distance is not generally related to heterosis. Presently, adaptation is likely to
be more important for sorghum than heterosis, but breeding for adaptation should be done
within heterotic pools from the very beginning.

4.4 Seeking the adequate sorghum ideotype

‘Eventually most plant breeding may be based on ideotypes’ (Donald 1968)

As outlined in 1.4, sorghum is an extremely versatile crop with numerous end-uses. Hence,
several ideotypes to optimize its agronomical properties have been developed according to the
concept of ideotype breeding described by Donald (1968). However, specific environmental
conditions require specific ideotypes, and for bioenergy generation in Central Europe, the
sorghum ideotype remains to be defined. In contrast to a mere selection for yield, the concept
of ideotype breeding involves also the understanding of physiological aspects, and how
morphological traits contribute to them.

Plant height is a genetically relative simple, but highly important agronomical trait. In
contrast to other cereals such as wheat and barley, for sorghum a shorter stature does not
imply significant changes in harvest index (George-Jaeggli et al. 2011), and grain yield is
positively correlated with plant height (Jordan et al. 2003, George-Jaeggli et al. 2011).
Though, this rule seems to apply rather for shorter genotypes, and a maximum productivity
for grain yield is assumed at heights of approx. 175 to 180 cm (Miller 1982, Rao & Rana
1982), which is around the upper limit for combine-harvestable grain types. Analyzing taller
genotypes, a negative correlation between plant height and grain yield was observed in the
present study. Nevertheless, taking into account the later anthesis of taller genotypes, this
relation can also be attributed to the short growing season in Central Europe which does not
allow for proper grain development of late cultivars. Provided that flowering is not too late for
grain maturity, days to anthesis and yield are positively correlated, but only if water supply is
not limited. However, sorghum is principally grown in drought-prone environments, so that earlier cultivars are often preferred since their water use matches better to the available water (Jordan et al. 2003). Tillering can be beneficial or not, also depending on water supply and flowering time. At early flowering with less incidence of drought, tillering is considered advantageous, while it is disadvantageous at late flowering, since tillers require water which would be better used for the grain development of the main stem (Jordan et al. 2015). Under a short growing season in temperate environments, tillering can also be problematic if seeds of later tillers do not ripe. On the other hand, tillering can compensate for poor seedling establishment which is critical under temperate conditions.

For biogas sorghum in Central Europe, no ideotype has been defined yet. This is not surprising, since even for maize there is no consensus among breeders with regard to the optimal phenotype after almost two decades of biogas use. To maximize methane yield per area unit in maize production, it is suggested to focus rather on dry matter yield than on energy density, and to use cultivars with a slightly later maturity than common varieties for the specific region, provided that a dry matter content of at least 28% to ensure a good silage quality is still attained (Grieder et al. 2012a). Further, digestibility traits are considered to be of much less importance, since the retention period in biogas plants is up to 60 days (Weiland 2006) compared to approx. 2 days in the rumen of cattle (Hartnell & Satter 1979), permitting also a fermentation of almost indigestible substances up to a certain degree (Grieder et al. 2012a). However, these assumptions are not directly transferable to sorghum. The methane potential of biogas maize is significantly higher than that of present biomass sorghum varieties grown in Central Europe (Zeise & Fritz 2012), due to the higher starch content even in later maize cultivars and the higher shoot digestibility as a result of a long-term selection for cattle feeding. Even at an equal biomass dry matter yield, the marginal returns of present commercial sorghum varieties are lower than of maize, due to the inferior energy density and lower dry matter contents which imply higher transportation costs (Theiß et al. 2013, Kornatz et al. 2014). These inconveniences may be overcome by a novel ideotype as described in this study, with an enhanced dry matter content and an energy density comparable to maize, which is significantly higher than in presently used sorghum biomass types. Though, to make this sorghum ideotype fully competitive, enhancements in biomass dry matter yield are strictly necessary. The negative correlation between panicle (grain) and shoot yield described in this study generally reflects the challenge to combine adequate energy density and dry matter content with high biomass yield under a short vegetation period. Hence, improvements in early-stage chilling tolerance to allow for earlier sowings and a prolonged period of vegetative
growth before flowering are mandatory. Flowering should not be later than mid of August, to avoid the risk of pollen sterility caused by cold nights, and allow for grain maturity to dough stage at harvest. If flowering is too late, lower temperatures and solar radiation imply a source limitation for grain filling, as was shown for maize (Borrás et al. 2004). For the grower, it would be advantageous to have more precise information regarding the maturity of different sorghum cultivars, based on a maturity group model as successfully established for maize. Sorghum is not only grown as a dedicated crop, but also as a catch crop in bioenergy rotations after rye or barley. In this case, earlier varieties are required, and while drought tolerance is more important than for dedicated sorghum, early-stage chilling tolerance becomes less vital due to the later sowing. Hence, breeding programs for biogas sorghum might diverge on the long term depending on the specific cultivation.

For a satisfying biomass yield, biogas sorghum must not be too short. In this study, a medium correlation between plant height and biomass dry matter yield ($r = 0.62^{**}$, means of Gross-Gerau and Moosburg 2013) was observed on a data basis of $n= 203$ experimental hybrids. However, this relation was mainly due to hybrids shorter than 210 cm. If only hybrids above 210 cm plant height were considered ($n= 71$), the correlation dropped to $r = 0.21^{ns}$. Hence, there is likely no need for biogas sorghum to be as high as present commercial varieties which reach up to 400 cm, especially since the incidence of lodging tends to increase with plant height. Further, an excessive tallness implies difficulties in chopper-harvesting, and experiences in biogas maize show that extremely late and tall varieties were not widely accepted by farmers. Based on these experiences, a plant height between 210 cm and 300 cm seems adequate, which would be in the same range as most maize varieties grown for biogas use. At this plant height, panicles can still contribute to 40-50% of total dry matter, resembling the plant architecture of maize, and therefore improve the methane yield significantly.

For maize, the strong yield increase during the last century is not only attributed to the success of hybrid breeding, but also to a steady increase in plant density (Dhugga 2007). This has been facilitated by changes in canopy architecture. Modern maize hybrids have a much more acute (erect) leaf angle than older ones, which allows them to capture more solar radiation also at higher plant densities (Duvick & Cassman 1999). In contrast, sorghum has an open canopy with wide leaf angles that almost parallel the ground (Xin et al. 2013), and this architecture has remained largely unchanged during the last 50 years (Assefa & Staggenborg 2011). A sorghum mutant with erect leaf angles was already found in 1973 (Singh & Drolsom 1974) and has then been used to improve leaf angle in sorghum breeding (Xin et al. 2013).
Nevertheless, in a recent study a lower grain yield of the erect-leaf mutant compared to the wild type was described (Gitz et al. 2015), but the field trials were conducted in a semi-arid environment in Texas where radiation was not a limiting factor, and the plant density was probably too low for the mutants. For higher latitudes as Central Europe with low solar radiation, a more acute leaf angle would probably be beneficial, avoiding lower leaves being shaded, but eventually its utility will also depend on the adequate genetic background.

4.5 Conclusions

Sorghum is considered as a ‘crop of the future’ and may gain importance also in temperate Europe, especially in the context of increasing temperature and drought events due to climate change. In spite of present drawbacks, its adaptation into cooler environments can be accomplished in the medium term, resembling the expansion of maize which can partially serve as a blueprint for this endeavor. This study has focused on two important aspects to facilitate the establishment of sorghum as a novel crop in temperate Europe. The design of a novel ideotype for biogas use with enhanced energy density and advanced agronomic properties may improve its acceptance by growers and accelerate its establishment also for other uses. A more detailed knowledge about the inheritance of chilling tolerance and the expression of heterosis in F₁ hybrids may help breeders to develop an efficient breeding strategy for this crucial and complex trait. Learning from maize breeding, (i) the continuous enhancement of inbred lines for adaptation, quality and per se performance, (ii) the establishment of heterotic pattern and development of genetically diverse heterotic pools for a systematic exploitation of maximum heterosis seem to be essential components of a successful breeding strategy. For both components, novel molecular breeding tools can be extremely helpful and accelerate breeding progress.
5 Summary

In temperate Europe, sorghum (*Sorghum bicolor* L. Moench) is considered a promising novel bioenergy and fodder crop. Principal advantages compared to maize besides drought tolerance include nutrient efficiency and *Diabrotica virgifera* tolerance. However, the history of temperate-adaptation is much younger for sorghum than for maize, and several drawbacks need to be overcome to make it fully competitive. Presently, its early-stage chilling sensitivity delays sowing and notably limits the available vegetation period and yield potential. Several studies have identified quantitative trait loci (QTL) for chilling tolerance related traits, but up to now very little is known about the inheritance of these traits in *F*<sub>1</sub> hybrids. Since due to the strong heterosis for grain and biomass yield almost all commercial varieties are hybrids, an efficient breeding strategy for the enhancement of chilling tolerance in hybrid cultivars requires a profound understanding of heterosis, combining ability and relation between parental line and hybrid performance for this trait. In this regard, a comprehensive approach comprising field trials and controlled environment experiments was conducted to analyze emergence and juvenile shoot- and root development of four sterile female lines, 16 male lines and their factorial hybrids. The results show that most traits are heterotic and that the mid-parent values are rather poor predictors of hybrid performance. Thus, hybrid breeding programs should focus on efficient general combining ability (GCA) tests rather than on a too strict selection among lines based on their *per se* performance. Nevertheless, to achieve substantial enhancements of sorghum chilling tolerance on the long-term, the development of better adapted inbred lines is essential. The medium to high heritabilities estimated for seedling emergence and juvenile biomass suggest that a robust breeding progress for these complex traits is feasible. Since emergence and early heterotrophic growth are rather determined by the seed parent than by the pollinator, a special focus should be laid on the development of cold tolerant female lines.

For biogas use, another principal shortcoming compared to maize is that current sorghum varieties fail to combine a high biomass dry matter yield with an adequate dry matter content for silage and satisfying methane yield, implying lower marginal returns even when maize biomass yields are matched. In the present study, early-maturing silage type *Sorghum bicolor* experimental hybrids which represent a novel, alternative variety type (ideotype) for biogas use were analyzed regarding their agronomic performance and energy density. The results show that under adequate conditions, they reach higher dry matter contents. Due to a higher ratio of starch containing grains, the methane yield per dry matter unit of silage types is significantly higher than that of existing biomass type standard varieties, which offsets their
presently slightly lower dry matter yield and allows for a methane yield per area unit similar or superior to existing biomass type varieties. However, due to the high importance of grains for yield and quality parameters, seed set and sufficient maturity (dough stage) under cold nights which can induce pollen sterility are critical factors.

Experiences in maize breeding show that both systematic exploitation of heterosis by using genetically distinct heterotic pools of hybrid parents and continuous improvements of line per se performance are crucial for an optimal breeding progress in yield and adaptation. Plant height and maturity of sorghum hybrids are strongly related to their parental lines, so that selection for these traits can be efficiently conducted on inbred lines. In contrast, for hybrid biomass yield the general combining ability (GCA) of inbred lines is a far better predictor than their per se performance, even though with ongoing enhancements of inbred lines their relation to hybrid yield is expected to increase. The observed predominance of general combining ability (GCA) over specific combining ability (SCA) effects for sorghum hybrid biomass yield facilitates the identification of superior combiners. Heterotic groups in sorghum are not clearly defined yet, since heterotic pattern were compromised by early line breeding methods as the intermating of cultivars from different origins and the subsequent use of cytoplasmic-male sterility (CMS) for hybrid seed production, due to which breeders preferred to group their material rather based on fertility reaction than on races (morphotypes) or geographic origin. In this study, the phylogenetic relatedness in a broad sorghum diversity set (n=470) has been shown to be predominantly based on geographic origin and races, confirming the results of previous studies and underlining the possibility to establish heterotic groups based on these characteristics. Generally, the observed correlation between genetic distance of hybrid parents and mid-parent heterosis and hybrid yield has been low or inexistent, possibly due to a higher importance of adaptation traits. However, the maximum level of mid-parent heterosis depended on the phylogenetic group of the male parent, and was comparatively low when both hybrid parents were from the same group, supporting the idea of genetically diverse pools which should be developed and maintained separately from each other.

The results of this thesis contribute to the design of efficient breeding strategies for the adaptation of sorghum as a novel crop in temperate Europe, which is expected to be accomplished in the medium term.
6 Zusammenfassung


Für die Biogasnutzung ist ein weiteres wesentliches Problem, dass es aktuellen Sorghumsorten im Gegensatz zu Mais nicht gelingt, hohe Trockensubstanz-Erträge in der


Insgesamt leisten die Ergebnisse dieser Arbeit einen Beitrag zur Gestaltung wirksamer Züchtungsstrategien zur Adaptation von Sorghum als neue Kulturpflanze in den gemäßigten Klimaregionen Europas, was mittelfristig realisierbar erscheint.
7 References


Assefa Y and Staggenborg SA (2011) Phenotypic changes in grain sorghum over the last five decades. *Journal of Agronomy and Crop Science* 197, 249-257.


REFERENCES


Conner AB and Karper RE (1927) Hybrid vigor in sorghum. *Texas Agricultural Experimental Station Bulletin* 359, Texas A & M University, College Station.


REFERENCES


Murphy RL, Morishige DT, Brady JA, Rooney WL, Yang S, Klein PE, Mullet JE (2014) Ghd7 (Ma₆) represses sorghum flowering in long days: Ghd7 alleles enhance biomass accumulation and grain production. The Plant Genome 7 (2).


REFERENCES


78
Quinby JR and Karper RE (1945) Inheritance of three genes that influence time of floral initiation and maturity date in Milo. *Journal of the American Society of Agronomy* 37, 916-936.


Shiringani AL and Friedt W (2011) QTL for fibre-related traits in grain x sweet sorghum as a tool for the enhancement of sorghum as a biomass crop. *Theoretical and Applied Genetics* 123, 999-1011.

REFERENCES


83


8 Eidesstaatliche Erklärung (Declaration of Honor)


(I declare that the dissertation here submitted is entirely my own work, written without any illegitimate help by any third party and solely with materials as indicated in the dissertation. I have indicated in the text where I have used texts from already published sources, either word for word or in substance, and where I have made statements based on oral information given to me. At all times during the investigations carried out by me and described in the dissertation, I have followed the principles of good scientific practice as defined in the ‘Statutes of the Justus Liebig University Giessen for the Safeguarding of Good Scientific Practice’.)
9 Acknowledgements

At first, I would like to express my deep gratitude towards my “Doktorvater” Prof. Dr. Dr. h.c. Wolfgang Friedt, who has been supporting me in many ways during the last years. I am very grateful that you gave me the opportunity to start as a PhD student almost directly after finalizing my master thesis. Throughout the PhD project, you have always helped me with good ideas and advices, especially regarding the writing and handling of the manuscripts, and it was very kind that your suggestions were always so prompt. I enjoyed very much that we could score many of the field trials together, since your experience was extremely valuable for me and your humor made everything more pleasant. It was very nice that you took your time to accompany me on trips as far away as Mexico. I wish you all the best and hope that we will keep in touch.

Dr. Benjamin Wittkop has done an excellent job as advisor. You have always given me useful recommendations, and simultaneously left me the freedom for my own decisions and experiences. Thank you for trusting in me all the time! I have learnt a lot from you, especially regarding field trials and practical agriculture. It has been a pleasure to be your office mate for the most time of my PhD project, and I am looking forward to continue that hopefully for some more time. It is great that we can laugh about almost everything and thereby alleviate the little day-to-day frustrations in science.

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This project has been carried out in cooperation with Norddeutsche Pflanzenzucht Hans-Georg Lembke KG (NPZ). I am very grateful to Dr. Gunhild Leckband for securing my funding at the end of the project and to Dr. Martin Frauen for his confidence in me to establish a sorghum breeding program and for facilitating seed production in Mexico as well as field trials in Moosburg and Malchow (Poel). At Malchow, I would like to thank Bärbel Frenz for overseeing the field trials and extensive scorings.

I would like to thank the many technicians who have helped me performing the numerous field trials and climate chamber experiments. Most of the work regarding sowing preparations and breeding activities was conducted at the experimental station of Gross-Gerau. Mario Tolksdorf has excellently organized and overseen the practical activities at our breeding
nursery, and instructed me very well in phenotyping. Your delicious coffee has given me new energy after hours of extensive selection and phenotyping and contributed its part to the success of this work. Mona Nehl has also helped me a lot, not only in phenotyping in Gross-Gerau during summer, but also in reliable scoring of the climate chamber experiments in Giessen during winter. All the field team in Gross-Gerau has been very kind and full of good spirit. Special thanks to Janja Andrijevic and Ivka Sander for their skills and patience in spikelet emasculation, which has been crucial to create new breeding material. At the experimental station of Rauischholzhausen, Dr. Lothar Behle-Schalk has always taken his time for extensive discussions and helpful recommendations, and Karlheinz Balzer has reliably scored the field trials, in spite of being threatened by wild boars in several occasions. Daniela Ebinger has been in charge of the batch trials which were vital to discriminate the different ideotypes regarding their methane yield. At the institute of plant breeding, almost all technicians have helped me in one or more occasions. Annette Plank, Birgit Keiner, Nelly Weis and Swetlana Renner have collaborated in the climate chamber trials, and Nelly Weis has given valuable advice regarding the design of rhizotron experiments. Stavros Tzigos has helped me a lot with his experience during DNA extraction and tedious quality controls as PCR. Isabelle Deppé and Christian Werner as motivated master students have contributed a substantial part to the results regarding analysis of sorghum chilling tolerance. All PhD student colleagues have created a good spirit in the institute, and I especially thank Andreas Stahl and Timm Bernhard for the nice time and discussions during coffee breaks and Kai Voss-Fels as R genius for his help in marker analysis.

Last but not least, I would like to express my deep gratitude towards my parents for their love and support, making possible all my education, and towards my wife Amelia for all her love, care and support.
Supplements

Suppl. 1: Genotypic variances (mean squares) from field trials in Giessen and Poel for all observed traits and years.

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<th>Emergence</th>
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<th>Days to 3-leaf-stage (D3L)</th>
<th>Days to 5-leaf-stage (D5L)</th>
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**Suppl. 2:** Descriptive statistical traits from field trials in Giessen and Poel for all observed traits and years.

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<th>Days to 5-leaf-stage (D5L)</th>
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Suppl. 3: Descriptive statistical traits from controlled environment experiments.

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<td>9.1 29.5</td>
<td>7.2 32.3 34.3</td>
<td>10.4 35.8 7.6 13.2</td>
<td>2.8</td>
<td>41.3</td>
<td>81.0</td>
</tr>
<tr>
<td>Hybrids</td>
<td>CDE: 64 CAE, Ctr: 51, CGA, WGA: 53</td>
<td>Mean</td>
<td>90.5</td>
<td>15.2</td>
<td>6.4</td>
<td>6.4</td>
<td>8.3 62.8 54.7</td>
<td>7.7 28.0</td>
<td>5.7 30.0 32.6</td>
<td>8.3 27.5 3.2 9.2</td>
<td>2.2</td>
<td>23.9</td>
<td>66.0</td>
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<tr>
<td></td>
<td>Min</td>
<td>68.8</td>
<td>12.0</td>
<td>4.9</td>
<td>2.8</td>
<td>5.2</td>
<td>46.1 39.9</td>
<td>5.4 22.1</td>
<td>2.5 22.2 18.7</td>
<td>5.0 15.4 0.4 3.6</td>
<td>0.8</td>
<td>6.3</td>
<td>53.5</td>
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</table>
|        | Max | 99.2                         | 19.8          | 8.1   | 9.0   | 10.7     | 77.0 67.9            | 10.0 31.7              | 8.9 48.4 44.6               | 10.4 38.3 7.0 16.0           | 4.2       | 65.0     | 84.3       | ³: emergence index; ²: leaf greenness; ³: chilling during emergence; ⁴: chilling after emergence; ⁵: control; ⁶: cold gel assay; ⁷: warm gel assay; ⁸: lateral root length; ⁹: lateral root number
Suppl. 4: Relative values [%] of different traits, comparing chilling during emergence and control experiment and early and normal sown field trials, respectively.

<table>
<thead>
<tr>
<th>Trait</th>
<th>Type of entry</th>
<th>Ratio chilling during emergence: control</th>
<th>Ratio GI 2013: GI 2014</th>
<th>Ratio PL 2013: PL 2014</th>
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</thead>
<tbody>
<tr>
<td>Shoot dry weight (SDW)</td>
<td>Lines and Hybrids</td>
<td>0.153</td>
<td>0.165</td>
<td>0.776</td>
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<td>Shoot length (SL)</td>
<td>Lines</td>
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<td>0.157</td>
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<td>Hybrids</td>
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<td>0.168</td>
<td>0.750</td>
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<tr>
<td></td>
<td>Lines</td>
<td>0.232</td>
<td>-</td>
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<tr>
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<td>Hybrids</td>
<td>0.283</td>
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<tr>
<td>Primary root length (PRL)</td>
<td>Lines and Hybrids</td>
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<td></td>
<td>Lines</td>
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<td>-</td>
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<td></td>
<td>Hybrids</td>
<td>0.176</td>
<td>-</td>
<td>-</td>
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