Development of Dual Use Maize Cultivars – Corn as Food and Stover for Biogas Production

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I. General Introduction

I.1 Maize cultivation in Germany

Maize (*Zea mays* L.) is one of the oldest cultivated plants in the world. It is the species with the highest grain yield potential in the family of grasses, where it is belonging to (Sangoi 2000). Caused by this fact, maize is one of the most important crops next to rice and wheat for food and feed production in the world (Lütke Entrup et al. 2011).

Within the last years, the area of maize cultivation in Germany has been increasing very fast, as Figure I.1 shows. The total cultivation area of maize was in 2016 around 2.5 million hectare (DMK e.V. 2016a). Maize cultivation thus added up to around 20 % of the total amount of agricultural area. Around 80 % of the maize grown in Germany is used as silage maize for feed and energy production. For about 20 % of the grown maize only the grains are harvested and used as feed (Neumann 2016). Comparing the different uses of maize, it is obvious that the total area used for grain and silage maize production as food and feed, stays nearly constant during the last years (Figure I.1). But through the new Renewable Energy Law 2000 the usage of maize as energy crop is increasing, and with it the area used for maize cultivation used for energy production (EEG 2000, FNR 2015b).

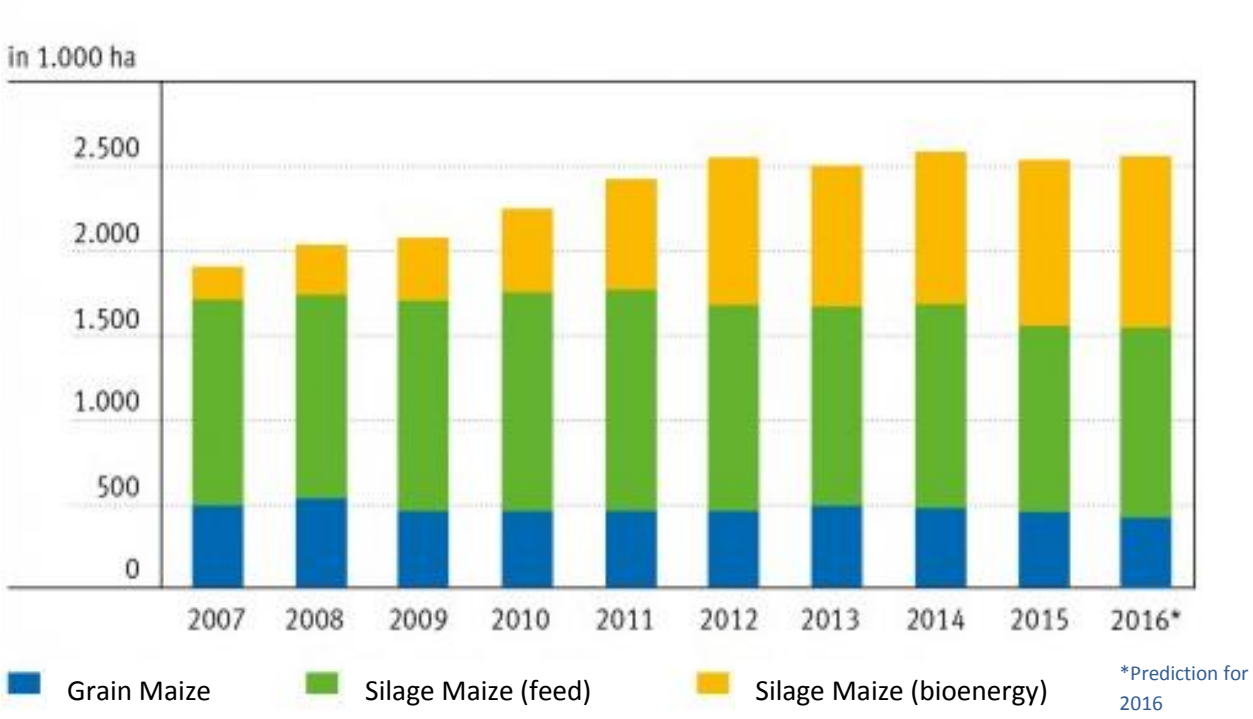

Figure I.1: Maize cultivation areas during the last ten years (FNR 2015b)

Main cropping areas of maize are the western and southern part of Germany. Climate change is causing higher temperatures in spring, which shifts the sowing date of maize. Therefore maize could also be sown in the northern part of Germany in late April, beginning of May, being no problem for the C_4-plant maize (Chmielewski et al. 2004). Especially the harvest date is important depending on the type of harvest, as grain or silage maize. Avoiding frost events is of great interest, especially in the colder northern part of Germany. Models are showing, that with an increasing annual mean temperature the risk of crop failure due to climate change and resulting drought stess is increasing

(Herrmann et al. 2005). In temperate regions, like in Germany, solar radiation around flowering time is a very important need in terms of maize production and amount of yield (Otegui et al. 1995).

Because of the fact, that the area used for maize cultivation has been increasing during the last decades, even so the area for grain and silage maize stays nearly constant, a public discussion came up about the usage and the need of the high amount of maize cultivation. The refusal in public is also caused by the fact that the cultivation area of maize is shifting between the different geographical areas in Germany (Linhardt and Dhungel 2013). For example in the area around Göttingen only 10 % of the grown cultivated plants is maize, whereas in the area Cloppenburg more than 50 % of the cultivated crops is maize (DMK e.V. 2010, Schütte 2013). Especially environmental associations discussing in public the cultivation of maize as energy crop. Compared to the opinion of farmers, ecological and economical facts are acting against each other (Linhardt and Dhungel 2013).

I.2 Food - Energy Conflict

To produce bioenergy many different substances can be used, like droppings, slurry or bio waste. The carbon inside the different substance is converted into biogas/ biomethane through fermentation.

The use of natural renewable resources as energy is a goal, defined in the Renewable Energy Law 2000 [REL] (EEG 2000). With the help of the REL the building and usage of biogas plants has been increasing very fast during the last 15 years (Figure I.2).

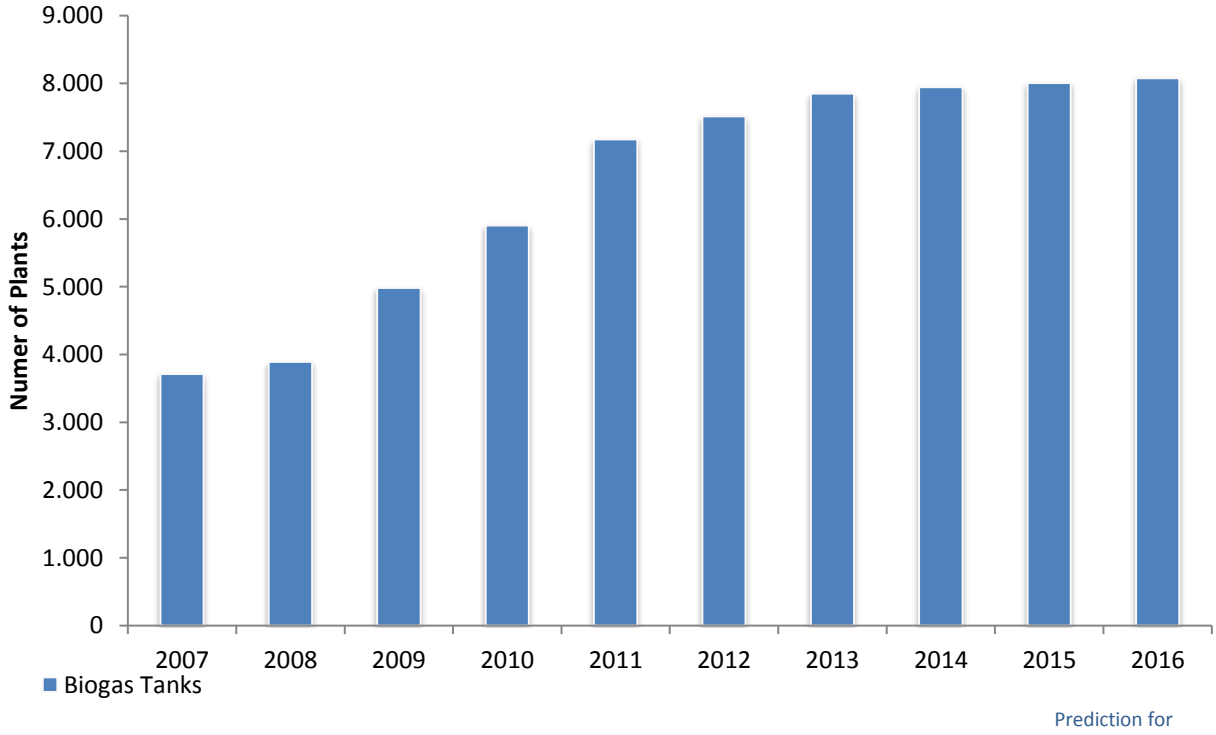

Figure I.2 Development of Biogas Plants form 2007-2016 (FNR2015a)

The total amount of electricity, produced by biogas plants in Germany was around 29,0 billion kWh in 2014 (FNR 2015a). Biogas production is very interesting, because next to electricity and heat, also fuel and natural gas substrate can be produced (Emmann et al. 2012).

With around 73 %, maize was the energy crop most used for bioenergy production in Germany (FNR 2015d). Most farmers decided to use maize because of its easy cultivation. They are used to cultivate silage maize already as feed for animals and the same kind of maize is usable in biogas plants. Besides, maize has a low need of pesticide agents, especially no fungicides and insecticides, and during one harvest already high amounts of yield can be reached. This is important because methane yield is closely correlated with dry matter yield (Oechsner 2005, Stolzenburg 2012). Especially maize can reach high amounts of methane yield (Falter et al. 2015).

Plant breeding companies developed energy maize breeding programs to answer the request. Italian genepools, with efficient genotypes have been crossed with cold-tolerant German genotypes. Finally, the short-day-gen coming from the Mexican genepool was integrated as well (Eder and Papst 2004). The new developed energy maize cultivars show lower cost per cubic meter methane (Table I.1).

Table I.1: Costs per methane yield for different substrates (Moeser 2013)

Substrate	Costs methane (€/m³)
Silage maize	0.30
Rye-total plant	0.34
Rye- total plant + forage (intermediate crop)	0.35
Green waste rye + maize	0.38
Rye grain	0.39
Barley - total plant + sorghum (intermediate crop)	0.39
Grassland	0.40
Cultivated grassland	0.42
Sugar beet	0.42

Zschache et al. (2009) discussed the different public opinions about bioenergy and its production. She used articles from 2006 – 2008 of the four biggest German newspapers, 'Süddeutsche Zeitung', 'Frankfurter Allgemeine Zeitung', 'Die Welt' and 'Frankfurter Rundschau'. The differentiation between ecological use and social interest as well as the financial aspect showed the multifacetedness of bioenergy (Zschache et al. 2009). From the ecological point of view, bioenergy can help to reduce the need of fossil energy resources, like gas or fuel which are limited resources. On the other hand, nobody knows if the building up and usage of biogas plants has a negative influence on the environment. The social aspect is the third big issue counting for the public. Not only the felt decreasing diversity of cultivated crops, but also the fact that developing countries are growing energy crops, that are then used in Europe for bioenergy is giving a negative impression on energy crops. Those areas are no longer available for growing crop plants for human consumption. People in developing countries are thus using their arable land for growing energy crops, but not to feed themselves (Zschache et al. 2009). Therefore the ethical issue is high, leading to a rejection of energy crop cultivation also in Germany. Another important drawback is the fact that caused by the use of first generation biofuels higher food prices come up. They are rising due to its competition for agricultural areas for energy of food/feed production (Bauer et al. 2010).

Comparing the maize cultivation in Germany and the production of energy maize in contrast to maize as food and feed, it is shown, that 65 % of the grown maize is used as food and feed, while 35 % is used for energy production (FNR 2015c). The public opinion is thus contrary to the real situation.

In 2012 there came an amendment for the REL responding to the discussion in public. It is reducing the subsidies for new biogas plants and giving a threshold to 60 % for the usage of silage maize as substrate in biogas plants (EEG 2014). This stopped the fast increase of building biogas plants. But the bioenergy industry is still industry influenced by the price level and opportunity costs of alternative land use (Emmann et al. 2012).

Right now around 10 % of agricultural areas are used for the production of energy crops like maize, forage and rye (BMWi 2016). Other land is used for settlements and infrastructure, growing every day with 73 hectare (Destatis 2014b). Therefore high yields are essential for energy crops to get highest output.

During the last years the usage of maize has been increasing in whole Europe (Eckner 2017). Even though not all countries are using the same amount of area, eventhough is the area used for maize cultivation increasing (DMK e.V. 2017a, DMK e.V. 2017b). This is not only caused by special breeding programs for energy crops (Eder and Papst 2004), adapting the plants to the European climate, but also caused by mechanization of cultivation and harvest and care of the cultivation areas (Krischke et al. 2011). On the other hand, comparing the average development of yield of silage and grain maize over the last 10 to 15 years a stagnation of yield on constant level is shown (Krischke et al. 2011, Tilman et al. 2011).

I.3 Dual Use Maize

The limited agricultural areas and the competition between energy and food/feed production as well as the public discussion about energy crop cultures show a need for a solution. But still, biomass is the only resource that is renewable and usable in all different energy parts (electricity, heat, fuel), and on the other hand a substrate for usage chains (Baur 2010). The problem itself could probably not be solved that easy, but there are several opportunities to mitigate the conflict.

At first, the cascade use is shown. Cascade use is defined as the use of a regrowing resource that is used substantially (probably several times) at first and then used energetically (Baur 2010) in the final step. This leads to a higher total use of the resource. Another positive effect has cascade use on the climate and it provides new jobs in the different fields that are taken (Arnold et al. 2009).

Maize can be used in a cascade. It could be used as packaging supplies or for padding. It is also possible to use maize in the cosmetic industry or for paper production (Grunert 2006). The use afterwards for energy production would sum up the cascade use. But due to the fact that the straw and spindle part are getting higher, during the vegetation period (Kurtz 2006, Zeller et al. 2009) there will not be any usage of the stover anymore after using maize grains as animal feed. A possibility for maize used in a cascade in Germany is the use of silage maize. At first, silage maize is used as animal feed, leading e.g. to milk and meat production. The digested silage maize then is used as resource for bioenergy production (Schmidt et al. 2016). The usage of the whole maize plant as silage maize for animal feed and bioenergy production still leads to a problem. The composition of the maize plant at

full maturation can be splitted in corn cob with the grains and the stover. The energy, in the form of starch is found in the corn cob and is lying around 7.5 to 8.5 MJ NEL/kg dry matter. The stover is containing a low amount of energy and mostly raw fiber with an energy density of 5.5 MJ NEL/kg dry matter (KWS SAAT SE 2014). By using just the stover as energy, the energy density should be increased while the energy density of the corn cob has to stay constant high.

There are agricultural areas, where just grain maize is cultivated. Here the stover will stay on the field, after harvest, because cascade use is not possible (Kurtz 2006, Zeller et al. 2009). The famers have to decide if they want to cultivate silage maize for energy production or grain maize for feeding their chicken and pigs. This is overall mostly an economical decision.

Another use that can lead to defusing the conflict between food and energy is the dual use of maize. Dual use means that the maize grain is used for feed and the stover (stem and leaves) is also used directly for bioenergy production and will not stay on the field, as common. This kind of use can help to mitigate the conflict and at the same time to improve the image of maize. Furthermore, it can be of economic interest for the farmer to sell the stover extra.

Right now the way of harvesting grain maize is different. At grain ripeness, around BBCH-State 89 (Weber and Bleiholder H. 1990), the maize grain is harvested. The stover is not harvested but will stay on the field. For dual use maize the stover will be harvested as well and for conservation reasons it is silage and can later on be used as substrate for the production of biomethane (Fleschhut 2015). So the stover is used profitably, too. As studies already show, the straw is usable for biogas production. But the total yield used to produce biomethane is lower because of the lack of grain in the production system (Bauer et al. 2010).

If all cultivation areas for grain maize production are used for dual use maize cultivation, the arable land that could potentially be used would be around 400.000 hectare (Destatis 2016a). Furthermore, if all areas that are used for silage maize production used for bioenergy would also cultivate dual use maize, the area would increase much more (Schmidt et al. 2016). There would be an increase of area usage leading to higher profit for famers because they can sell maize grain for feed and maize straw for biomethane production. In 2010 around 14 % of the agricultural undertakings used natural renewable resources as an extra source of earnings (Destatis 2011). In 2013 already more than 16 % did it (Destatis 2014a). One idea for this development is the fact that through the use of biogas plants, the farmers are able to pay more rent which makes them more competitive (Theuvesen, L. and Emmann, C.H 2012). A second fact taken into account is the amendment 2014 and 2017. From 2016 on the subsidy for new biogas plants is just for small plants, with a maximum output of 100kWh. If the biogas plants are bigger, the owner has to sell the produced output by himself (EEG 2014). Depending on the new laws, it is not sure how the agricultural business will react.

During the breeding programs for grain maize and silage maize, different traits become important. Traits like frost tolerance or fast maturation are important, independent of the use. For grain maize the grain yield is important. The maturation of stem and leaves are neglectable. For silage maize the total yield and its digestibility is important. The energy maize should have a high amount of biomass to put into the biogas plants.

The useful traits for dual use maize are a combination from grain maize and energy maize breeding goals. Due to the fact that dual use maize is harvested during grain maturation and as a first step the grain is used, its grain yield should be high. As a second use the stover biomass is taken. So the stover

biomass yield should be high as well because stover, stem and leaves are used as substrate for biomethane production. Caused by this, the stover should have a high water and sugar content to keep the stover able to silage and guarantee a stable biogas production.

Especially high sugar contents in the stover are necessary, because sugar is the limiting factor in producing high-quality fermented products (Seale et al. 1986). Lactic acid bacteria need the sugar in the stover to produce lactic acid that decreases the pH-level. Caused by the low pH-level aerobe lactic acid bacteria and yeast are not coming up (Gross and Riebe 1974). For silage maize high sugar contents in stem and leaves are not necessary because the whole plant including the grain, which contains a lot of starch and sugar, is used for the production of bioenergy. Silage maize is harvested with a total dry matter content of 28 % to 35 % and when the grain is showing a black layer, indicating the end of the grain filling phase (Weissbach 2000).

On the other hand the sugar content in stem and leaves is not important for grain maize, because just the grain is harvested. So it is favorable that all assimilates are filled in the grain (Hugger 2005). The sugar content in the stover is declining during grain filling because of a translocation of metabolites (Widstrom et al. 1988). Furthermore the dry matter content of the grain should be high to reduce the costs of drying. The optimal dry matter content is 60 % or higher (Hugger 2005).

For dual use maize, high sugar contents are necessary to make sure that the silage of maize stover runs stable, even without the grain. The dry matter content of the stover should be low enough to have a still usable bioenergy substrate. Also is the risk for losses by rewarming after opening the silage higher, if the dry matter content of the stover is too high (Gross and Riebe 1974).

A second important trait is the stay-green character of maize plants (Figure I.3). There is a positive correlation between late senescence and yield of maize. It is also important that the trait stay-green for some crop plants might be just beneficial under stress situations (Xu et al. 2000, Gregersen et al. 2013). The stover starts drying off and there is no production and storage of sugars in the stover anymore. To identify the maturation of the plants, maize is classified in different maturation classes, depending on the use as silage maize or grain maize. The maturity classification for silage maize depends on the amount of days the plant needs to reach total dry matter content between 32% and 35 % in the plant. For grain maize the maturity classification depends on the amount of days, the grain needs to become fully ripe. Depending on the ripening of the stover three types are known, showing a different ripeness behavior.

‘Dry down’ types are showing an almost dead stover at grain maturity (Figure I.3) Especially if there is drought stress or high Fusarium pressure, a fast riping of the whole plant is visible. Harvest time is really short and the amount of days the plants need to reach silage maize maturity is lower than the amount of days the plants need to reach grain maize maturity. The second group shows a parallel maturation. Here grain and stover are riping nearly at the same time and the maturity classification for silage maize and grain maize equals each other. This group is in between ‘dry down’ and ‘stay-green’. The last group is the stay-green type (Figure I.3). They show still green leaves and stems after maturaty of the grain (DMK e.V. 2016c). A genotype shows the stay-green trait if its contribution of green plant tissue is above the average and its grain moisture is below or equal to the average. If the stay-green and grain moisture are higher than the population average, the genotype is not considered as showing stay-green but having a longer vegetation period (Bekavac et al. 1998, Bekavac et al. 2007). If the maize is used as silage, the harvest time is not longer compared to ‘dry down’ types, with still high yields and feed quality. ‘Stay-green’ types have a higher maturity number

for silage maize than the for grain maize (DMK e.V. 2016c). Also they are more resistant against stem rot. As a favorable effect, the stay-green character is indicating good plant health later in the season (Bekavac et al. 2007, Zheng et al. 2009).

Figure I.3 Stay-green characteristic of maize genotype (right), compared to a dry down genotype (left) © W. Schmidt

There are different types of stay-green that are known, differing in the photosynthetic activity. Some are showing a delayed or later starting senescence, but they are still showing photosynthetic activity. Others show green leaves and stem but CO_2-fixation and photosynthesis is no longer provided (Thomas and Howarth 2000, Bekavac et al. 2007). But a particular stay-green characteristic can be a combination from more than two different functional traits (Thomas and Howarth 2000). During the last years progress has been made to identify the genetic background of the stay-green characteristic (Bekavac et al. 2007, Zheng et al. 2009, Thomas and Ougham 2014). In maize a positive correlation between stay-green and grain yield was found (Bekavac et al. 2007). Furthermore positive correlation has been found between thousand seed weight, grain cob diameter, yield and stay-green (Zheng et al. 2009). These results are still controversial (Bekavac et al. 2007). Caused by the fact that plants with a slower senescence also have a slower transportation of micro nutrients and nitrogen from the leaves, stay-green is a disadvantage for them. But maize stores starch with high-carbon compound in the grain. So a longer assimilation period could be advantageous and with it the stay-green character (Thomas and Ougham 2014).

By using modern techniques to identify the genetic background of plants, there are already studies that show QTLs (Quantitative Trait Loci) for the sugar content of the stem in maize plants and the stay-green behavior of maize (Zheng et al. 2009, Wang et al. 2012a, Belícuas et al. 2014, Bian et al. 2014, Bian et al. 2015, Kante et al. 2016). With help of genome wide association mapping different breeding material and wild populations have been studied to identify associations between genotypic and phenotypic data. Therefore genotypic data, coming from marker analysis and phenotypic data, coming from field trails are compared with each other and alleles are checked for their association with different traits. On the other hand the identified alleles are probably closely

related to QTLs (Becker 2011). A disadvantage of genome wide association mapping is the large number of markers needed for getting results that are significant. During the last year the technique for sequencing has rapidly been changing and the cost for analysis decreased. The identification of small associations and QTLs hard and false positive results are still common due to the used populations and thier close relationship between the genotypes. To decrease the weaknesses of the method, general linaer model, genome wide association mapping made progress in the analysis methods and developed the mixed linear model, which is taking population structure and familial relatedness in to account (Zhu et al. 2008, Larsson et al. 2013).

The sugar content in the stover of maize has not been studied a lot before. Sugar contents of other crops like sorghum have already been studied earlier. Bian et al. (2015) studied the sugar content in maize steams. They showed that the sugar content has dynamic changes during the whole ontogeny. The heritability varies during the ripening process of the maize plants and the found QTLs indicate, that major genes and polygenes are controlling the sugar content simultaneously (Bian et al. 2015). Furthermore QTLs are found on nearly each chromosome.

The stay-green characteristic of plants has already been studied for a long time. Especially stay-green and its correlation to nitrogen uptake and yield has been studied (Wood et al. 1993, Subedi and Ma 2005, Zheng et al. 2009). Zheng et al. (2009) identified ten linkage groups, of these nearly all contain a QTL for stay-green behavior (Zheng et al. 2009).

I.4 Objectives of the study

The main goal of this study is to investigate methods of breeding for dual use maize cultivars, switching from grain maize **or** energy maize production to grain maize **and** energy maize production. An efficient use of environmental resources and a higher economic value for the farmers are favorable effects. It is primarily stated that the conflict between food and energy production can be mitigated. Furthermore the genetic background of the traits stay-green behavior and sugar content in the stem of the current material are of great interest. A genome-wide association mapping should identify significant associations between marker alleles and QTL if relevant for dual use maize.

Therefore the three main objectives of the study are:

1. Testing different maize genotypes for the usage as dual use maize (preformance tests)
2. Developing dual use maize cultivars (selection)
3. Identify significant associations between SNPs and stay-green behavior and sugar content (genome-wide association mapping)

The study is divided into two parts. The first part (performance test and selection) is focusing on classical breeding approaches. Therefore the first and second main objectives are tried to answer. The second part (genome-wide association mapping) is focusing on the genetic background of the traits stay-green behavior and sugar content of the stover and is focusing on the third main objective.

For the study, different maize genotypes of the KWS SAAT SE are tested. Testcrosses with lines from the Dent and Flint pool are evaluated. In the second year factorial testcrosses were made from the selected parental lines and tested.

II. Performance test and selection

1. Introduction

The combination of a crop plant, which can be used as energy source on the one hand and on the other hand being food or feed at the same time is indicating a dual use. At the same time could this help to mitigate the conflict between food/feed and bioenergy production.

Dual use of maize describes the usage of maize grain as feed and the maize stover (stem and leaves) as source for biomethane production. Right now it is common that the stover stays on the field after grain harvest and is decomposed in spring again. The dual use maize harvest is different. As a first step the maize grain is harvested around BBCH-State 89 (Weber and Bleiholder H. 1990) as a second step the stover is taken from the field, chopped and stored as silage for further use in biogas plants as energy source (Fleschhut 2015).

Requirements for dual use maize are differing from the requirements for silage or grain maize, where the whole plant is used for only one purpose. Depending on the growing areas frost tolerance and fast maturation are important traits. Also, especially are grain dry matter yield and stover dry matter yield, making the profit for the farmer, are the most important traits for dual use maize. To guarantee a stable silage and biogas production, the sugar content of the stover and a high water content is needed as well (Seale et al. 1986). For reaching high sugar contents a photosynthetic active stover could be an indicator, so a stay-green characteristic is wanted. The combination of all traits would indicate a dual use maize variety.

This study is focused on the different traits that are necessary for breeding a dual use maize variety and a way to select promising genotypes. The tested genotypes are coming from two different genepools, Flint and Dent. The two pools are showing differences to others in their cold tolerance and their grain morphology (Brown et al. 1985).

The maize stover has a high potential to be used as energy resource in biogas plants, even though its cellulose, hemicellulose and lignin is high (Menardo and Balsari 2012, Przybyl et al. 2013, Li et al. 2016). If the amount easily dismantle products is high, the methane yield is high as well (Amon et al. 2004). The dismantling of cellulose, hemicellulose and lignin is not easy (Menardo and Balsari 2012, Przybyl et al. 2013, Li et al. 2016), therefore a stable production has to be guaranteed, even so the methane yield would be lower. Kaiser (2007) showed that there is a negative correlation between methane yield and high dry matter contents. Late mature genotypes are of interest, showing a stay-green characteristic. Within the stay-green characteristic a long photosynthetic activity is indicated, resulting in a higher sugar content in the stover plant. Water content and sugar content of the stover are important traits to guarantee a stable bioenergy production.

The objectives of the study are to test maize genotypes for their usage as dual use maize and to develop dual use maize varieties. The most important breeding traits are grain dry matter yield and total dry matter yield, as well as stover dry matter yield. Furthermore the sugar content of the stover, water content in the stover and stay-green characteristic are important.

The correlations between the different traits and its heritability are important to optimize the selection methodology. Interactions between the type of harvest, as grain maize or silage maize and traits, as well as genotype-environment interactions are of interest for the applicability of dual use maize varieties.

2. Material and Methods

2.1 Experimental design and Plant material

The used maize genotypes are genotypes from the breeding program of the KWS SAAT SE, consisting of the Dent- Genepool and Flint-Genepool. Also check varieties are included.

The first experiment contains 89 different mother Dent lines, that have been crossed with one Flint line (G14-155/23 = G14-155/3), as a tester and pollen donor, resulting in 89 testcrosses that are further mentioned as Dent genotypes [experiment 1]. The second experiment contains 89 mother Flint lines, that have been crossed with one Dent line (G14-156/95 = G14-156/94) as tester and pollen donor, further mentioned as Flint genotypes [experiment 2]. The total number of genotypes in the field was 100 per experiment, because also 11 check varieties are included. The testcrosses Dent and Flint have been sown in the field during all three years for observation tests and in 2014 for performance tests.

After the first season, 2014, 7 Dent testcrosses and 13 Flint testcrosses were selected. The selected parental lines have been crossed with each other in the KWS SAAT SE winter nursery, resulting in 88 factorial testcrosses [experiment 3] (Figure II.1/Table II.1). Because of poor seed quality and missing crosses, only 88 factorial crosses have been available instead of 91. The factorial crosses have been sown in the field during two years for observation tests and in 2015 for performance tests.

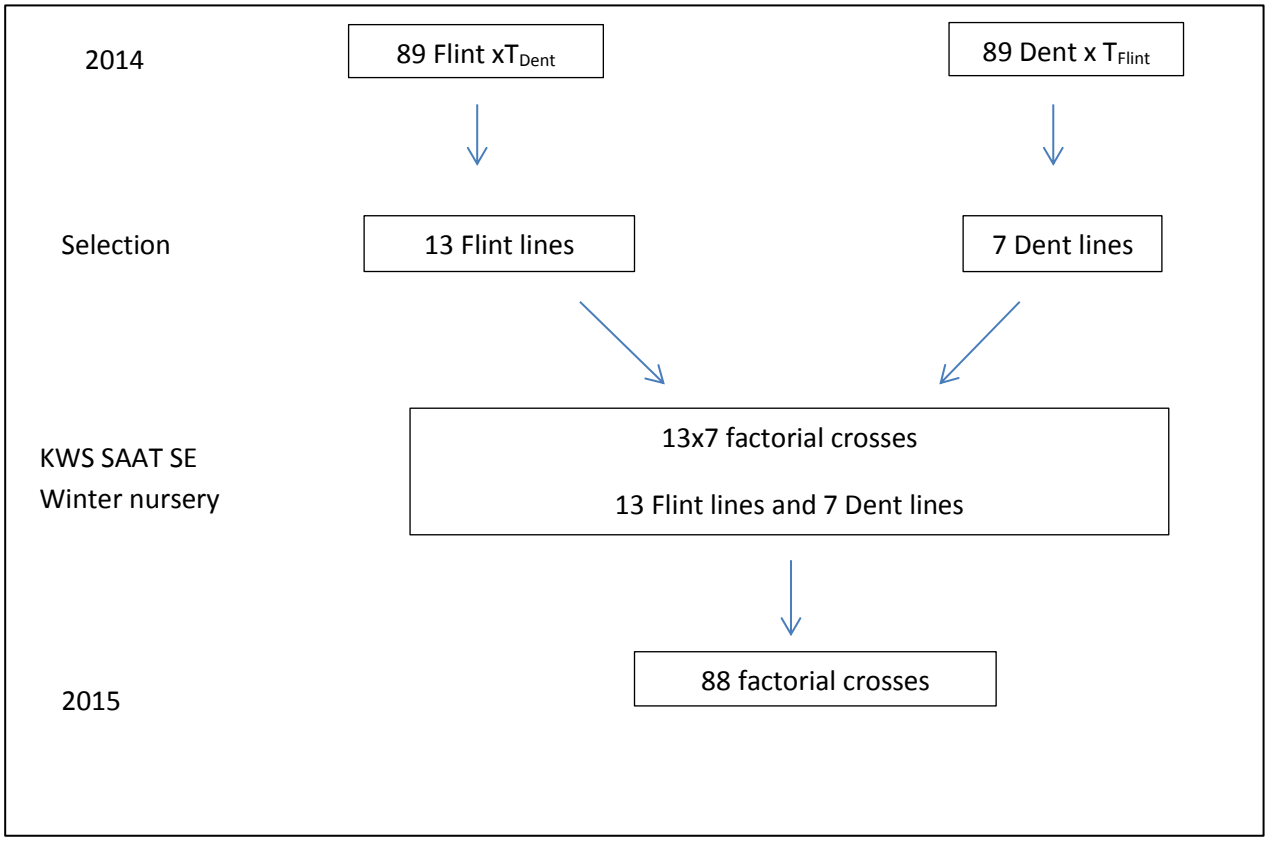

Figure II.1: Experimental design (T=Tester)

Table II.1: Entries of the factorial crosses in 2015 (Parental lines are selected lines of tested Flint and Dent Genepools in 2014)

Entry Number	Mother Line	Father Line	Entry Number	Mother Line	Father Line
3	$Dent_{7}$	$Flint_{86}$	**52**	$Dent_{80}$	$Flint_{78}$
4	$Dent_{89+96}$	$Flint_{86}$	**53**	$Dent_{82}$	$Flint_{78}$
5	$Dent_{45+97}$	$Flint_{86}$	**54**	$Dent_{7}$	$Flint_{90}$
6	$Dent_{33}$	$Flint_{86}$	**55**	$Dent_{94+95}$	$Flint_{90}$
7	$Dent_{80}$	$Flint_{86}$	**56**	$Dent_{89+96}$	$Flint_{90}$
8	$Dent_{82}$	$Flint_{86}$	**57**	$Dent_{45+97}$	$Flint_{90}$
9	$Dent_{7}$	$Flint_{3+23}$	**58**	$Dent_{33}$	$Flint_{90}$
10	$Dent_{89+96}$	$Flint_{3+23}$	**59**	$Dent_{80}$	$Flint_{90}$
12	$Dent_{45+97}$	$Flint_{3+23}$	**60**	$Dent_{82}$	$Flint_{90}$
13	$Dent_{33}$	$Flint_{3+23}$	**62**	$Dent_{7}$	$Flint_{77}$
14	$Dent_{80}$	$Flint_{3+23}$	**63**	$Dent_{94+95}$	$Flint_{77}$
15	$Dent_{82}$	$Flint_{3+23}$	**64**	$Dent_{89+96}$	$Flint_{77}$
16	$Dent_{7}$	$Flint_{85}$	**65**	$Dent_{45+97}$	$Flint_{77}$
17	$Dent_{94+95}$	$Flint_{85}$	**66**	$Dent_{33}$	$Flint_{77}$
18	$Dent_{89+96}$	$Flint_{85}$	**67**	$Dent_{80}$	$Flint_{77}$
19	$Dent_{45+97}$	$Flint_{85}$	**68**	$Dent_{82}$	$Flint_{77}$
20	$Dent_{33}$	$Flint_{85}$	**69**	$Dent_{7}$	$Flint_{40}$
22	$Dent_{80}$	$Flint_{85}$	**70**	$Dent_{94+95}$	$Flint_{40}$
23	$Dent_{82}$	$Flint_{85}$	**72**	$Dent_{89+96}$	$Flint_{40}$
24	$Dent_{7}$	$Flint_{100}$	**73**	$Dent_{45+97}$	$Flint_{40}$
25	$Dent_{94+95}$	$Flint_{100}$	**74**	$Dent_{33}$	$Flint_{40}$
26	$Dent_{89+96}$	$Flint_{100}$	**75**	$Dent_{80}$	$Flint_{40}$
27	$Dent_{45+97}$	$Flint_{100}$	**76**	$Dent_{82}$	$Flint_{40}$
28	$Dent_{33}$	$Flint_{100}$	**77**	$Dent_{7}$	$Flint_{29}$
29	$Dent_{80}$	$Flint_{100}$	**78**	$Dent_{94+95}$	$Flint_{29}$
30	$Dent_{82}$	$Flint_{100}$	**79**	$Dent_{89+86}$	$Flint_{29}$
32	$Dent_{7}$	$Flint_{79}$	**80**	$Dent_{45+97}$	$Flint_{29}$
33	$Dent_{89+96}$	$Flint_{79}$	**82**	$Dent_{33}$	$Flint_{29}$
34	$Dent_{45+97}$	$Flint_{79}$	**83**	$Dent_{80}$	$Flint_{29}$
35	$Dent_{33}$	$Flint_{79}$	**84**	$Dent_{82}$	$Flint_{29}$
36	$Dent_{80}$	$Flint_{79}$	**85**	$Dent_{7}$	$Flint_{53}$
37	$Dent_{82}$	$Flint_{79}$	**86**	$Dent_{94+95}$	$Flint_{53}$
38	$Dent_{7}$	$Flint_{94}$	**87**	$Dent_{89+96}$	$Flint_{53}$
39	$Dent_{94+95}$	$Flint_{94}$	**88**	$Dent_{45+97}$	$Flint_{53}$
40	$Dent_{89+96}$	$Flint_{94}$	**89**	$Dent_{33}$	$Flint_{53}$
42	$Dent_{45+97}$	$Flint_{94}$	**90**	$Dent_{80}$	$Flint_{53}$
43	$Dent_{33}$	$Flint_{94}$	**91**	$Dent_{82}$	$Flint_{53}$
44	$Dent_{80}$	$Flint_{94}$	**92**	$Dent_{7}$	$Flint_{97}$
45	$Dent_{82}$	$Flint_{94}$	**93**	$Dent_{94+95}$	$Flint_{97}$
46	$Dent_{7}$	$Flint_{78}$	**94**	$Dent_{89+96}$	$Flint_{97}$
47	$Dent_{94+95}$	$Flint_{78}$	**95**	$Dent_{45+97}$	$Flint_{97}$
48	$Dent_{89+96}$	$Flint_{78}$	**96**	$Dent_{33}$	$Flint_{97}$
49	$Dent_{45+97}$	$Flint_{78}$	**97**	$Dent_{80}$	$Flint_{97}$
50	$Dent_{33}$	$Flint_{78}$	**98**	$Dent_{82}$	$Flint_{97}$

2.2 Locations and years

The experiments have been provided as observation tests and performance tests. The experiments were conducted as lattice design during all three years, with two replications per experiment.

The oberservation tests, for the Dent testcrosses, Flint testcrosses and factorial crosses, including the check varities, have been conducted in Göttingen during the years 2014, 2015 and 2016. The second location, where observations test have been conducted, was Einbeck in 2014 and 2015. In 2016 the field trails have been conducted in Stöckheim near Einbeck. Stöckheim is handled in the following as Einbeck because there are no differences in field conditions. The plots are consisting of two rows and have been 6m long while the row spacing was 75cm. At both locations the sugar content of the stover (BRIX) has been measured as well as the chlorophyll content of the leaves (SPAD) during the season. In 2014 only the testcrosses have been analyzed, while in 2015 and 2016 the testcrosses and the factorial crosses have been observed. At the location Göttingen a storm event damaged the experiments in 2016. The location was no longer used for data evaluation for chlorophyll content (SPAD).

The performance tests have been provided at five different locations in Baden-Wuerttemberg (Eutingen, Gondelsheim/Pforzheim, Langenau bei Ulm, Heilbronn) and Rhineland-Palatinate (Neupotz). All locations have clay soil with outstanding qualities. The average annual temperature was in a range between 8.3°C and 10.5°C, whereas the average annual rainfall was between 644mm/m^2 to 889mm/m^2. The locations, Eutingen, Gondelsheim/Pforzheim and Neupotz have been used for performance tests in 2014 for the Dent testcrosses and Flint testcrosses. The locations Gondelsheim/Pforzheim, Langenau bei Ulm and Heilbronn have been used for the performance tests in 2015 for the factorial crosses. All experiments have been filled up to a total number of 100 entries with check varieties of KWS SAAT SE. The experimental design was a lattice design with two replications per experiment. All experiments have been set up twice, containing all genotypes, for two different types of harvests (silage maize harvest and dual use maize harvest, see chapter 2.3).

2.3 Seeding and harvest

All locations are under conventional use and have been prepared in the generally accepted way before sowing. In spring there was a nitrogen fertilization (220kg minus N_{min} value). The seed-bed cultivation took place a few days before sowing. In all experiments and at all locations ten grains per m^2 have been sown with a pneumatic precision seed drill. The sowing of the experiments was in 2014 and 2015 during the middle of April in the locations in Southern Germany (15.04.-20.04). In Göttingen and Einbeck the sowing took place at the end of April (20.04.-08.05.) in 2014, 2015 and 2016.

The harvests of the performance tests for silage maize and dual use maize differ from each other. For both trials 9m^2 have been harvested, the extra rows are taken as board rows to avoid neighboring effects.

Harvest of silage maize was done at a BBCH- state 75 (Weber and Bleiholder H. 1990). The trail of the silage maize harvest, contains four rows of 6m length, 75cm spacing and a total plot size of 18m^2. The two rows in the middle of the plot have been harvested. During the harvest, the whole plant was cut

around 15-20cm above ground. In 2014 and 2015 KWS SAAT SE harvested at all three locations of the performance tests with an automatic maize chopper (Baural) and a chaff system that was used together with a carrier machine (Haldrup). The harvest was done during one day per location between the 05.09.-07.09. each year.

The location Heilbronn was not used for the analysis of the performance tests silage maize in 2015. Long drought stress had lead to a fast ripening of the maize plants that the silage harvest was actually too late compare to the wanted BBCH-state 75 (Weber and Bleiholder H. 1990). The dual use maize harvest took place a few days later, whch also proved the too late silage maize harvest.

Dual use maize performance tests were harvested at a BBCH-State 89 (Weber and Bleiholder H. 1990). The plot was containing six rows of 6m length, 75cm row spacing and a total plot size of 27m^2. The board rows to the next plots, left and right were not used and the four rows in the middle were harvested in two steps. At first two (9m^2) of the four rows were harvested as whole plant. Second, two neighboring rows (9m^2) were harvested as grain maize. The harvest was done by KWS SAAT SE. The grain was harvested with a C-85 plot threshing machine (Firma Haldrup) and the whole plant with the same machines used for silage maize harvest. The whole harvest was done during one day per location and took place between 24.09.-04.11. The only exception was Heilbronn in 2015. Long drought stress had lead to a fast riping of the maize plants. The harvest for the dual use maize performance tests was already at the beginning of September (08.09.2015).

2.4 Traits

Some traits are collected directly; others are calculated from the collected ones.

2.4.1 Total fresh matter (TFM), Total dry matter content (TDC) Total dry matter yield (TDY)

During silage maize harvest and dual use maize harvest the whole maize plants have been weighed on the combine harvester to evaluate the total fresh matter (TFM) per plot (kg/9m^2) and converted into dt/ha. The total dry matter content (TDC) was measured during the harvest at the machine with a near infrared spectroscopy (NIRS).

The total dry matter yield (TDY) was calculated with help of the total fresh matter and the total dry matter content (TDC) and is given in dt/ha.

$$TDY = \left[\frac{TFM * TDC}{100}\right] * 11.1111$$

TDY = Total dry matter yield
TFM= Total fresh matter
TDC= Total dry matter content

Equation II.1

2.4.2 Total grain fresh matter (GFM), Grain dry matter content (GDC), Grain dry matter yield (GDY)

For the performance tests of dual use maize the experiments have been harvested as grain maize. The total grain fresh matter (GFM) was weighed at the combine harvester for each plot (kg/9m^2) and converted into dt/ha. The grain dry matter content (GDC) was measured by a near infrared spectroscopy (NIRS) during harvest at the combine harvester.

The grain dry matter yield (GDY) has been adjusted to a grain dry matter content (GDC) of 86 % and is given in dt/ha.

$$\mathrm{GDY} = \left(\frac{\frac{10000}{9}}{86}\right) * \mathrm{GFM} * \left(\frac{\mathrm{GDC}}{100}\right)$$

GDY = Grain dry matter yield
GFM= Total grain fresh matter
GDC= Grain dry matter content

Equation II.2

2.4.3 Stover fresh matter (SFM), Stover dry matter content (SDC), Stover dry matter yield (SDY), Water content of the stover (RH_2O)

The four different traits for the stover have all been calculated.

For calculating the stover fresh matter (SFM), the total fresh matter (TFM) and the grain fresh matter (GFM) have been substracted from each other and are giving the SFM in dt/ha.

$$\mathrm{SFM} = (\mathrm{TFM} - \mathrm{GFM}) * 11.1111$$

SFM= Stover fresh matter
TFM= Total fresh matter
GFM= Grain fest matter

Equation II.3

To calculate the dry matter yield of the stover (SDY), the grain dry matter yield (GDY) was subtracted from the total dry matter yield (TDY) and is given in dt/ha.

$$\mathrm{SDY} = \mathrm{TDY} - \mathrm{GDY}$$

SDY = Stover dry matter yield
TDY= Total dry matter yield
GDY= Grain dray matter yield

Equation II.4

The stover dry matter content (SDC) is calculated from of the stover dry matter yield (SDY) and the stover fresh matter (SFM) and is given in %.

$$SDC = \left(\frac{SDY}{SFM}\right) * 100$$

SDC= Stover dry matter content
SDY= Stover dry matter yield
SFM= Stover fresh matter

Equation II.5

The last trait analyzed, is the water content of the stover (RH_2O). This trait is the complementary to the stover dry matter content (SDC). The water content is given in %.

$$RH_2O = 100 - SDC$$

RH_2O = Water content of the stover
SDC= Stover dry matter content

Equation II.6

2.4.4 Sugar content in the stover (BRIX-method)

The sugar content in the stem is measured with help of the BRIX method. With an electrical refractometer Pocket PAL 1 (ATAGO 2016) the BRIX-value in °BRIX is given, showing the sucrose content of the sample. Per plot three plants were cut into two parts.

The first part was taken directly above the fully formed corn cob, while the second part was taken from below the fully formed corn cob (Figure II.2). In total six samples per plot of around 10-15cm were taken.

Sample above corn cob **Sample below corn cob**

Figure II.2: Cutting the samples for the sugar (BRIX) measurement

The samples were put into a bench vise to squeeze out the maize sap. The sap was put into the electrical refractometer Pocket PAL 1 and analyzed (ATAGO 2016). The refractometer Pocket PAL 1

was calibrated with tap water. The values are averaged for each part of the plant (above and below corn cob). An overall average were calculated to get a value for the genotype of one plot. After measuring a sample the bench vise and the refractometer were cleaned with water before using it again.

The measurement was done twice each year for all plots and experiments. The first measurement took place around eight weeks before harvest, while the second measurement was done close to harvest (around one week before, until a few days earlier). After cutting the samples they were put into cooling boxes and squeezed during the following three days. The samples had been stored in a cooling chamber.

2.4.5 Stay-green characteristic (SPAD-method)

The stay-green characteristic means a high chlorophyll content of the leaves while the grain is already mature. The chlorophyll content is measured indirectly with Chlorophyllmeter SPAD 502 (Konica Minolta Optics, Inc. 2009). The SPAD-value is not directly the Chlorophyll content but is proportional to it (Konica Minolta Optics, Inc. 2009).

Ten plants were measured per plot, five per row. The leaf at the corn cob was taken, around 10 cm away from the connection between the leaf sheath and the leaf blade at the leaf blade (Figure II.3). An average was taken of all ten measured plants.

Figure II.3: Position of the Chlorophyllmeter SPAD 502 while measuring

The measurement was done several times during the season. In the middle of August the first measurement took place, around eight weeks before harvest. Weekly the SPAD-values were measured to see how the chlorophyll content was changing during the season. The last measurement was done before harvest. Because of early frost, the last measurement in 2015 at the locations Einbeck and Göttingen was already in the middle of October. In 2016 the last measurement was already done at the beginning of October, because of a long drought stress in September and October at the location Einbeck. The location Göttingen was destroyed by a storm event in August 2016 and not usable for data collection anymore.

2.5 Selection and Response to Selection

At the end of the experimental year 2014 a selection of the best testcrossess of the Dent-genepool and the Flint-genepool was done. To identify the best genotypes in both testcrosses separately, the total dry matter yield (TDY), the grain dry matter yield (GDY) and the grain dry matter content (GDC) as well as the water content of the stover (RH_2O), the sugar content of the stover and the 'stay-green' behavior have been analyzed. The results have been visualized to easily identify the best genotypes.

Before selecting the genotypes with highest yield, their 'stay-green' behavior of the plants was studied. Genotypes, showing a high stay-green behavior, have been selected first. Afterwards, the different traits: total dry matter yield (TDY), the water content of the stover (RH_2O) and the grain dry matter yield (GDY) have been plotted against grain dry matter content (GDC) and total dry matter content (TDC) at the time of dual use maize harvest. Here the genotypes showing a good stay-green behavior have been studied again, for their yield performance.

Already the pre-selected genotypes, based on their stay-green behavior, needed to show a moderate to high yield and water content, to be used for further selection. Finally, the sugar content of the selected genotypes was checked. If the sugar content of the stover was also within the range the genotype was selected. Finally the last check was done by the company KWS SAAT SE to avoid selection of genotypes showing unexpected weakness.

The response to selection can be calculated for the different traits. Moreover the expected response to selection is categorized in two classes. The direct response to selection is the phenotypic difference between the mean of the population and the mean of the selected fraction after selection for a wanted trait. The direct response to selection is calculated with the following equation:

$$R_D = i_D * h_D * \sigma_D$$

R_D = direct response to selection
i_D= selection intensity of the wanted trait (direct trait)
h_D = square root of the heritability of the wanted trait
σ_D = genetic standard deviation of the wanted trait

Equation II.7

The second category is the indirect response to selection when selection is based on a secondary trait. For calculation of the indirect response to selection a different equation is used:

$$R_I = i_I * h_I * \sigma_D * r_G$$

R_I = indirect response to selection
i_I = selection intensity of the assistant trait (indirect trait)
h_I = square root of the heritability of the assistant trait
r_G= genetic correlation of wanted trait and assistant trait

Equation II.8

The response of selection is calculated for the total dry matter yield of maize during the dual use maize harvest. As assistant trait the total dry matter yield of the silage maize harvest is used. The calculation is made separately for the two testcrosses, Flint and Dent. The selection intensity is taken from the selection intensity table (Kearsey and Pooni 1996, unknown 2016).

2.6 Statistical analysis

For the statistical analysis the software PlabStat (Plant Breeding Statistical program, Version 3A) was used (Utz 2011). The experiments have been analyzed at first as a lattice design for each year and each location separately, including all checkvarieties.

The standard error of the genetical correlation coefficients was calculated after Mode and Robinson 1959. Here the check varieties are not included. For the single environments the experimental error is calculated with help of the lattice analysis. Also the means of the different experiments are calculated with the lattice analysis. The experimental errors as well as the calculated means are taken for further ANOVA analysis.

Depending on the trait and the location, respectively the environmental conditions of the locations, some locations have been excluded of the analysis Table II.2.

Table II.2 Overview over the used locations and years for each test and experiment

Experiment	Location	Test	Year
Experiment 1: Dent testcrosses	Neupotz Gondelsheim/Pforzheim	Silage Maize Performance test	2014
Experiment 1: Dent testcrosses	Eutingen Neupotz Gondelsheim/Pforzheim	Dual Use Maize Performance test	2014
Experiment 1: Dent testcrosses	Einbeck Göttingen	BRIX Measurement Observation test	2014 2015 2016
Experiment 1: Dent testcrosses	Einbeck Göttingen	SPAD Measurement Observation test	2014 2015 2016 (only Einbeck)
Experiment 2: Flint testcrosses	Eutingen Neupotz Gondelsheim/Pforzheim	Silage Maize Performance test	2014
Experiment 2: Flint testcrosses	Eutingen Neupotz	Dual Use Maize Performance test	2014
Experiment 2: Flint testcrosses	Einbeck Göttingen	BRIX Measurement Observation test	2014 2015 2016
Experiment 2: Flint testcrosses	Einbeck Göttingen	SPAD Measurement Observation test	2014 2015 2016 (only Einbeck)
Experiment 3: Factorial crosses	Gondelsheim/Pforzheim Langenau (bei Ulm) Heilbronn	Silage Maize Performance test	2015
Experiment 3: Factorial crosses	Gondelsheim/Pforzheim Heilbronn	Dual Use Maize Performance test	2015
Experiment 3: Factorial crosses	Einbeck Göttingen	BRIX Measurement Observation test	2014 2015 2016
Experiment 3: Factorial crosses	Einbeck Göttingen	SPAD Measurement Observation test	2014 2015 2016 (only Einbeck)

For the analysis different statistical models are used, depending on the analyzed trait and the available data for the trait.

For all yields, grain dry matter yield (GDY), total dry matter yield (TDY) and stover dry matter yield (SDY), the following statistical model is used:

$$x_{ij} = \mu + l_i + g_j + lg_{ij} + m_{ij}$$

x_{ij} = yield of the genotype j in environment i
μ = general mean
l_i= effect of location i
g_j= effect of genotype j
lg_{ij}= interaction between location i and genotype j
m_{ij}= experimental error, estimated from lattice analysis of single locations

Equation II.9

To analyze the sugar content of the stover (BRIX) all years and locations are taken into account. Therefore the following statistical model was used:

$$x_{ijk} = \mu + y_k + l_i + yl_{ki} + g_j + lg_{ij} + gy_{jk} + gly_{jik} + m_{ijk}$$

x_{ijk} = observation value of genotype j in location i and year k
μ = general mean
y_k= effect of year k
l_i = effect of location i
yl_{ki}= interaction between year k and location i
g_j = effect of genotype j
lg_{ij} = interaction between location i and genotype j
gy_{jk} = interaction between genotype j and year k
gly_{jik} = interaction between genotype j, location I and year k
m_{ijk} = experimental error, estimated from lattice analysis of single locations

Equation II.10

For analyzing the chlorophyll content of leaves (SPAD) some locations could not be used in all years. Therefor location-year combinations were considered as environments and the following statistical model was used:

$$x_{ij} = \mu + e_i + g_j + eg_{ij} + m_{ij}$$

x_{ij} = observation value of the genotype j in environment i
μ = general mean
e_i= effect of environment i
g_j= effect of genotype j
eg_{ij}= interaction between environment i and genotype j
m_{ij}= experimental error, estimated from lattice analysis of single environments

Equation II.11

The heritability was calculated with the following equation (Falconer and Mackay 2009) for all traits:

$$h^2_{iws} = \frac{\sigma^2_g}{\sigma^2_P} = \frac{\sigma^2_g}{(\sigma^2_g + \left(\frac{\sigma^2_{ge}}{e}\right) + \left(\frac{\sigma^2_m}{er}\right)}$$

h^2_{iws} = heritability
σ^2_g = genotypic variance of the average
σ^2_P=phenotypic variance of the average
σ^2_{ge}=variance of the genotype-environment interaction
σ^2_m=variance of error
e = number of environments
r = number of replications

Equation II.12

3. Results

3.1 Performance test Silage maize harvest

The harvest as silage maize was done in the experimental years 2014 and 2015. During the experimental year 2014 two different types of testcrosses, with Dent and Flint lines, were grwon. In the experimental year 2015 the factorial crosses of selected lines have been tested.

The different genotypes including the check varieties got entry numbers from 1 to 100. It has to be taken into account that the numbers were given for each experiment separately. The silage maize harvest took place during the beginning and middle of September when the maize plants reached the maturity of silage maize with a dry matter content between 32 % and 35 %.

3.1.1. Dent testcrosses

Figure II.4 shows the total dry matter content (%) plotted against the total dry matter yield (dt/ha). Genotype 11 and 31 showed high yield with also an high dry matter content. Genotype 35 and 76 showed a low total dry matter content and a low total dry matter yield. Most genotypes showed a moderate total dry matter yield with an total dry matter content between 30 % and 36 %.

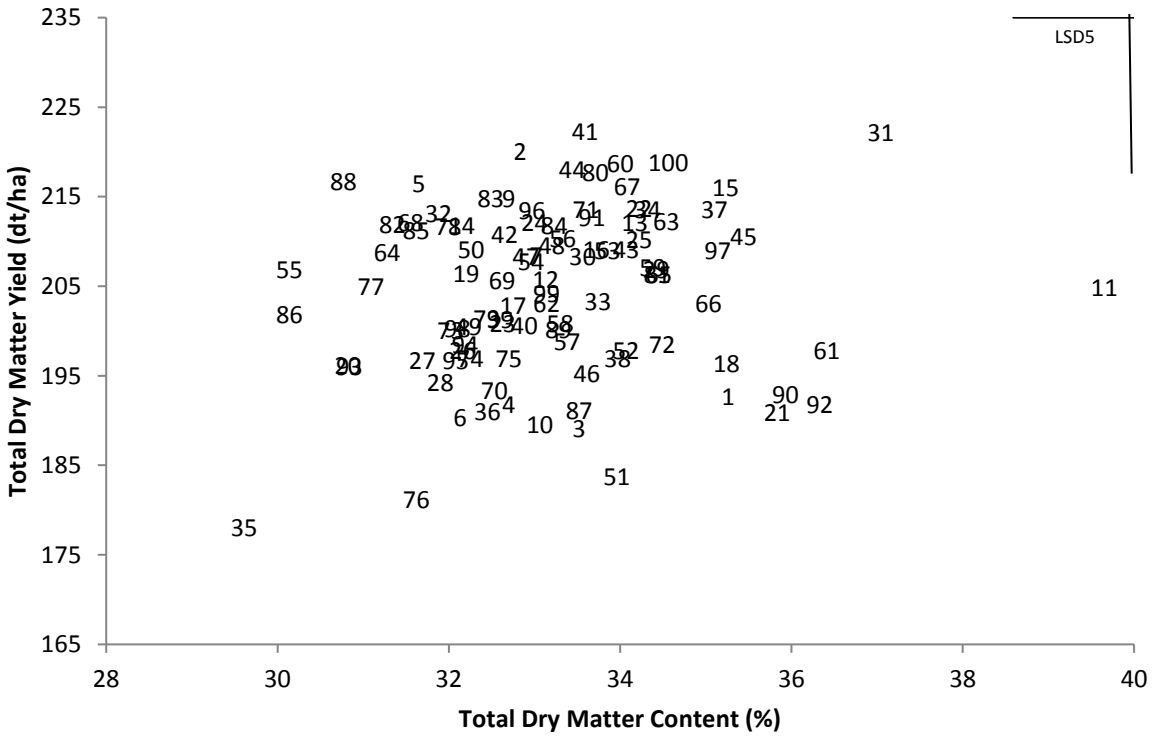

Figure II.4 Total dry matter yield (TDYs) in dt/ha against total dry matter content (TDCs) in % of the Dent testcrosses for the performance test silage maize in year 2014. Numbers are the entry numbers of the testcrosses. Coefficient of determination R^2: 0.0157

As Table II.3 is showing, is the correlation between total dry matter yield and total dry matter content (0.13) low as well. The correlation between total dry matter yield and total fresh matter was moderate significant (0.66**). The correlation between total dry matter yield and total fresh matter was negative and significant (-0.66**).

Table II.3 Table of correlation of the traits for the Dent testcrosses

Total dry matter content	0.41**		
Total fresh matter	-0.08	-0 66**	
Total dry matter yield	0.30**	0.13	0.66**
	Number of plants per plot	**Total dry matter content**	**Total fresh matter**

significance level *p=0.05, **p=0.01; +p=0.1

The heritability for the trait total dry matter content (TDCs) was high, with 89 %. The interaction between genotype and location was significant, but the variance and the variance component was low. The genotypes showed the highest variance component, while the variance is of the total dry matter content was given by the genotypes (Table II.4).

Table II.4 Analysis of Variance for the trait total dry matter content (TDCs) in % of the Dent testcrosses

Source	DF	MS	Var.cp	F-value	LSD5
Location	1	32.9974	0.3282	65.07**	0.20
Genotype	98	4.8050	2.1489	9.48**	1.41
Location-Genotype	98	0.5071	0.2286	1.82**	1.47
Error	156	0.2785	0.0313		
Heritability 89 %					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

Table II.5 is showing that the trait total dry matter yield (TDYs) had a lower heritability (64 %) compared to the total dry matter content. The interaction between location and genotypes was just significant at a level of 10 %. The location was showing a high variance component, while the genotypes were also showing a lower variance component. Therefore the variation of the total dry matter yield was based on the locations. Location-Genotype interactions were showing a low variance component.

Table II.5 Analysis of Variance for the trait total dry matter yield (TDYs) in dt/ha of the Dent testcrosses

Source	DF	MS	Var.cp	F-value	LSD5
Location	1	7508.3010	75.2198	122.00**	2.21
Genotype	98	173.3283	55.8922	2.82**	15.57
Location-Genotype	98	61.5439	13.4101	1.28+	19.37
Error	174	48.1338	48.1338		
Heritability 64 %					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

3.1.2. Flint testcrosses

Comparing the total dry matter yield (dt/ha) with the total dry matter content (%) (Figure II.5) it was shown that some genotypes, like entry number 89, have a high total dry matter content with a moderate total dry matter yield. Genotype 41 showed a moderate total dry matter content but had a high total dry matter yield. The most genotypes were in a moderate range of total dry matter yield and total dry matter content.

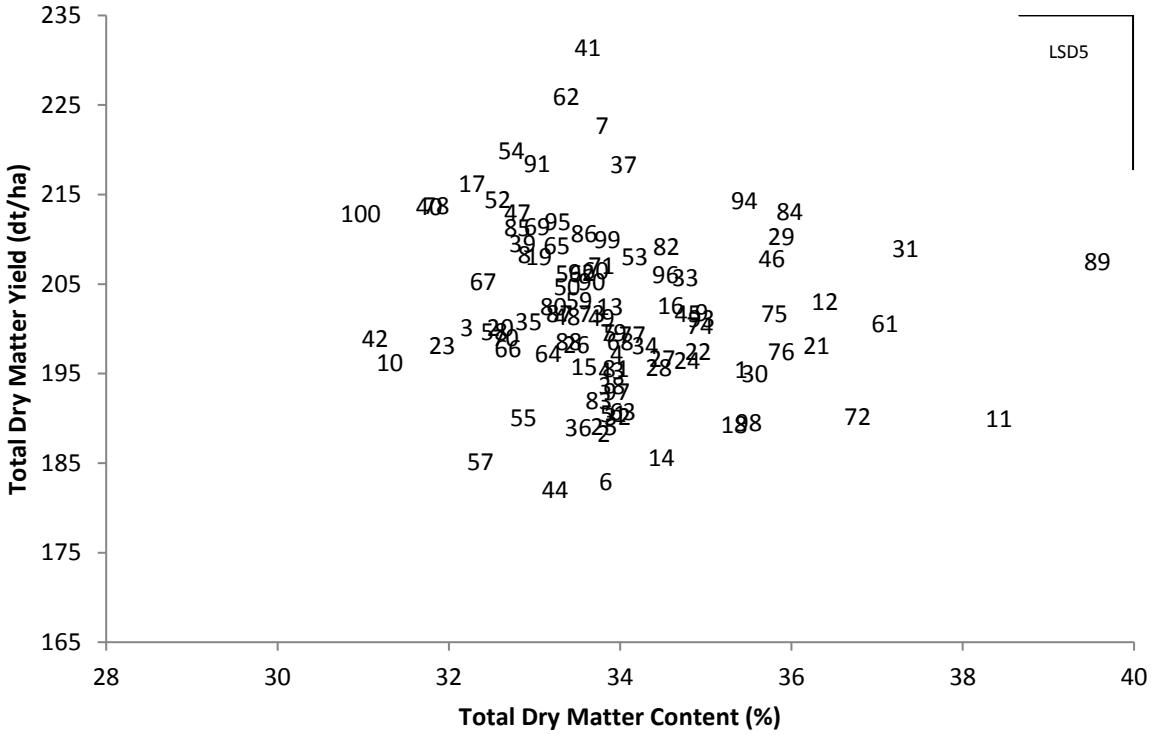

Figure II.5 Total dry matter yield (TDYs) in dt/ha plotted against total dry matter content (TDCs) in % of the Flint testcrosses for the performance test silage maize harvest in year 2014. Number are the entry numbers of the testcrosses. Coefficient of determination R^2: 0.0169

The correlation between both traits was negative and not significant (-0.15/Table II.6). Comparing the total fresh matter with the total dry matter yield, the correlation between both traits was high and significant (0.80**). On the other hand was the correlation between total fresh matter and total dry matter content highly negative and significant (-0.72**).

Table II.6 Table of correlation of the traits for the Flint testcrosses

Total dry matter content	0.10		
Total fresh matter	0.13	-0.72**	
Total dry matter yield	0.29**	-0.15	0.80**
	Number of plants per plot	**Total dry matter content**	**Total fresh matter**

significance level *p=0.05, **p=0.01; +p=0.1

The heritability of the total dry matter content was high (88 %). Table II.7 is showing that the variance component of the genotypes was highest, while the interaction between location and genotype was showing the lowest variance component. The interaction between location and genotype was significant on a level of 5 %. Therefore it was shown that the genotypes themselves are causing the variability of the trait total dy matter content.

Table II.7 Analysis of Variance for the trait total dry matter content (TDCs) in % of the Flint testcrosses

Source	DF	MS	Var.cp	F-value	LSD5
Location	2	44.2619	0.4357	64.31**	0.23
Genotype	99	6.1837	1.8318	8.98**	1.34
Location-Genotype	198	0.6883	0.1805	1.36*	1.99
Error	241	0.5078	0.5078		
Heritability 88 %					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

Table II.8 is showing the analysis of variance for the trait total dry matter yield. The heritability was with 68 % high. The location-genotype interaction was low significant on a level of 5 % . The locations were showing the highest variance component, resulting in high significance. The genotypes were also showing a high variance as well, resulting in a significant F-value.

Table II.8 Analysis of Variance for the trait total dry matter yield in (TDYs) in dt/ha of the Flint testcrosses

Source	DF	MS	Var.cp	F-value	LSD5
Location	2	16086.2750	160.0283	192.78**	2.55
Genotype	99	263.5960	60.0511	3.16**	14.71
Location-Genotype	198	83.4426	20.0402	1.32*	22.18
Error	241	53.4025	63.4025		
Heritability 68 %					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

3.1.3. Factorial crosses

Figure II.6 is showing the total dry matter yield (dt/ha) compared to the total dry matter content (%). The distribution of all genotypes was wide. Genotype 5 was showing a higher total dry matter content, with a low total dry matter yield. On the other hand was genotype 24 showing a low total dry matter content with a higher total dry matter yield.

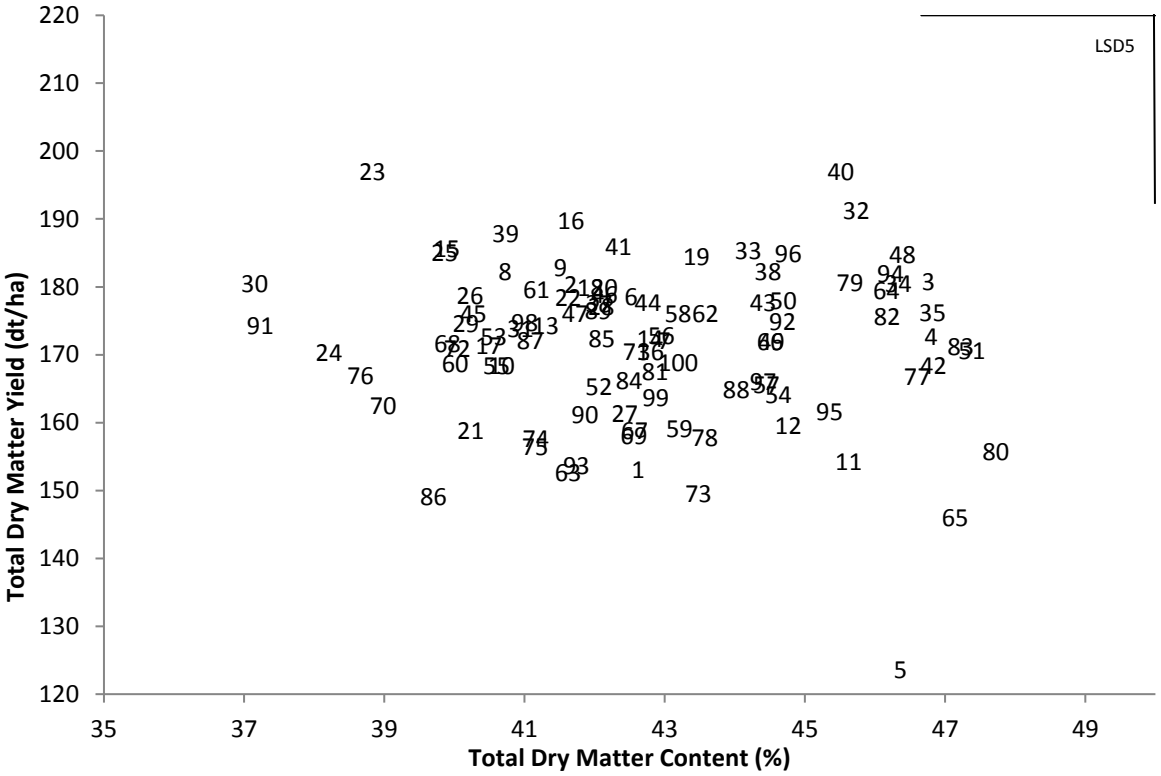

Figure II.6 Total dry matter yield (TDYs) in dt/ha plotted against total dry matter content (TDCs) in % of the factorial crosses for the performance test silage maize harvest in year 2015. Numbers are the entry numbers of the testcrosses. Coefficient of determination R^2: 0.0087

As Table II.9 is showing, that no correlation between total dry matter content and total dry matter yield (-0.09) was found. On the other hand was the correlation between total dry matter yield and total fresh matter significant (0.72**). Total dry matter content and total fresh matter were showing a negative significant correlation (-0.69**).

Table II.9 Table of Correlation of the traits for the factorial crosses

Total dry matter content	-0.14		
Total fresh matter	0.34**	-0.69**	
Total dry matter yield	0.44**	-0.09	0.72**
	Number of plants per plot	**Total dry matter content**	**Total fresh matter**

significance level *p=0.05, **p=0.01; +p=0.1

Table II.10 is showing the analysis of variance for the trait total dry matter content. The heritability of the trait was high (75 %). Also was the interaction between the location and genotype significant, with a low variance component. The locations were showing the highest variance component and a significant high F-value. The genotypes were as well significant but its variance component was lower.

Table II.10 Analysis of Variance for the trait total dry matter content (TDCs) in % of the factorial crosses

Source	DF	MS	Var.cp	F-value	LSD5
Location	2	3651.5029	36.4721	851.46**	0.58
Genotype	99	17.7503	4.4872	4.14**	3.34
Location-Genotype	190	4.2885	2.4425	2.32**	3.79
Error	200	1.8461	1.8461		
Heritability 75 %					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

Comparing the heritability of the total dry matter content with the heritability of the total dry matter yield, the total dry matter yield had a lower heritability (35 %). The locations were showing the highest significance, with causing also most variation. The genotypes were also significant with the lowest F-value. The interaction between location and genotype was significant and showed a high variance component. Therefore the interaction was also causing a lot of variation within the total dry matter yield (Table II.11).

Table II.11 Analysis of Variance for the trait total dry matter yield (TDYs) in dt/ha of the factorial crosses

Source	DF	MS	Var.cp	F-value	LSD5
Location	2	30899.1538	306.4324	120.74**	4.46
Genotype	99	394.3058	46.1315	1.54**	25.76
Location-Genotype	190	255.9112	161.4014	2.71**	27.11
Error	200	94.5098	94.5098		
Heritability 35 %					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

3.2. Performance test dual use maize harvest

The harvest as use maize harvest was done in the experimental years 2014 and 2015. During the experimental year 2014 two different types of testcrosses, Dent and Flint, have been tested. In the experimental year 2015 the factorial crosses have been tested.

The different genotypes, including the check varieties, got entry numbers from 1 to 100. It has to be taken into account that the numbers are given for each experiment separately. The dual use maize harvest took place during the beginning and middle of October.

3.2.1. Dent testcrosses

Figure II.7 is comparing the grain dry matter content (%) to the grain dry matter yield (adjusted to 86 % GDC in dt/ha). The variation of the genotypes was wide. Genotype 1 and 11 (both are check varieties) were showing a high grain dry matter content, while their grain dry matter yield was low. Comparing genotype 41, it was showing a low grain dry matter content, with a high grain dry matter yield.

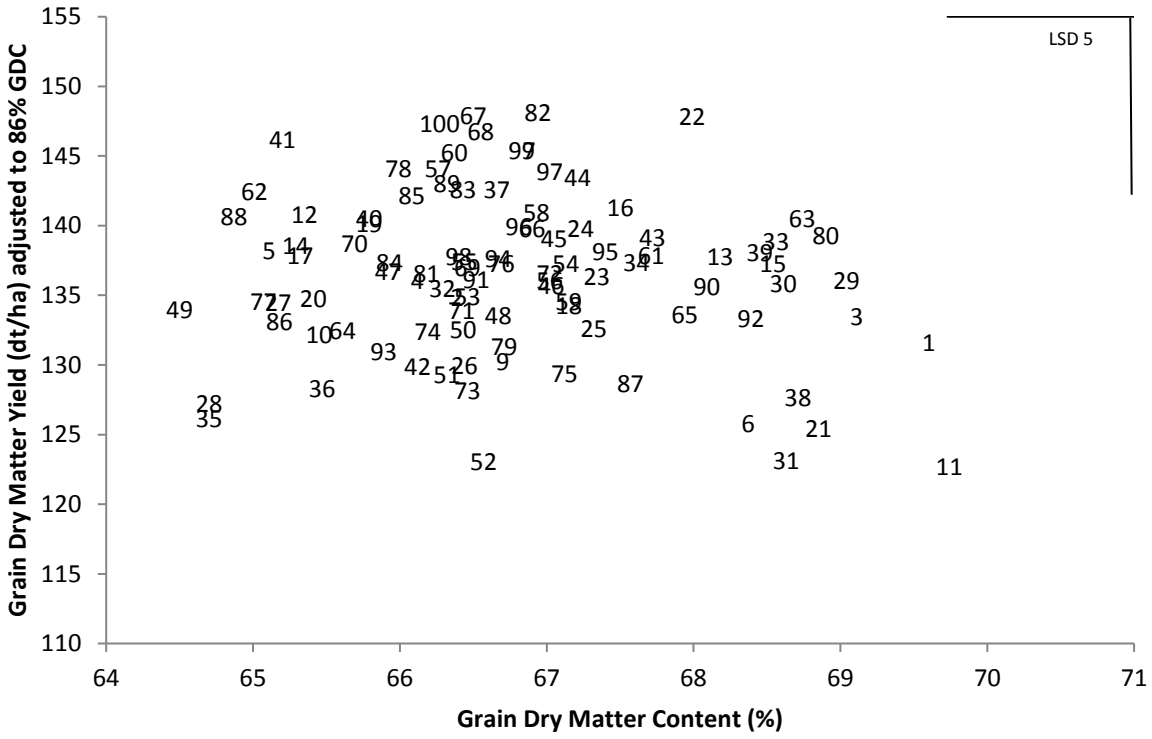

Figure II.7 Grain dry yield (GDY) in dt/ha (adjusted to 86 % GDC) plotted against grain dry matter content (GDC) in % of the Dent testcrosses for the performance test dual use maize harvest in year 2014. Numbers are the entry numbers of the testcrosses. Coefficient of determination: R^2: 0.0212

Table II.12 is showing the correlations between the different traits. The total dry matter yield and the stover dry matter yield were highly positive correlated with each other (0.75**), while the grain dry matter yield and the stover dry matter yield were not correlated with each other (-0.04). The grain dry matter yield and the total dry matter yield were significantly correlated with each other (0.63**).

Table II.12 Table of Correlation of the traits for the Dent testcrosses

	Total fresh matter	Total dry matter content	Grain fresh matter	Grain dry matter content	Grain dry matter yield	Total dry matter yield	Stover dry matter yield	Stover fresh matter
Total dry matter content	-0.82**							
Grain fresh matter	0.57	-0.31**						
Grain dry matter content	-0.58**	0.66**	-0.45**					
Grain dry matter yield	0.43**	-0.11	0.95**	-0.15				
Total dry matter yield	0.65**	-0.12	0.62**	-0.16	0.63**			
Stover dry matter yield	0.49**	-0.07	-0.01	-0.08	-0.04	0.75**		
Stover fresh matter	0.97**	-0.85**	0.36**	-0.53**	0.21*	0.56**	0.56**	
Water content of stover	0.57**	-0.86**	0.44**	-0.53**	0.30**	-0.13	-0.40**	0.52**

Significance level *p=0.05, **p=0.01; +p=0.1

As Table II.13 shows, had the grain dry matter yield a heritability of 64 %. The locations were showing significant differences and a high variance component. The genotypes were also significant at a signficiance level of 1 %. The interaction between location and genotype was significant, and its variance component as well. Most variation was explained by the location, while the genotype-location interaction was explaining lowest.

Table II.13 Analysis of Variance for the trait Grain dry matter yield (GDY) in dt/ha of the Dent testcrosses

Source	DF	MS	Var.cp	F-value	LSD5
Location	2	6565.9199	65.1904	140.06**	1.91
Genotype	99	132.7871	28.6355	2.83**	11.03
Location-Genotype	196	46.8805	19.4865	1.71**	14.59
Error	222	27.3941	27.3941		
Heritability 64 %					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

The heritability of the grain dry matter content was high with 83 %. Table II.14 shows that the location differ significantly from each other and furthermore, were having the lowest variance component. The genotypes were also showing a significant high F-value, explained most of the variation. Furthermore was the interaction between genotype and location significant.

Table II.14 Analysis of Variance for the trait Grain Dry Matter Content (GDC) in % of the Dent Genepool

Source	DF	MS	Var.cp	F-value	LSD5
Location	2	8.3484	0.0773	13.44**	0.22
Genotype	99	4.0998	1.1596	6.60**	1.27
Location-Genotype	196	0.6209	0.4566	3.78**	1.13
Error	204	0.1643	0.1643		
Heritability 83 %					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

Comparing the stover dry matter yield with the grain dry matter content, no correlation was shown between the traits. The stover dry matter content was significant low correlated with the stover dry matter yield (0.40**). The total dry matter yield was not correlated with the grain dry matter content and the stover dry matter content (Table II.12).

Figure II.8 is plotting the stover dry matter yield (dt/ha) against the grain dry matter content (%). The total variation of all genotypes was not high, most genotypes were showing a moderate grain dry matter content (65 % - 69 %) and a moderate stover dry matter yield (60 dt/ha - 80 dt/ha). Genotype 1 and 11 were showing a low grain dry matter content and also their stover dry matter yield was low (Figure II.8) Genotype 29 had a high stover dry matter yield and was containing a high grain dry matter content.

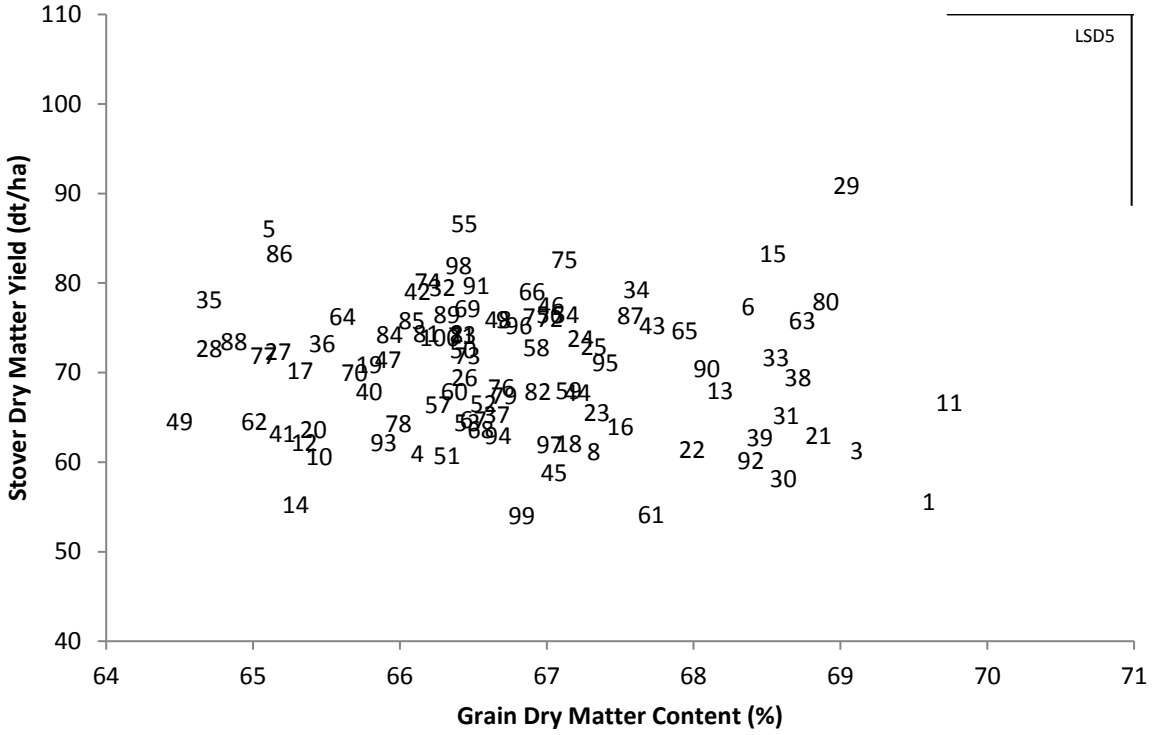

Figure II.8 Stover dry Matter Yield (SDY) in dt/ha plotted against grain dry matter content (GDC) in % of the Dent testcrosses of the performance test dual use maize harvest in year 2014. Numbers are the entry numbers of the testcrosses. Coefficient of determination R^2: 0.0067

Table II.15 shows, that the interaction between location and genotype was significant, with also a high variance component. The genotypes each by themselves did not show any significance, containing the lowest variance component. The locations differed significantly from each, explaining most of the variation. The heritability of the stover dry matter yield was low (16 %).

Table II.15 Analysis of Variance for the trait Stover dry matter yield (SDY) in dt/ha of the Dent testcrosses

Source	DF	MS	Var.cp	F-value	LSD5
Location	2	5342.3655	51.9716	36.79**	3.36
Genotype	99	174.0780	9.6245	1.20	19.40
Location-Genotype	196	145.2044	44.0155	1.43**	28.08
Error	240	101.1889	101.1889		
Heritability 16 %					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

Comparing the two traits water content in the stover (RH_2O) and the grain dry matter content a negative correlation was shown (Figure II.9/Table II.12). Figure II.9 shows, that genotype 11, which had a high grain dry matter content, was having a low water content in the stover on the other hand. Genotype 1, contained a high grain dry matter content as well was showing a moderate water

content in the stover. Comparing genotype 82, had high water content in the stover with a moderate grain dry matter content.

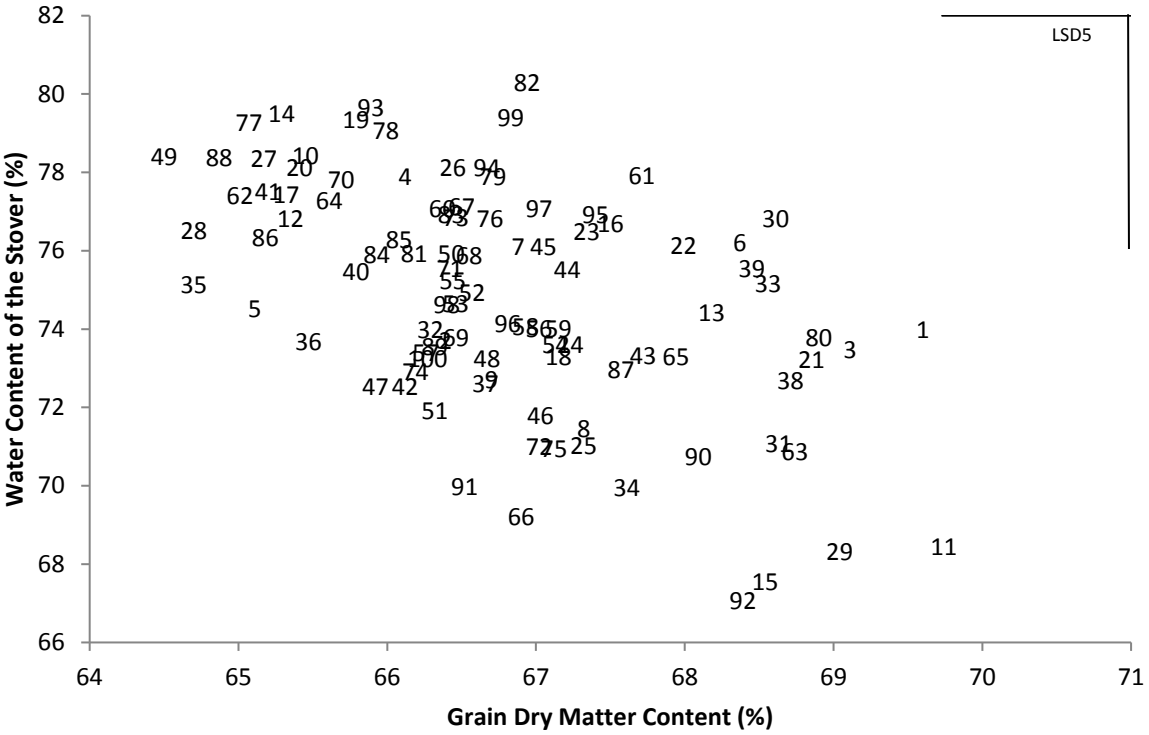

Figure II.9 Water content of the stover (RH_2O) in % plotted against Grain dry matter content (GDC) in % of the Dent testcrosses for the performance test dual use maize harvest in year 2014. Numbers are the entry numbers of the testcrosses. Coefficient of determination R^2: 0.2821

The heritability of the water content of the stover was 54 %. The interaction between location and genotype was significant. While its variance component was not differeing a lot to the variance component of location and genotype. All sources explained nearly the same of the variation (Table II.16).

Table II.16 Analysis of Variance for the trait Water content in the stover (RH_2O) in % of the Dent testcrosses

Source	DF	MS	Var.cp	F-value	LSD5
Location	2	529.8094	5.1833	46.15**	0.94
Genotype	99	25.0531	4.5242	2.18**	5.46
Location-Genotype	196	11.4803	4.2187	1.58**	7.51
Error	240	7.2617	7.2617		
Heritability 54 %					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

The water content was low negative significant correlated with the stover dry matter yield (-0.4**) and had a low positive significant correlation with the grain dry matter content (0.30**). There was no correlation between water content of the stover the total dry matter yield (Table II.12).

3.2.2. Flint testcrosses

Comparing the grain dry matter yield (adjusted to 86 % GDC in dt/ha) with the grain dry matter content (%) it was shown that there is a distribution around the average of 68 % grain dry matter content (Figure II.10). Genotype 30 laid outside of the group as seen in figure II.10, with a high grain dry matter content and a low grain dry matter yield. The lowest grain dry matter yield was owned by genotype 12 and 75. The highest grain dry matter yield was owned by genotype 100.

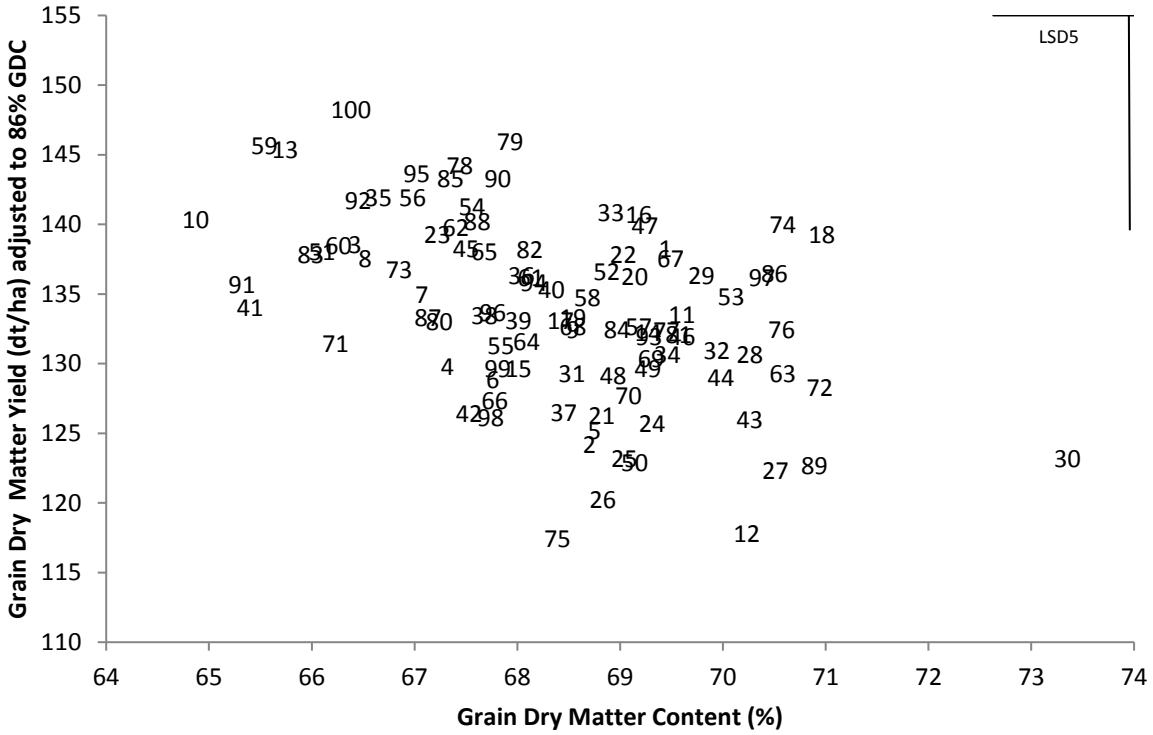

Figure II.10 Grain dry matter yield (GDY) in dt/ha (adjusted to 86 % GDC) plotted against Grain dry matter content (GDC) in % of the Flint testcrosses for the performance test: dual use maize harvest in year 2014. Numbers are the entry numbers of the testcrosses. Coefficient of determination R^2: 0.2231

The correlation between grain dry matter yield and grain dry matter content was negative (Figure II.10/Table II.17).

Table II.17 Table of Correlation of the traits for Flint testcrosses

	Total fresh matter	Total dry matter content	Grain fresh matter	Grain dry matter content	Grain dry matter yield	Total dry matter yield	Stover dry matter yield	Stover fresh matter
Total dry matter content	-0.76**							
Grain fresh matter	0.64**	-0.39**						
Grain dry matter content	-0.52**	0.40**	-0.73**					
Grain dry matter yield	0.60**	-0.33**	0.94**	-0.47**				
Total dry matter yield	0.82**	-0.26**	0.61**	-0.44**	0.60**			
Stover dry matter yield	0.63**	-0.12	0.14	-0.21*	0.14	0.84**		
Stover fresh matter	0.97**	-0.77**	0.43**	-0.38**	0.41**	0.77**	0.69**	
Water content of stover	0.29**	-0.76**	0.32**	-0.16	0.32**	-0.25*	-0.52**	0.24*

significance level *p=0.05, **p=0.01; +p=0.1

The total dry matter yield and the stover dry matter yield were highly positive correlated with each other (0.84**), while the grain dry matter yield and the stover dry matter yield were not correlated with each other (0.14), as Table II.17 shows. The grain dry matter yield and the total dry matter yield were correlated with each other but not significant (0.60).

The analysis of variance for the trait grain dry matter yield (Table II.18) was showing that the locations and genotypes are differing significantly from each other. The interaction between location and genotype was also significant. Most variation was explained by the loctaions. The heritability of the grain dry matter yield was 37 %.

Table II.18 Analysis of Variance for the trait Grain dry matter yield (GDY) in dt/ha of the Flint testcrosses

Source	DF	MS	Var.cp	F-value	LSD5
Location	1	6436.6538	63.8493	124.44**	2.02
Genotype	99	82.4127	15.3442	1.59*	14.27
Location-Genotype	98	51.7242	28.0636	2.19**	13.58
Error	162	23.6607	23.6607		
Heritability 37 %					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

The grain dry matter content had a high heritability (89 %) and the analysis of variance was showing a small error (Table II.19). The interaction between location and genotype was showing a small variance component, resulting in a low significant F-value. The locations were differing from each other significantly, but were having a small variance component as well. The genotypes also showed significance and explained most of the found variation.

Table II.19 Analysis of Variance for the trait Grain dry matter content (GDC) in % of the Flint testcrosses

Source	DF	MS	Var.cp	F-value	LSD5
Location	1	28.6043	0.2813	60.41**	0.19
Genotype	99	4.4143	1.9704	9.32**	1.37
Location-Genotype	98	0.4735	0.2886	2.56**	1.20
Error	144	0.1849	0.1849		
Heritability 89 %					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

Figure II.11 is comparing the stover dry matter yield (dt/ha) and the grain dry matter content. Genotype 30 were showing a moderate stover dry matter yield but a high grain dry matter content. Genotype 37 was showing a high stover dry matter yield and a moderate grain dry matter content. While genotype 91, 41 and 10 were showing a low grain dry matter content and a high stover dry matter yield.

No correlation between the traits stover dry matter yield (dt/ha) and grain dry matter content (%) was found (Figure II.11/Table II.17). The stover dry matter yield was showing a stronger correlation with the stover dry matter content (0.518**). The total dry matter content was showing no correlation with the stover dry matter yield (0.248) (Table II.17).

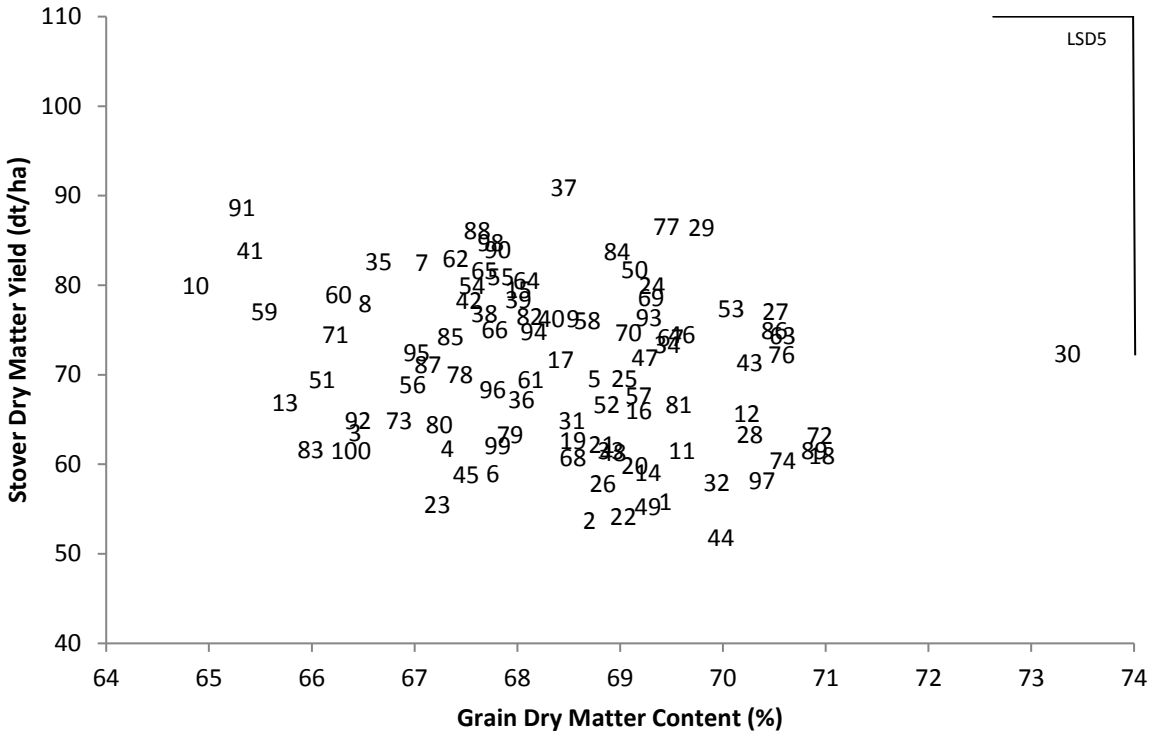

Figure II.11 Stover Dry Matter Yield (SDY) in dt/ha plotted against Grain dry matter content (GDC) in % of the Flint testcrosses for the performance test: dual use maize harvest in year 2014. Numbers are the entry numbers of the testcrosses. Coefficient of determination R^2: 0.0426

Table II.20 is showing the analysis of variance for the trait stover dry matter yield. The heritability was low with 24 %. The locations showed no significance, while its variance component was very low as well. The interaction between genotype and location was also not significant, but explaining most of the variation with the highest variance component. Only the genotypes were showing a significant differences on a level of 10 %.

Table II.20 Analysis of Variance for the trait Stover dry matter yield (SDY) in dt/ha of the Flint hybrid

Source	DF	MS	Var.cp	F-value	LSD5
Location	1	161.9818	0.0527	1.03	3.51
Genotype	99	207.4484	25.3692	1.32+	24.84
Location-Genotype	98	156.7100	27.9255	1.22	31.72
Error	144	128.7845	128.7845		
Heritability 24 %					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

Figure II.12 is comparing the two traits water content in the stover (RH_2O) and the grain dry matter content. The correlation between the traits was low. Also the water content in the stover was high enough to be silage.

Genotype 30 had the highst grain dry matter content and a low water content in the stover. Genotype 23 was showing the highest water content in the stover, with a moderate grain dry matter content. But also genotype 100 and 83 had a high water content in the stover but a slightly lower grain dry matter content, compared to genotype 23 (Figure II.12).

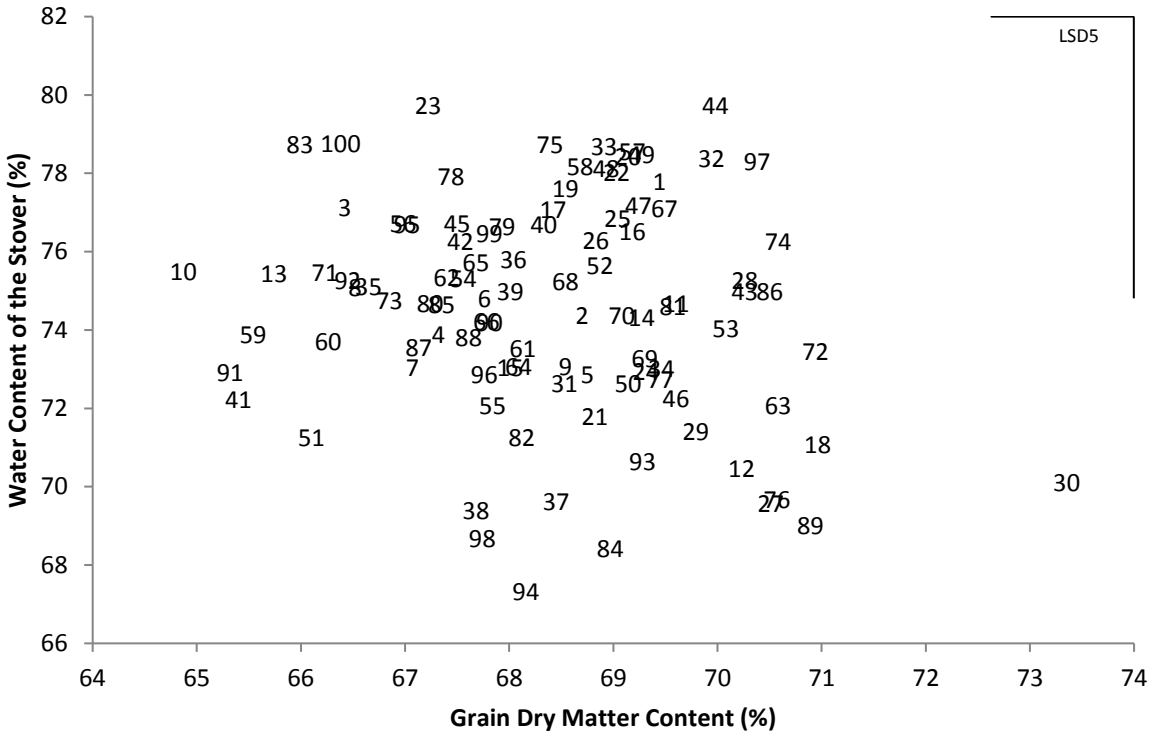

Figure II.12 Water Content of the Stover (RH_2O) in % plotted against Grain dry matter content (GDC) in % of the Flint testcrosses for the performance test: dual use maize harvest in the year 2014. Numbers are the entry numbers of the testcrosses. Coefficient of determination R^2: 0.0256

The analysis of variance for the trait water content in the stover was showing a significance between location and genotype, explaining most of the variation with the highest variance component. Also the locations differed significantly from each other, showing the lowest variance component. The genotypes were showing significance as well, with the second highest variance component. The heritability was moderate with 31 % (Table II.21).

Table II.21 Analysis of Variance for the trait Water content in the stover (RH_2O) in % of the Flint testcrosses

Source	DF	MS	Var.cp	F-value	LSD5
Location	1	85.9505	0.7535	8.11**	0.91
Genotype	99	15.5584	2.4779	1.47*	6.46
Location-Genotype	98	10.6026	4.2261	1.66**	7.05
Error	162	6.3765	6.3765		
Heritability 31 %					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

The water content was low negative significant correlated with the stover dry matter yield (-0.52**) and low negative correlated with the grain dry matter content (-0.16). There was a low significant correlation between water content of the stover the total dry matter yield (-0.25*/Table II.17).

3.2.3 Factorial crosses

Figure II.13 is showing the grain dry matter yield (da/ha) adjusted to 86 % GDC plotted against the grain dry matter content (%).Genotype 80 was showing a low grain dry matter yield with a high grain dry matter content. The grain dry matter content of genotype 67 and 83 were higher but also the grain dry matter yield was higher compared to genotype 80. Genotype 24 had a low grain dry matter content with a moderate grain dry matter yield.

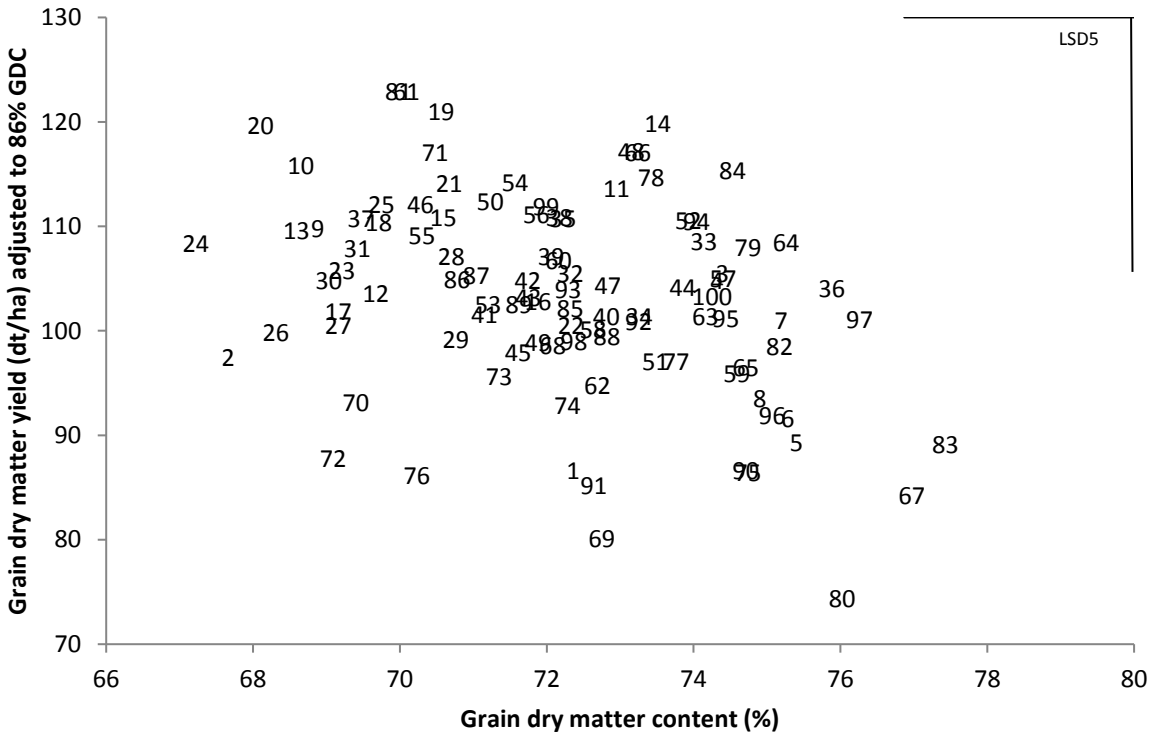

Figure II.13 Grain dry matter yield (GDY) in dt/ha plotted against Grain dry matter content (GDC) in % of the Factorial crosses for the performance test: dual use maize harvest in year 2015. Numbers are the entry numbers of the testcrosses. Coefficient of determination R^2: 0.1288

The correlation between grain dry matter content and grain dry matter yield was low as the coefficient of determination and the table of correlation were showing (Figure II.13/Table II.22).

Table II.22 Table of Correlation of the traits for factorial crosses

	Total fresh matter	Total dry matter content	Grain fresh matter	Grain dry matter content	Grain dry matter yield	Total dry matter yield	Stover dry matter yield	Stover fresh matter
Total dry matter content	-0.68**							
Grain fresh matter	0.52**	-0.28**						
Grain dry matter content	-0.60**	0.61**	-0.59**					
Grain dry matter yield	0.42**	-0.14	0.96**	-0.36**				
Total dry matter yield	0.76**	-0.14	0.51**	-0.29**	0.49**			
Stover dry matter yield	0.62**	-0.11	-0.09	-0.12	-0.16	0.77**		
Stover fresh matter	0.96**	-0.68**	0.26**	-0.49**	0.14	0.69**	0.74**	
Water content of stover	0.29**	-0.79**	0.44**	-0.43**	0.40**	0.29**	-0.46**	0.18

significance level *p=0.05, **p=0.01; +p=0.1

Table II.22 shows that the total dry matter yield and the stover dry matter yield were highly positive correlated with each other (0.77**), while the grain dry matter yield and the stover dry matter yield were not correlated with each other (-0.16). The grain dry matter yield and the total dry matter yield were signficintly correlated with each (0.49**).

The heritability of the grain dry matter yield was moderate with 31 % (Table II.23). The locations were showing a high variance component and a high F-value, explaining most of the variation. The differences between the genotypes were also highly significant, while they were showing the smalles variance component. Furthermore was the interaction between location and genotype significant with the second highest variance component (Table II.23).

Table II.23 Analysis of Variance for the trait Grain dry matter yield (GDY) in dt/ha of the factorial crosses

Source	DF	MS	Var.cp	F-value	LSD5
Location	1	50991.2520	508.6603	407.22**	3.14
Genotype	99	182.1397	28.4611	1.45*	22.20
Location-Genotype	99	125.2174	60.5080	1.94*	22.47
Error	160	64.7094	64.7094		
Heritability 31 %					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

The grain dry matter content was showing a high heritability (75 %). As Table II.24 shows, was the interaction between location and genotype significant, but explained nearly nothing of the variations. The variance component of the location was highest, explained most of the variation, with a significant high F-value. The genotypes were also showing a signficiant high F-value, but its variance component was second lowest (Table II.24).

Table II.24 Analysis of Variance for the trait Grain dry matter content (GDC) in % of the factorial crosses

Source	DF	MS	Var.cp	F-value	LSD5
Location	1	1064.0346	10.6154	426.83**	0.44
Genotype	99	10.0067	3.7569	4.01**	3.13
Location-Genotype	99	2.4929	1.2888	2.07**	3.06
Error	160	1.2041	1.2041		
Heritability 75%					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

Figure II.14 is showing the stover dry matter yield (dt/ha) compared to the grain dry matter content (%). There was no correlation of the traits (Figure II.14 / Table II.22).

The stover dry matter yield was significant correlated with the stover dry matter content (0.46**). The total dry matter yield was showing a strong positive correlation with the stover dry matter yield (0.77**/ Table II.22).

Genotype 11 showed the lowest stover dry matter yield with a moderate grain dry matter content, while genotype 4 was showing the highest stover dry matter yield and having a higher grain dry matter content compared to genotype 11 (Figure II.14). Genotype 42 and 83 had a low stover dry matter yield as well, but their grain dry matter content differed from each other. Genotype 42 had a moderate grain dry matter content, while genotype 83 had a high grain dry matter content (Figure II.14).

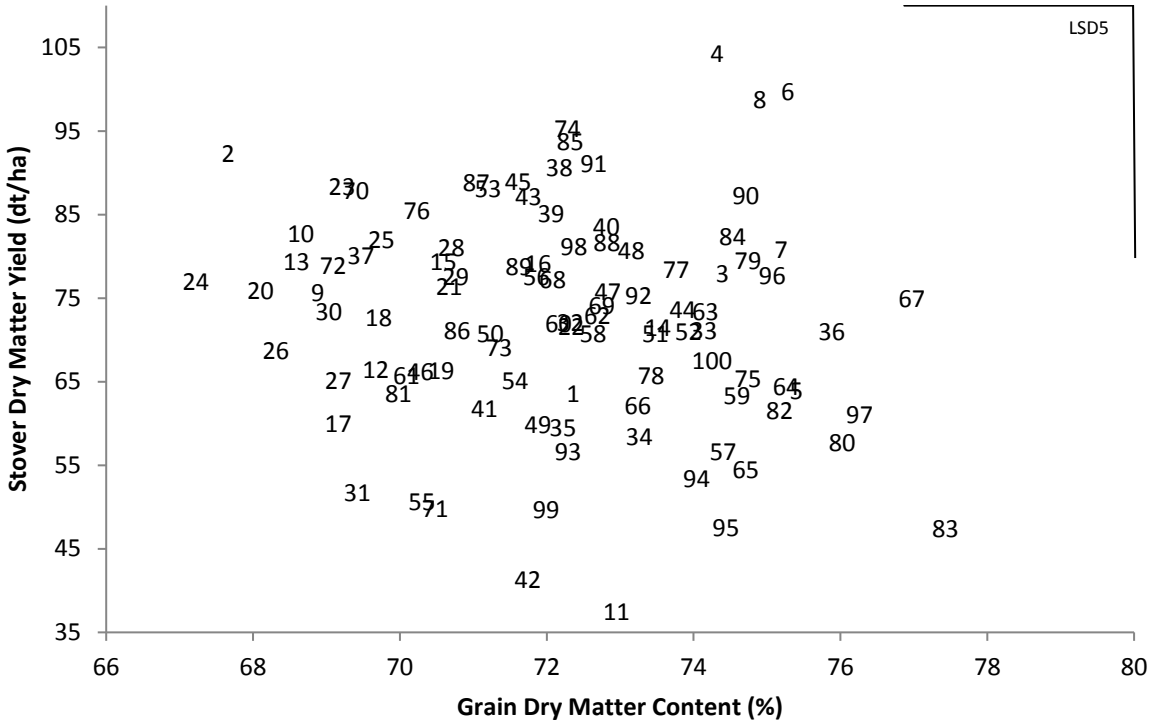

Figure II.14 Stover dry matter yield (SDY) in dt/ha plotted against Grain dry matter content (GDC) in % of the factorial crosses for the performance test: dual use maize harvest in year 2015. Numbers are the entry numbers of the testcrosses. Coefficient of determination R^2: 0.015

The heritability of the stover dry matter yield was moderate (43 %). The locations differed significantly from each other at a level of 5 %, showing the lowest variance component. The genotypes differed significant form each other, explained most of the variation with the highest variance component. The interaction between location and genotype was also significant and explained also a lot of the variation (Table II.25).

Table II.25 Analysis of Variance for the trait Stover dry matter yield (SDY) in dt/ha of the factorial crosses

Source	DF	MS	Var.cp	F-value	LSD5
Location	1	1062.1349	8.7218	5.59*	3.87
Genotype	99	336.2816	73.1621	1.77**	27.35
Location-Genotype	97	189.9574	69.0541	1.57**	30.75
Error	136	120.9033	120.9033		
Heritability 43 %					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

The traits water content in the stover and grain dry matter content were plotted against each other in Figure II.15. The correlation between the traits was negative (Figure II.15/ Table II.22). Also the water content in the stover was high enough to be silage.

Genotype 40 still had the lowest water content in the stover, while the grain dry matter content was moderate. Genotype 24 was showing a high water content in the stover, with the lowest grain dry matter content. Genotype 71 had the highest water content in the stover and a moderate low grain dry matter yield. The genotypes 97 and 83 had a high grain dry matter content and a high water content in the stover (Figure II.15).

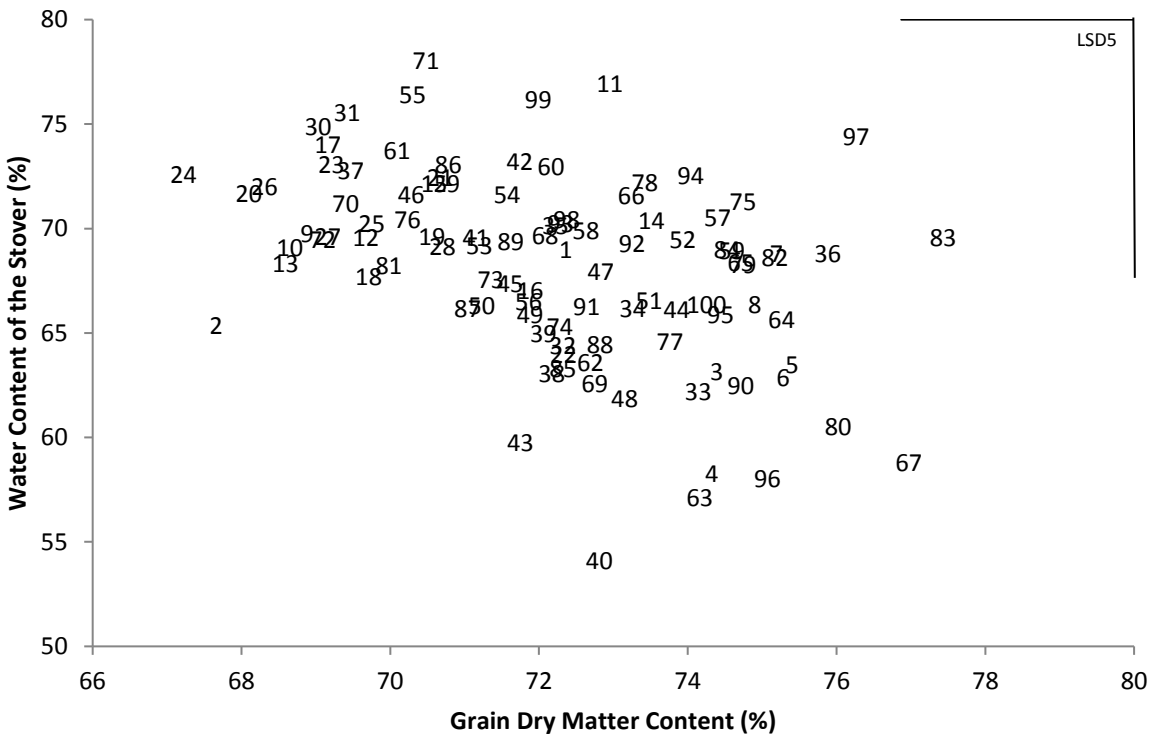

Figure II.15 Water content of the stover (RH_2O) in % plotted against Grain dry matter content (GDC) in % of the factorial crosses for the performance test: dual use maize harvest in year 2015. Numbers are the entry numbers of the testcrosses. Coefficient of determination R^2: 0.183

Table II.26 was showing the analysis of variance for the trait water content in the stover. The genotypes did not differ significantly from each other, and showed the lowest variance component. The locations showed a high F-value and were explaining most of the variation with the highest variance component. The interaction between location and genotype was significant. The heritability of the water content in the stover was low (20 %).

Table II.26 Analysis of Variance for the trait water content in the stover (RH_2O) in % of the factorial crosses

Source	DF	MS	Var.cp	F-value	LSD5
Location	1	8418.5340	83.8571	256.45**	1.61
Genotype	99	41.0762	4.1244	1.25	11.37
Location-Genotype	96	32.8273	17.2355	2.11**	11.04
Error	136	15.5918	15.5918		
Heritability 20 %					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

The water content was low negative significant correlated with the stover dry matter yield (-0.46**) and low negative significant correlated with the grain dry matter content (-0.43**). There was a small correlation between water content of the stover and total dry matter yield (-0.18/ Table II.22).

3.3. Comparing silage maize harvest and dual use maize harvest

3.3.1 Dent testcrosses

Table II.27 was showing the correlations between all traits analyzed for the performance test silage maize and dual use maize. There was no significant correlation between total dry matter yield of silage maize and the stover dry matter yield for dual use maize (0.20).

Table II.27 Table of Correlation for the Dent testcrosses of the traits measured during dual use maize harvest and the traits measured during silage maize harvest

	Total fresh matter[a]	Total dry matter content[a]	Total dry matter yield[a]	Grain fresh matter[a]	Grain dry matter content[a]	Grain dry matter yield[a]	Stover dry matter yield	Stover fresh matter	Water content of stover	Total dry matter content[b]	Total fresh matter[b]
Total dry matter content[a]	-0.82**										
Total dry matter yield[a]	0.65**	-0.11									
Grain fresh matter[a]	0.57	-0.31**	0.62**								
Grain dry matter content[a]	-0.58**	0.66**	-0.16	-0.47**							
Grain dry matter yield[a]	0.43**	-0.11	0.63**	0.95**	-0.14						
Stover dry matter yield	0.49**	-0.04	0.75**	0.01	-0.08	-0.04					
Stover fresh matter	0.97**	-0.85**	0.56**	0.36**	-0.52**	0.21*	0.56**				
Water content of stover	0.57**	-0.86**	-0.13	0.44**	-0.53**	0.23**	-0.40**	0.52**			
Total dry matter content[b]	-0.80**	0.80**	-0.31**	-0.33**	0.65**	-0.12	-0.29**	-0.80**	-0.58**		
Total fresh matter[b]	0.74**	-0.57**	0.55**	0.55**	-0.53**	0.41**	0.35**	0.68**	0.40**	-0.72**	
Total dry matter yield[b]	0.23*	0.03	0.47**	0.45**	-0.08	0.47**	0.20	0.13	-0.04	0.02	0.67**

[a] traits are taken during dual use maize harvest/[b] traits are taken during silage maize harvest/Significance level: **p=0.01, *p=0.05, +p=0.1

The total dry matter yield (TDY) of maize, harvested as dual use maize is plotted against the total dry matter yield (TDYs) of maize, harvested as silage maize in Figure II.16. Many genotypes showing a high total dry matter yield during dual use maize harvest were also showing a high total dry matter yield for silage maize harvest, like genotype 100, 5 and 2. On the other hand was the total dry matter yield during dual use maize of the genotypes 1, 21 and 51 low and also the total dry matter yield during silage maize harvest was low. Genotype 14 was showing a high total dry matter yield during silage maize harvest but the total dry matter yield during dual use maize harvest was low. The correlation between total dry matter yield of the performance test silage maize and the total dry matter yield of the performance test dual use maize was significant (Table II.27).

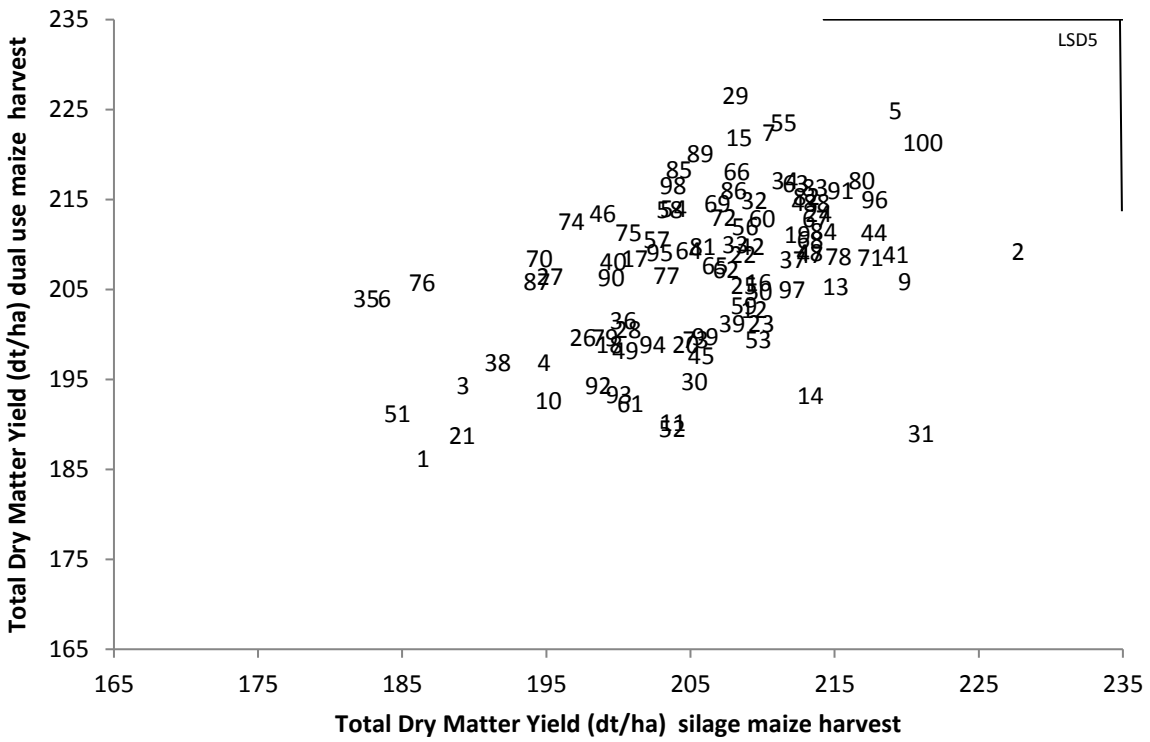

Figure II.16 Total dry matter yield of the performance test dual use maize (TDY) in dt/ha plotted against the total dry matter yield of the performance test silage maize (TDYs) in dt/ha of the Dent testcrosses. Number are the entry numbers of the testcrosses. Coefficient of determination R^2: 0.2187

Figure II.17 is showing the stover dry matter yield (SDY) plotted against the total dry matter yield of silage maize harvest (TDYs).

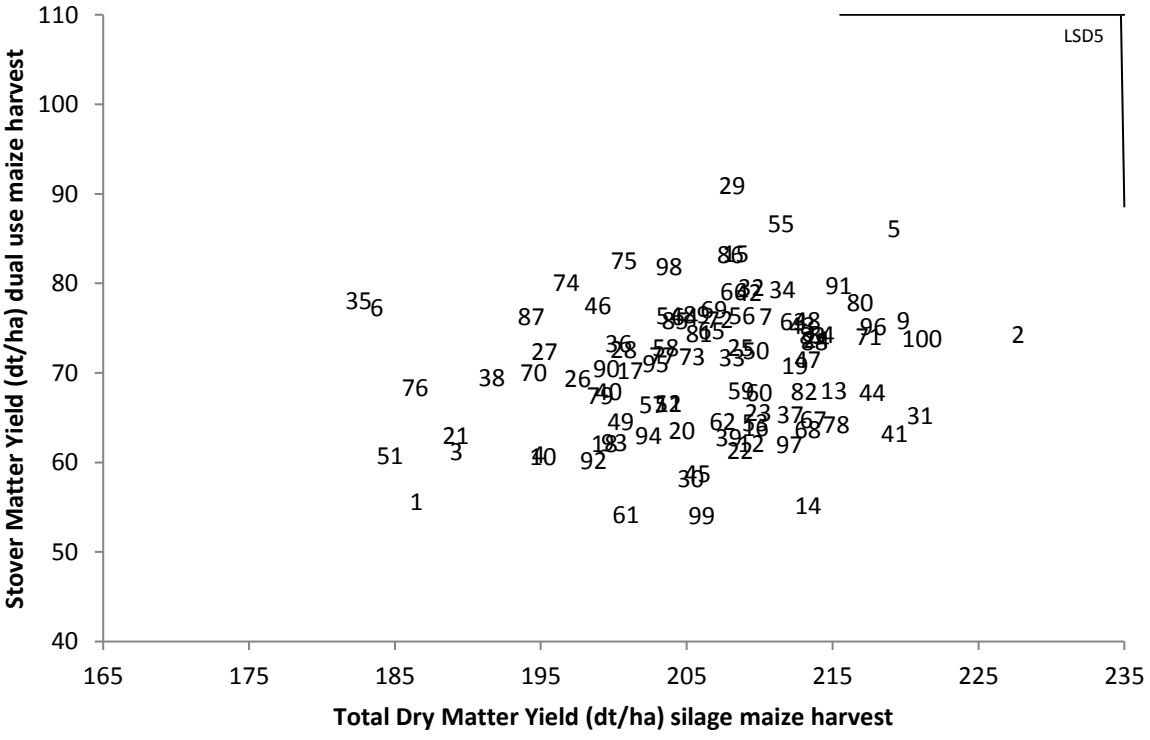

Figure II.17 Stover dry matter yield of the performance test dual use maize (SDY) in dt/ha plotted against total dry matter yield of the performance test silage maize (TDYs) in dt/ha of the Flint testcrosses Numbers are the entry numbers of the testcrosses. Coefficient of determination R^2: 0.0389

Genotype 1 and 51 showed a low total dry matter yield as silage maize and the stover dry matter yield was low as well. On the other hand showed genotype 2 and 5 a high total dry matter yield (performance test silage maize harvest) while the stover dry matter yield was moderate to high. Genotype 29 and 55 were showing the highest stover dry matter yield and their total dry matter yield was high as well. The correlation between grain dry matter yield and total dry matter yield (silage maize) was high (0.63**/Table II.27).

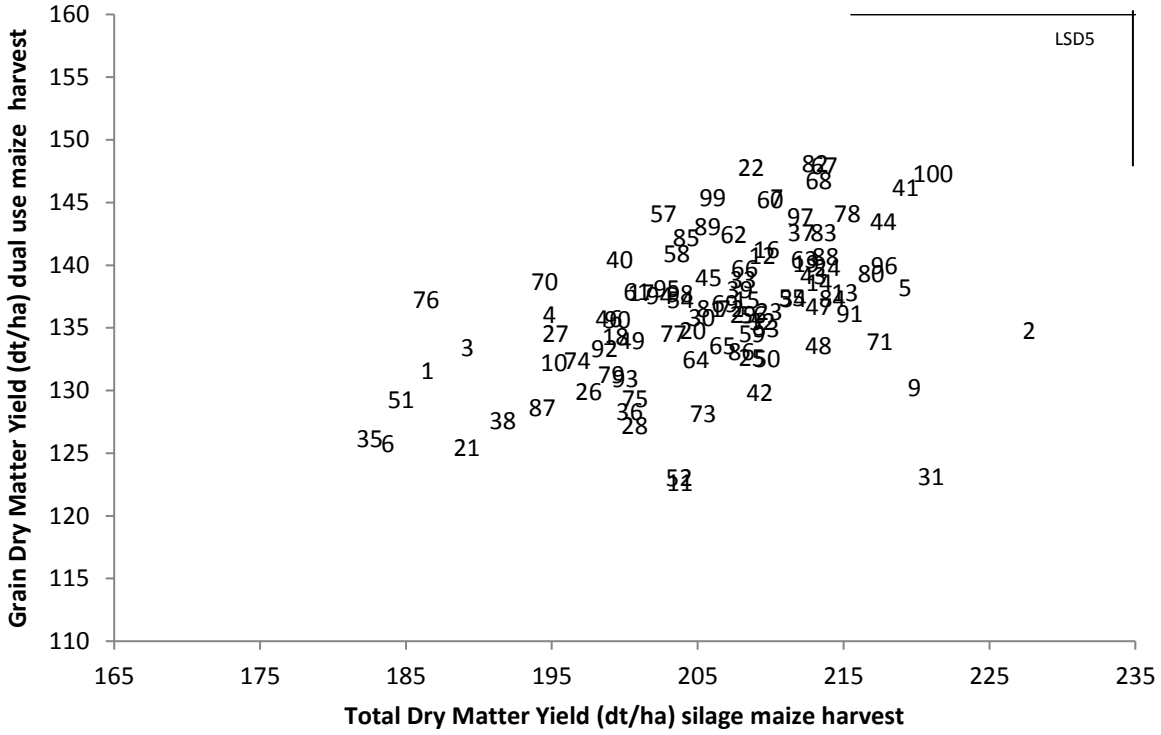

Figure II.18 Grain dry matter yield of the performance test dual use maize (GDY) in dt/ha plotted against total dry matter yield of the performance test silage maize (TDYs) in dt/ha of the Dent testcrosses Numbers are the entry numbers of the testcrosses. Coefficient of determination R^2: 0.2188

Figure II.18 is showing the grain dry matter yield (GDY) for the performance test dual use maize plotted against the grain dry matter yield for the performance test silage maize (TDYs). Some genotypes, like genotype 31, 21 and 35 were showing a low grain dry matter yield. Genotype 31 had on the other hand a high total dry matter yield (silage maize), while genotype 21 and 35 also had a low total dry matter yield (silage maize). Genotype 100 and 41 had a high grain dry matter yield and a high total dry matter yield for the performance test silage maize. Genotype 9 and 71 were showing a moderate grain dry matter yield but a high total dry matter yield for the performance test silage maize.

3.3.2. Flint testcrosses

Table II.28 is showing the correlations between all traits analyzed for the performance tests silage maize and dual use maize. There was a significant correlation between total dry matter yield of the performance test silage maize and the stover dry matter yield for the performance test dual use maize (0.41**).

Table II.28 Table of Correlation for the Flint testcrosses of the traits measured during dual use maize harvest and the traits measured during silage maize harvest

	Total fresh matter[a]	Total dry matter content[a]	Total dry matter yield[a]	Grain fresh matter[a]	Grain dry matter content[a]	Grain dry matter yield[a]	Stover dry matter yield	Stover fresh matter	Water content of stover	Total dry matter content[b]	Total fresh matter[b]
Total dry matter content[a]	-0.76**										
Total dry matter yield[a]	0.82**	-0.26**									
Grain fresh matter[a]	0.64**	-0.39**	0.61**								
Grain dry matter content[a]	-0.52**	0.40**	-0.44**	-0.73**							
Grain dry matter yield[a]	0.60**	-0.33**	0.60**	0.94**	-0.47**						
Stover dry matter yield	0.63**	-0.12	0.84**	0.14	-0.21*	0.14					
Stover fresh matter	0.97**	-0.77**	0.77**	0.43**	-0.38**	0.41**	0.69**				
Water content of stover	0.29**	-0.76**	-0.25*	0.32**	-0.16	0.32**	-0.52**	0.24*			
Total dry matter content[b]	-0.66**	0.76**	-0.31**	-0.41**	0.46**	-0.38**	-0.20*	-0.64**	-0.50**		
Total fresh matter[b]	0.72**	-0.59**	0.55**	0.51**	-0.51**	0.43**	0.40**	0.68**	0.25	-0.71**	
Total dry matter yield[b]	0.44**	-0.17	0.51**	0.36**	-0.31**	0.30**	0.41**	0.41**	-0.08	-0.15	0.80**

[a] traits are taken during dual use maize harvest/[b] traits are taken during silage maize harvest/Significance level: **p=0.01, *p=0.05, +p=0.1

The total dry matter yield (TDY) of maize, harvested as dual use maize is plotted against the total dry matter yield (TDYs) of maize, harvested as silage maize (Figure II.19). Many genotypes showing a high total dry matter yield during dual use maize harvest were also showing a high total dry matter yield for silage maize harvest. A high total dry matter yield at dual use maize harvest and a high total dry matter yield during silage maize harvest were showing genotype 41, 62 and 91. On the other hand was the total dry matter yield as dual use maize of the genotypes 75,26 and 2 low, and the total dry matter yield during silage maize harvest was low. Genotype 89 was showing a moderate total dry matter yield during silage maize harvest but the total dry matter yield during dual use maize harvest was low. The correlation between total dry matter yield of silage maize and the total dry matter yield of dual use maize was significant (0.51**/Table II.28).

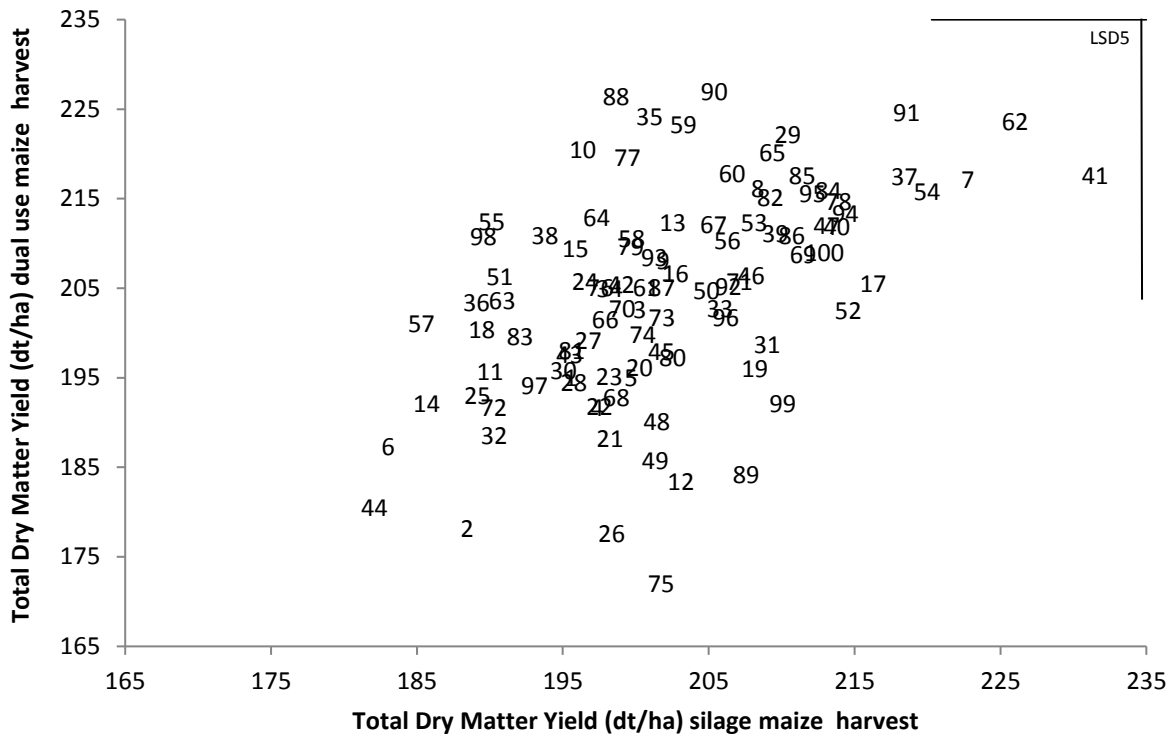

Figure II.19 Total dry matter yield of the performance test dual use maize (TDY) in dt/ha plotted against total dry matter yield of the performance test silage maize (TDYs) in dt/ha of the Flint testcrosses Numbers are the entry numbers of the testcrosses. Coefficient of determination R^2: 0.2629

Figure II.20 is showing the stover dry matter yield for dual use maize plotted against the total dry matter yield for silage maize.

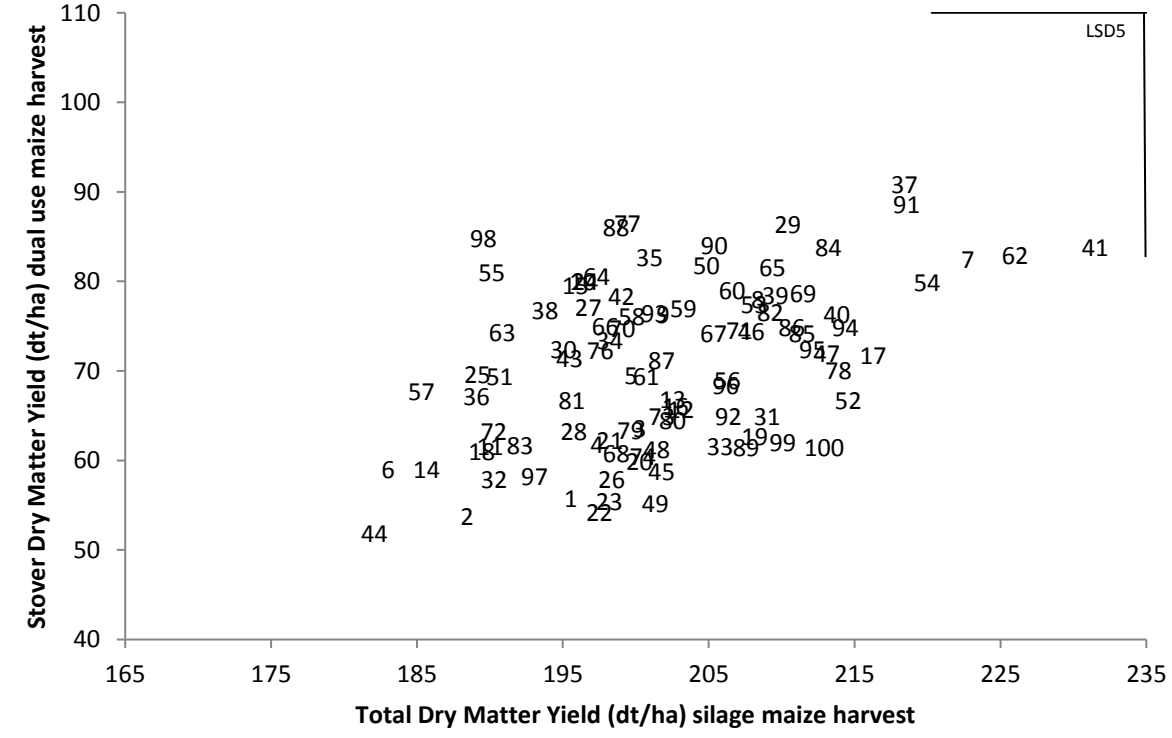

Figure II.20 Stover dry matter yield of the performance test dual use maize (SDY) in dt/ha plotted against the total dry matter yield of the performance test silage maize (TDYs) in dt/ha of the Flint testcrosses Numbers are the entry numbers of the testcrosses. Coefficient of determination R^2: 0.1643

Genotype 41, 62 and 7 contained a high stover dry matter yield and also the total dry matter yield of silage maize was high. On the other hand was genotype 44, 2, 14 and 6 showing a low total dry matter yield for silage maize and the stover dry matter yield was low.

Figure II.21 is showing the grain dry matter yield plotted against the total dry matter yield of silage maize. Some genotypes, like genotype 62,54 and 100 were showing a moderate to high grain dry matter yield and a high total dry matter yield of silage maize. On the other hand showed genotype 44, 25 and 98 a low grain dry matter yield and a low total dry matter yield for silage maize harvest. The correlation between grain dry matter yield and total dry matter yield (silage maize) was moderate (0.30**/Table II.28).

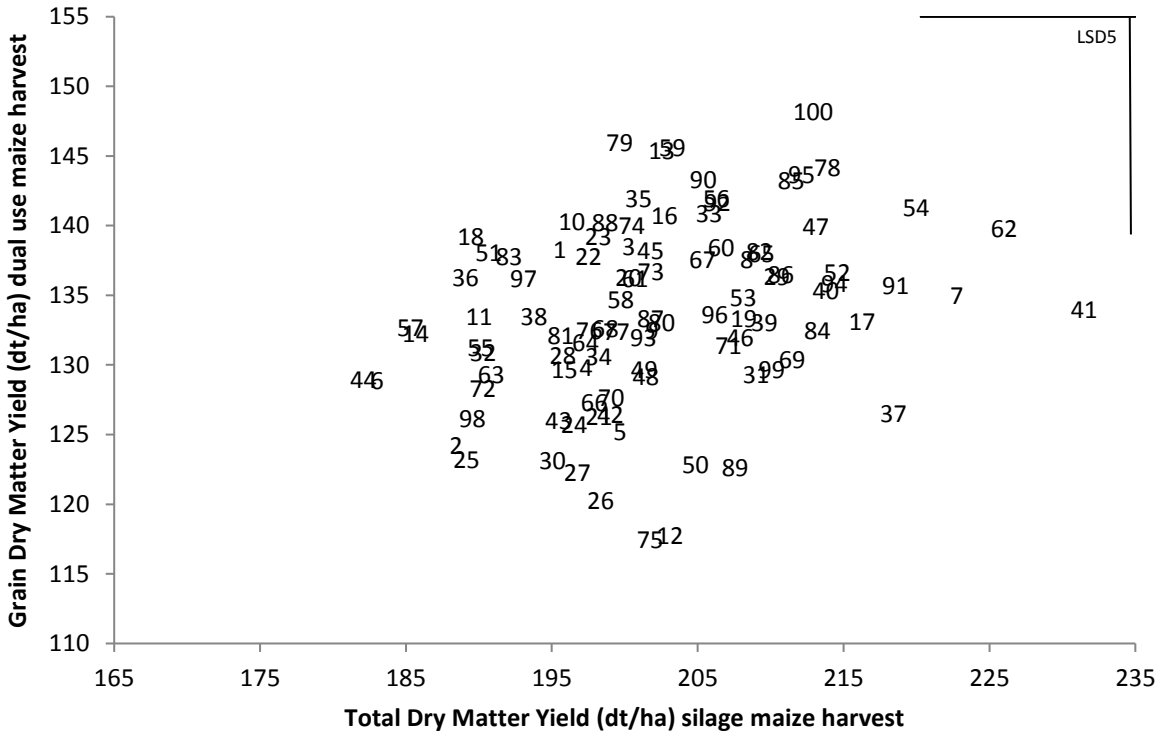

Figure II.21 Grain dry matter yield of the performance test dual use maize (GDY) in dt/ha plotted against total dry matter yield of the performance test silage maize (TDYs) in dt/ha of the Flint testcrosses Numbers are the entry numbers of the testcrosses. Coefficient of determination R^2: 0.0907

3.3.3 Factorial crosses

Figure II.29 is showing the correlations between all traits analyzed for the performance test silage maize and dual use maize. There was a significant correlation between total dry matter yield of the performance test silage maize and the stover dry matter yield for the performance test dual use maize (0.30*).

Table II.29 Table of Correlation for the factorial crosses of the traits measured during dual use maize harvest: and the traits measured during silage maize harvest:

	Total fresh matter[a]	Total dry matter content[a]	Total dry matter yield[a]	Grain fresh matter[a]	Grain dry matter content[a]	Grain dry matter yield[a]	Stover dry matter yield	Stover fresh matter	Water content of stover	Total dry matter content[b]	Total fresh matter[b]
Total dry matter content[a]	-0.68**										
Total dry matter yield[a]	0.76**	-0.13									
Grain fresh matter[a]	0.52**	-0.28**	0.51**								
Grain dry matter content[a]	-0.60**	0.62**	-0.29**	-0.59**							
Grain dry matter yield[a]	0.42**	-0.14	0.49**	0.96**	-0.36**						
Stover dry matter yield	0.62**	-0.11	0.77**	-0.09	-0.12	-0.16					
Stover fresh matter	0.96**	-0.68**	0.69**	0.26**	-0.49**	0.14	0.74**				
Water content of stover	0.29**	-0.79**	0.18	0.44**	-0.43**	0.40**	-0.49**	0.18			
Total dry matter content[b]	-0.59**	0.67**	-0.25*	-0.17	0.60**	-0.01	-0.34**	-0.61**	-0.35**		
Total fresh matter[b]	0.64**	-0.57**	0.38**	0.26*	-0.55**	0.12	0.41**	0.64**	0.24*	-0.75**	
Total dry matter yield[b]	0.36**	-0.12	0.38**	0.25*	-0.23*	0.21*	0.30**	0.33**	-0.06	-0.11	0.70**

[a] traits are taken during dual use maize harvest/[b] traits are taken during silage maize harvest/Significance level: **p=0.01, *p=0.05, +p=0.1

Figure II.22 is showing the total dry matter yield (TDY) of dual use maize plotted against the total dry matter yield (TDYs) of silage maize. The correlation between total dry matter yield of the performance test silage maize and the total dry matter yield of the performance test dual use maize was significant (0.38**/Table II.29). Most genotypes showing a high total dry matter yield as dual use maize were also showing a high total dry matter yield for silage maize harvest. Genotype 40 and 23 were showing a high total dry matter yield at silage maize harvest and a moderate total dry matter yield during dual use maize harvest. For genotype 80 and 83 was the total dry matter yield during dual use maize harvest low, while the total dry matter yield during silage maize harvest was moderate to low. Genotype 65 was showing a low total dry matter yield for silage maize harvest and a moderate total dry matter yield during dual use maize harvest. Genotype 81 and 61 were showing the highest total dry matter yield during dual use maize harvest, while their total dry matter yield during performance test silage maize harvest was moderate. Furthermore were genotype 19 and 20 also showing a high yield for the total dry matter during silage maize and dual use maize harvest.

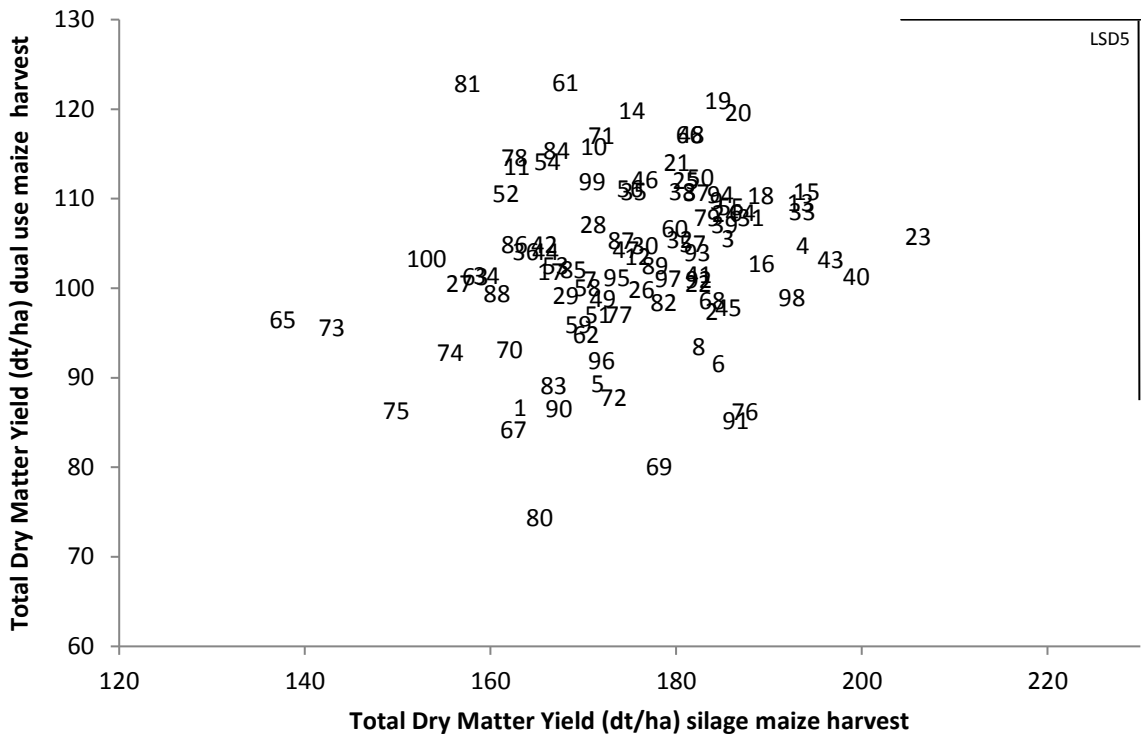

Figure II.22 Total dry matter yield of the performance test dual use maize (TDY) in dt/ha plotted against total dry matter yield of the performance test silage maize (TDYs) in dt/ha of the Flint testcrosses Numbers are the entry numbers of the testcrosses. Coefficient of determination R^2: 0.147

Figure II.23 is plotting the stover dry matter yield of the dual use maize harvest against the total dry matter yield of the silage maize harvest.

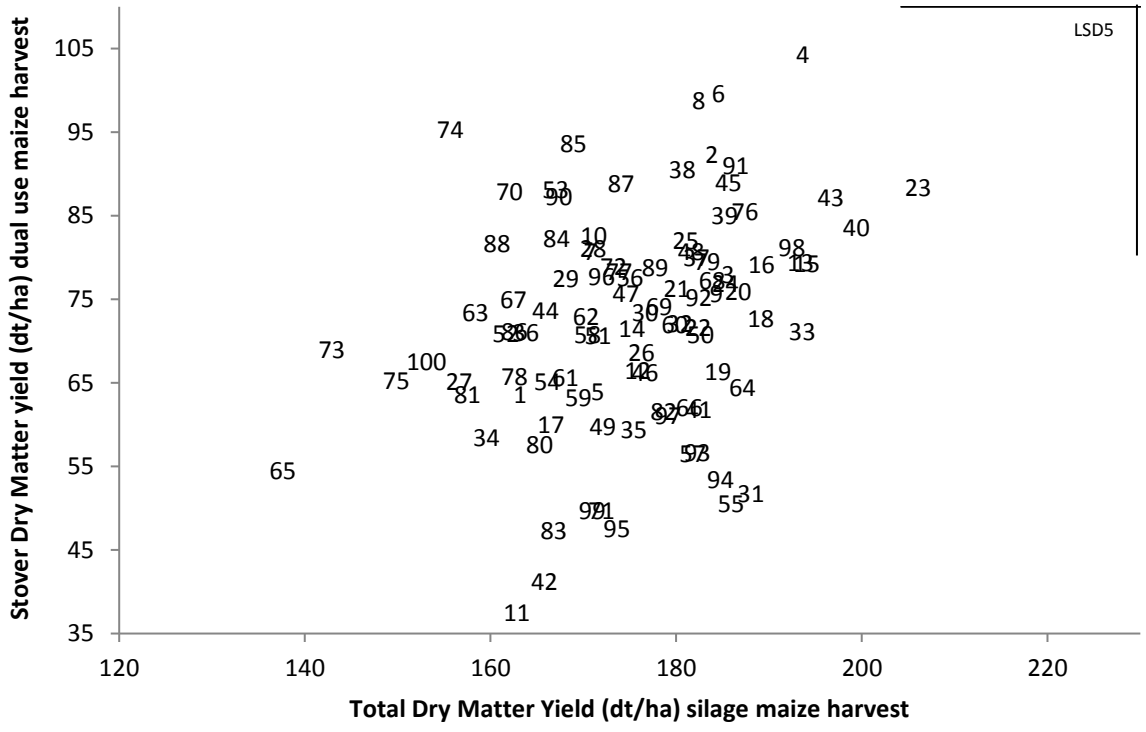

Figure II.23 Stover dry matter yield of the performance test dual use maize (SDY) in dt/ha plotted against total dry matter yield (TDYs) of the performance test silage maize harvest in dt/ha of the Flint testcrosses. Numbers are the entry numbers of the testcrosses, Coefficient of determination R^2: 0.092

Genotype 4 was showing the highest stover dry matter yield and a high total dry matter yield for the silage maize. Genotype 23 had a lower stover dry matter yield but on the other hand was the total dry matter yield during silage maize harvest even higher. Genotype 63 and 73 were showing a low stover dry matter yield and a low total dry matter yield for the silage maize harvest. The lowest stover dry matter yield contained genotype 11, 42,83 while their total dry matter yield for silage maize harvest was low to moderate (Figure II.23).

Figure II.24 is showing the total dry matter yield for dual use maize plotted against the total dry matter yield for silage maize. There was no correlation between total dry matter yield (silage maize) and grain dry matter yield (0.21/Table II.29). Genotype 80, 69 and 67 were showing a low grain dry matter yield and a low to moderate total dry matter yield for the performance test silage maize. The lowest total dry matter yield for silage maize contained genotype 73 and 65, while the total grain dry matter yield was moderate. Genotype 61 and 81 were showing a high grain dry matter yield and the total dry matter yield silage maize was moderate to low. Genotype 23, 40 and 43 were showing the highest total dry matter yield for silage maize and also the grain dry matter yield was moderate.

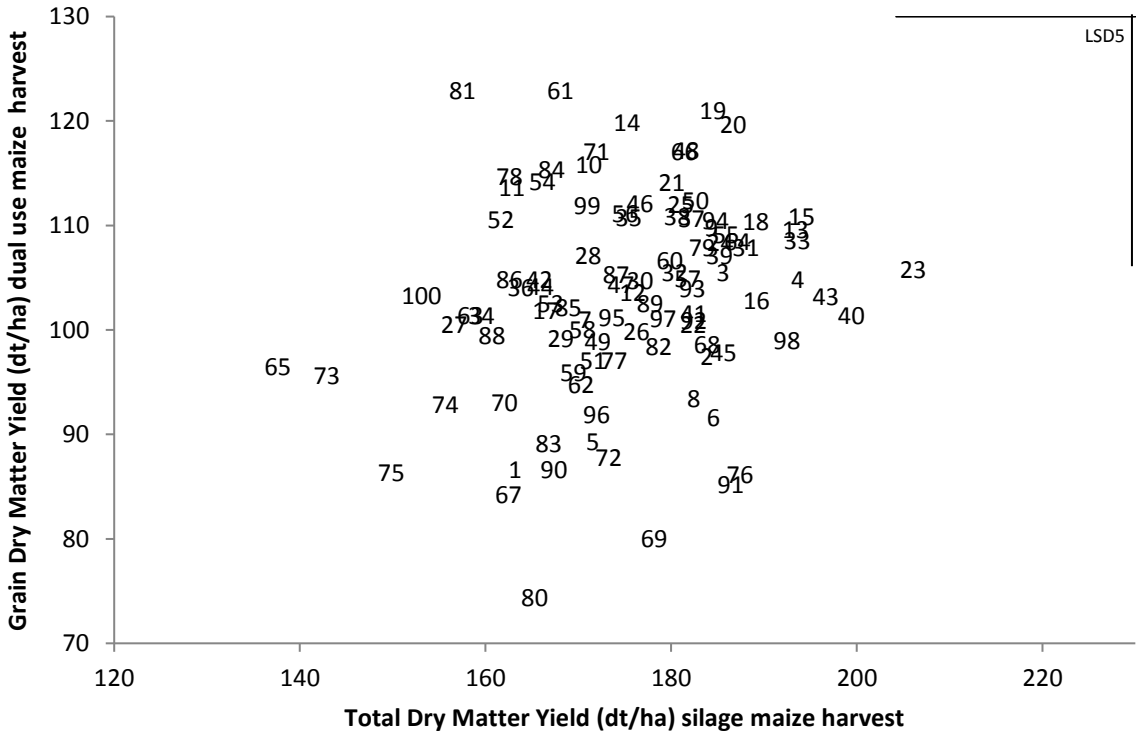

Figure II.24 Grain dry matter yield of the performance test dual use maize (GDY) in dt/ha plotted against the total dry matter yield of the performance test silage maize (TDYs) in dt/ha of the Flint testcrosses Numbers are the entry numbers of the testcrosses. Coefficient of determination R^2: 0.427

3.4 BRIX measurement

The BRIX measurement was done to analyzed the sugar content in the stover. With % BRIX the sucrose content in the sample is measured.

The Dent and Flint testcrosses have been tested during three years on their sugar content. In the first year (2014), just one time BRIX was measured one week before harvest. In year two (2015) and three (2016) BRIX was measured twice; the first time eight weeks before harvest, the second time one week before harvest. The factorial crosses have been measured twice during two years (2015/2016), eight weeks before harvest and one week before harvest.

3.4.1 Dent testcrosses

Figure II.25 shows how the BRIX vaule differs at the two measurement times. Eight weeks before harvest % BRIX was higher in both parts that have been measured, compared to one week before harvest. Furthermore was the sucrose content higher in the part below the corn cob, compared to the part above the corn cob.

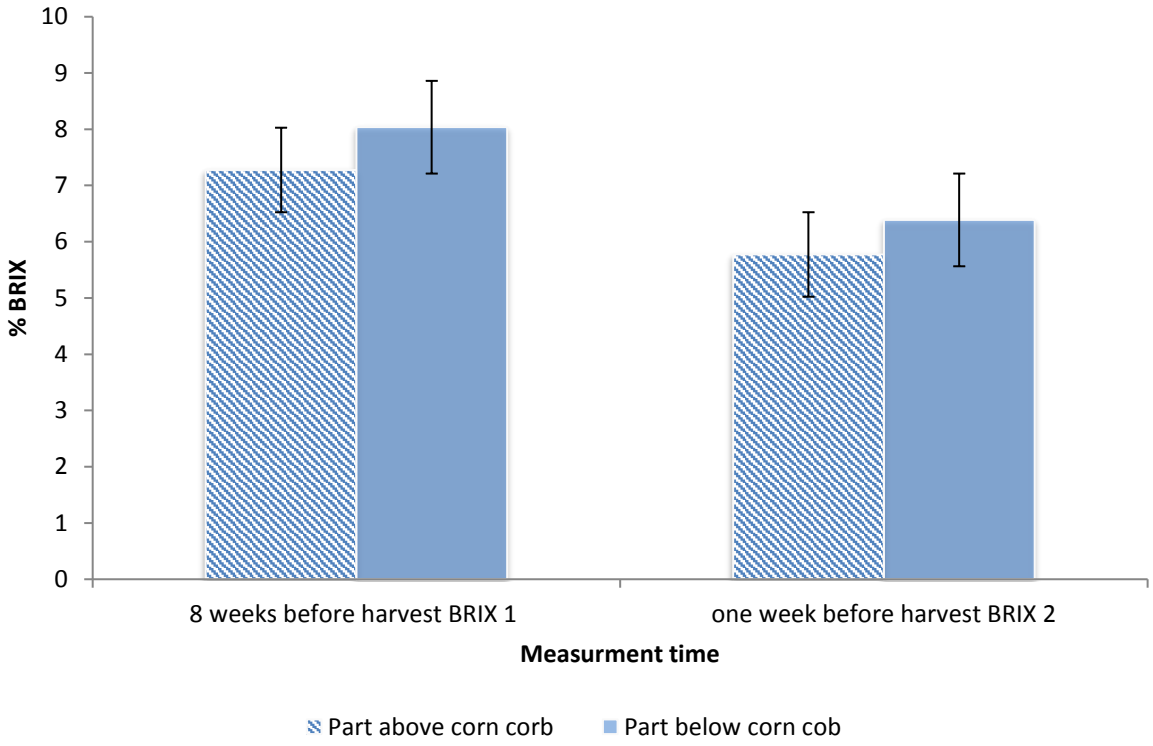

Figure II.25 Comparison between sucrose content of the Dent testcrosses at measuring time one (eight weeks before harvest) and measuring time two (one week before harvest) and the different cut parts (above and below corn cob) in % BRIX (mean over three years)

Figure II. 26 is plotting % BRIX against the grain dry matter content. The genotypes are variation form each other. Around an average between 5.5 to 8 most genotypes were found. Genotype 77 was showing the highest % BRIX but had a low grain dry matter content. Genotype 51 ws showing the lowest % BRIX with a low to moderate grain dry matter content.

There was a small negative significant correlation between % BRIX and grain dry matter content of the genotypes (-0.23**/Table II.30).

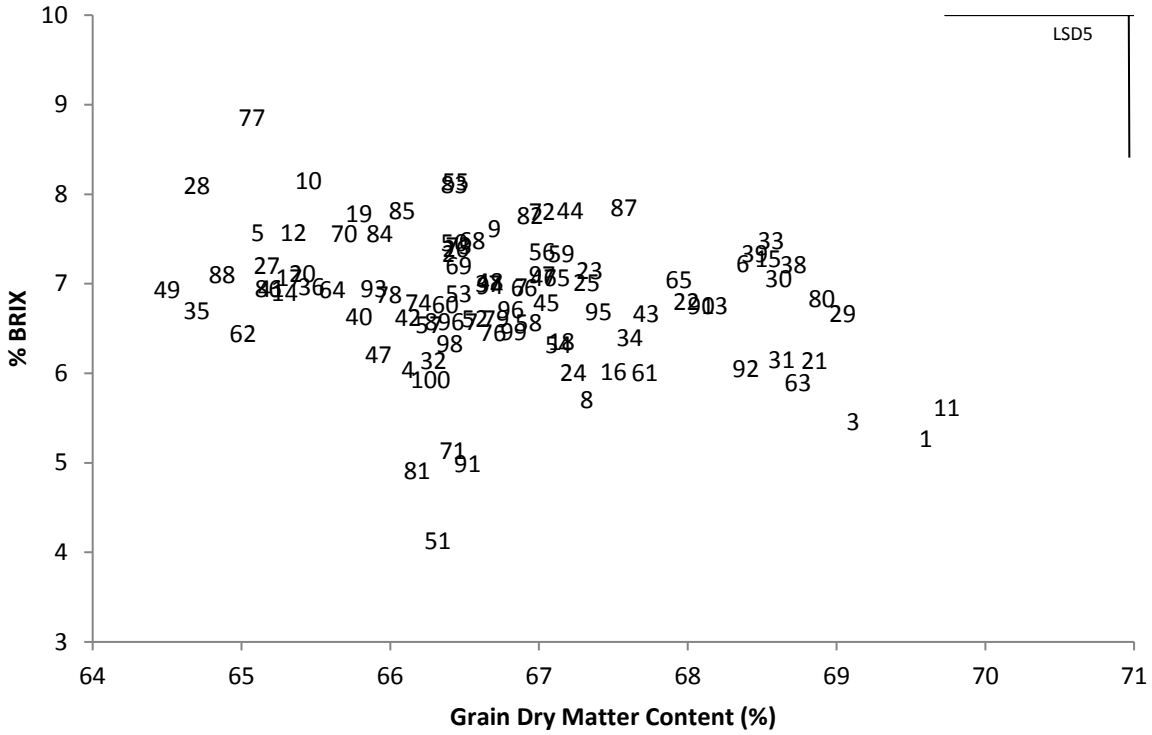

Figure II.26 % BRIX (one week before harvest [BRIX2]) plotted against grain dry matter content (GDC) in % of the Dent testcrosses. Numbers are the entry numbers of testcrosses. Coefficient of determination R^2: 0.0792

Table II.30 shows the correlation between the different traits of interest. There was a significant correlation between the two BRIX measurements, at the different times. BRIX 1 and BRIX 2 were correlated significantly (0.72**). Furthermore was BRIX 1 high significantly correlated with BRIX 1 above corn cob and BRIX 1 below corn cob (0.93**/0.94**) as well as BRIX 2 with BRIX 2 above corn cob and BRIX 2 below corn cob (0.93**/0.92**). The correlation of the stover dry matter content was not correlated with the BRIX 1, BRIX 1 above corn cob and BRIX 1 below corn cob (Table II.30). On the other hand was the stover dry matter content low significantly correlated with BRIX 2 above corn cob (-0.27**), BRIX 2 below corn cob (-0.33**) and BRIX 2 (-0.30**).

Table II.30 Table of Correlation of for the Dent testcrosses

	Grain dry matter content	Stover dry matter content	BRIX 1 above corn cob [a]	BRIX 1 below corn cob	BRIX 1	BRIX 2 above corn cob	BRIX 2 below corn cob
Stover dry matter content	0.51**						
BRIX 1 above corn cob	-0.12	-0.15					
BRIX 1 below corn cob	-0.11	-0.13	0.72**				
BRIX 1	-0.12	-0.16	0.93**	0.92**			
BRIX 2 above corn cob	-0.18	-0.27*	0.38**	0.36**	0.40**		
BRIX 2 below corn cob	-0.27**	-0.33**	0.31**	0.28**	0.31**	0.82**	
BRIX 2	-0.23*	0.30**	0.36**	0.32**	0.36**	0.94**	0.96**

Numbers are indicating the time of measuring, 1: eight weeks before harvest, 2: one week before harvest/ Significance level *p=0.05, **p=0.01; +p=0.1

Figure II.27 is showing % BRIX plotted against the stover dry matter content. The variation of the genotypes was wide again. Genotype 77 had the highest % BRIX but was showing a low stover dry matter content. Genotype 92 and 15 had a high stover dry matter content, with a moderate % BRIX. Genotype 51 contained a moderate to high stover dry matter content but with a low % BRIX.

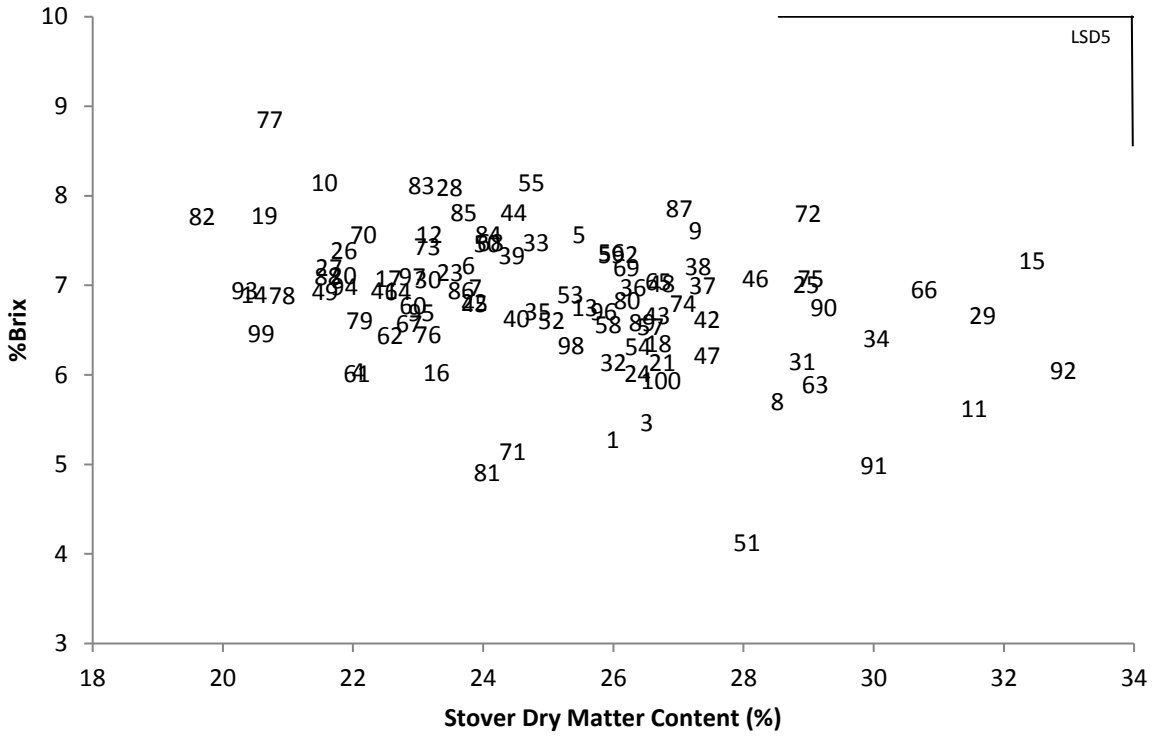

Figure II.27 % BRIX (one week before harvest [BRIX2]) plotted against Stover dry matter content (SDC) in % of the Dent genepool, Numbers are the entry numbers of the testcrosses. Coefficient of determination R^2: 0.1107

The heritability of BRIX at the second measuring time was moderate with 58 %. The years were differing significantly from each other, while the locations were not significant different. The interaction between years and locations was high significant and explained most of the variation. The genotypes were differing significantly from each other, but its interaction with the locations was not significant. The Genotype-Year interaction was significant and the interaction between genotype, years and locations was significant as well (Table II.31).

Table II.31 Analysis of Variance for the trait BRIX total measuring time 2 (BRIX2) in % BRIX of the Dent genepool

Source	DF	MS	Var.cp	F-value	LSD5
Year	2	354.8793	1.7691	334.26**	0.20
Location	1	329.8785	0.2411	1.28	5.64
Location-Year	2	257.5552	2.5649	242.59**	0.29
Genotype	99	3.4386	0.3359	2.42**	1.36
Genotype-Location	99	1.2999	0.0794	1.22	1.66
Genotype-Year	198	1.4230	0.1806	1.34*	2.03
Genotype-Location-Year	198	1.0617	0.4094	1.63**	2.24
Error	485	0.6523	0.6523		
Heritability 58 %					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

3.4.2 Flint testcrosses

Comparing the measurement times with each other, Figure II.28 shows that the sucrose content at the first time point was higher in both parts compared to the second measuring time for the Flint testcrosses. Moreover was the total content of sucrose higher in the part below the corn cob compared to the part above the corn cob.

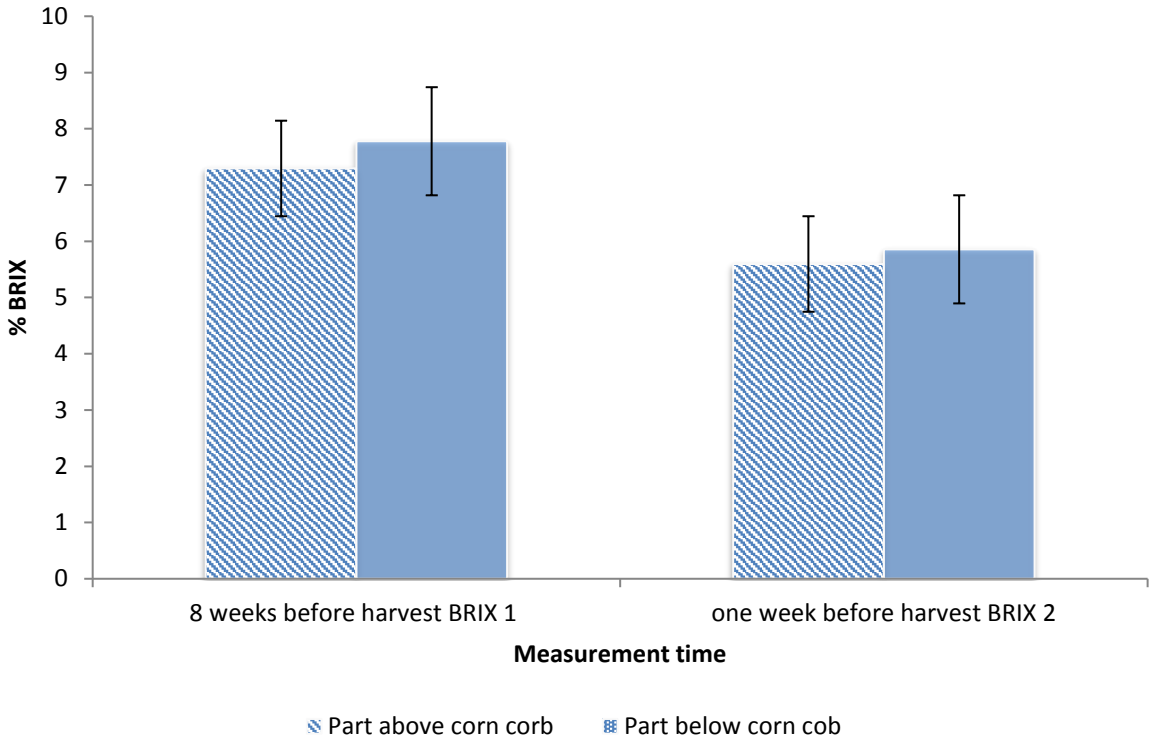

Figure II.28 Comparison between sucrose content of the Flint testcrosses at measuring time one (eight weeks before harvest) and measuring time two (one week before harvest) at different cut parts (above and below corn cob) in % BRIX (mean over three years)

Figure II. Figure II.29 is showing % BRIX with the grain dry matter content. The genotypes are variation form each other. The range all genotypes were found in is lying between 4.5 and 8 % BRIX.

Genotype 30 laid outside the group with a high grain dry matter content and a higher % BRIX. On the other hand contained genotype 51 and 81 a low % BRIX and a low and higher grain dry matter content respectively. Genotype 44 had a high amount % BRIX and its grain dry matter content was high. Genotype 10, 91 and 13 had a low grain dry matter content with a high % BRIX.

There was no correlation between % BRIX and grain dry matter content of the genotypes (0.09/Table II.32).

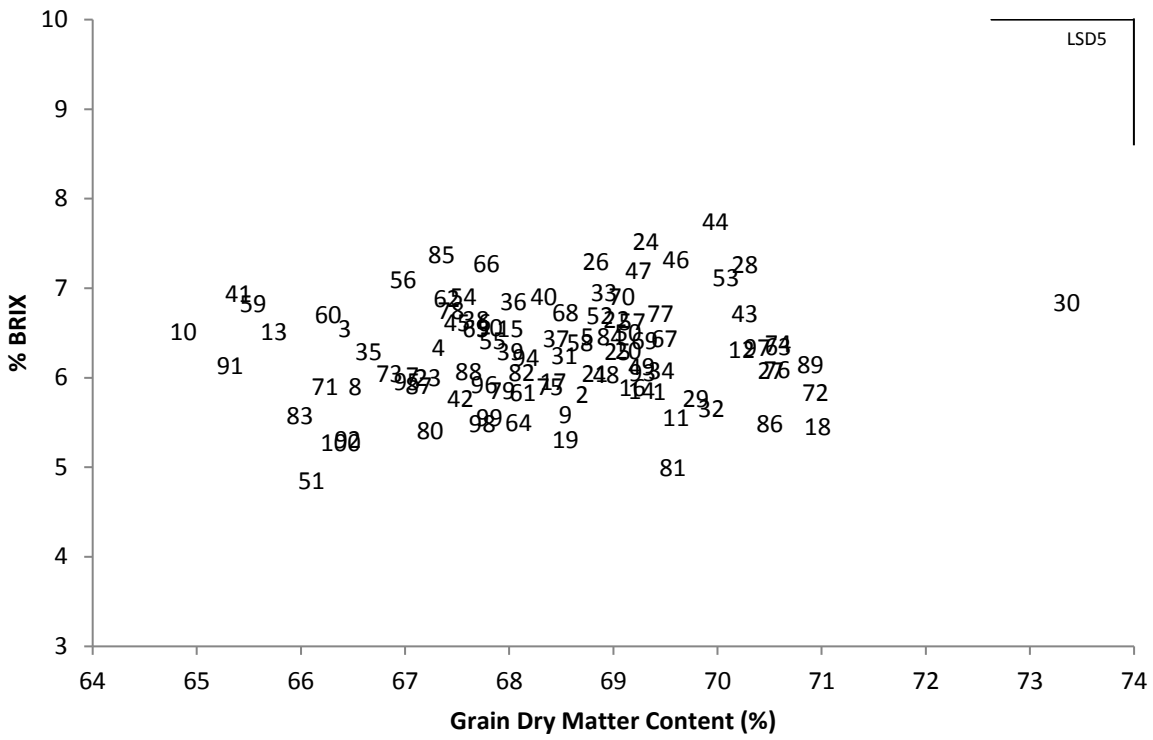

Figure II.29 % BRIX (one week before harvest [BRIX2]) plotted against Grain dry matter content (GDC) in % of the Flint testcrosses, Numbers are the entry numbers of the testcrosses. Coefficient of determination R^2: 0.0073

Table II.32 shows the correlation between the different traits of interest. As already mentioned, there was no correlation between the grain dry matter content and the BRIX 2 (0.09). Also no correlation was shown between BRIX 2 and the stover dry matter content (-0.07). BRIX 1 and BRIX 2 were correlated moderate significantly (0.47**). Moreover BRIX 1 was high significantly correlated with BRIX 1 above corn cob and BRIX 1 below corn cob (0.94**/0.94**) as well as BRIX 2 with BRIX 2 above corn cob and BRIX 2 below corn cob (0.94**/0.95**). The correlation of the stover dry matter content was not correlated with the BRIX 1, BRIX 1 above corn cob and BRIX 1 below corn cob or with BRIX 2 above corn cob, BRIX 2 below corn cob and BRIX 2 (Table II.32).

Table II.32 Table of Correlation for the Flint testcrosses

	Grain dry matter content	Stover dry matter content	BRIX 1 above corn cob[a]	BRIX 1 below corn cob	BRIX 1	BRIX 2 above corn cob	BRIX 2 below corn cob
Stover dry matter content	0.16						
BRIX 1 above corn cob	0.04	0.01					
BRIX 1 below corn cob	-0.04	0.00	0.78**				
BRIX 1	-0.01	0.01	0.94**	0.94**			
BRIX 2 above corn cob	0.13	-0.08	0.47**	0.32**	0.42**		
BRIX 2 below corn cob	0.05	-0.05	0.49**	0.41**	0.48**	0.79**	
BRIX 2	0.09	-0.07	0.50**	0.39**	0.47**	0.94**	0.95**

BRIX 1: eight weeks before harvest, BRIX 2: one week before harvest/ Significance level *p=0.05, **p=0.01; +p=0.1

The stover dry matter content is plotted against % BRIX in Figure II.30. The distribution of the genotypes was wide, and no correlation was found between the traits plotted against each other (R^2: 0.0043). Genotype 44 was containing the highest % BRIX with a low stover dry matter content. On the other hand had genotype 94 the highest stover dry matter content with a moderate % BRIX.

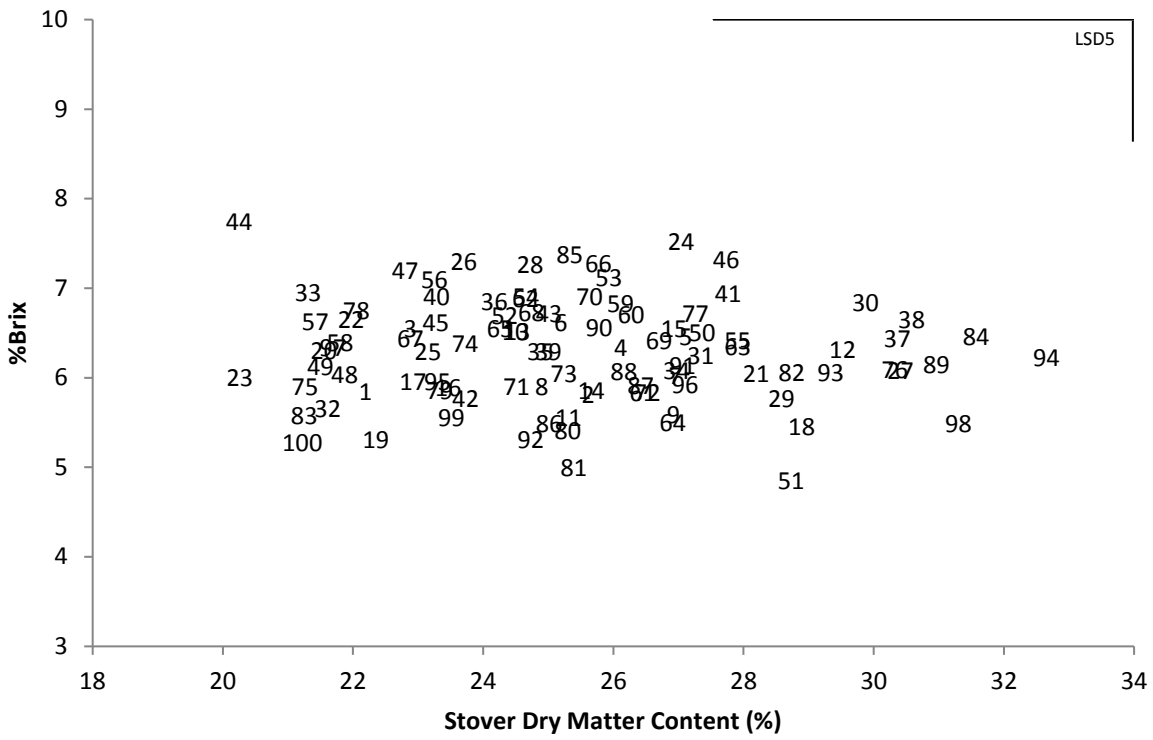

Figure II.30 % BRIX (one week before harvest [BRIX2]) plotted against Stover dry matter content (SDC) in % of the Flint genepool, Numbers are the entry numbers of the testcrosses. Coefficient of determination R^2: 0.0043

The trait BRIX at second measuring time had a moderate heritability with 41 %. The years were differing significantly from each other, and explained most of the variation with the highest variance component. The locations were not significant different. The interaction between years and locations was high significant, with the second highest variance component. The genotypes were differing significantly from each other, but its interaction with the locations was not significant. The Genotype-Year interaction was significant, as well as the interaction between genotype, year and location (Table II.33).

Table II.33 Analysis of Variance for the trait BRIX total measuring time 2 (BRIX2) in %BRIX of the Flint genepool

Source	DF	MS	Var.cp	F-value	LSD5
Year	2	984.8589	4.9201	1167.77**	0.18
Location	1	246.6953	0.3324	1.68	4.26
Location-Year	2	146.9683	1.4612	174.26**	0.26
Genotype	99	1.9793	0.1359	1.70**	1.23
Genotype-Location	99	0.7402	-0.0344	0.88	1.48
Genotype-Year	198	1.1636	0.1601	1.38**	1.81
Genotype-Location-Year	198	0.8434	0.2299	1.37**	2.18
Error	486	0.6135	0.6135		
Heritability 41 %					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

3.4.3 Factorial crosses

Figure II.31 shows that the sucrose content of the two measuring times before harvest. At the first time point (eight weeks before harvest) was the % BRIX higher in both parts compared to the second measuring time (one week before harvest). Besides was the total content of sucrose higher in the part below the corn cob compared to the part above the corn cob.

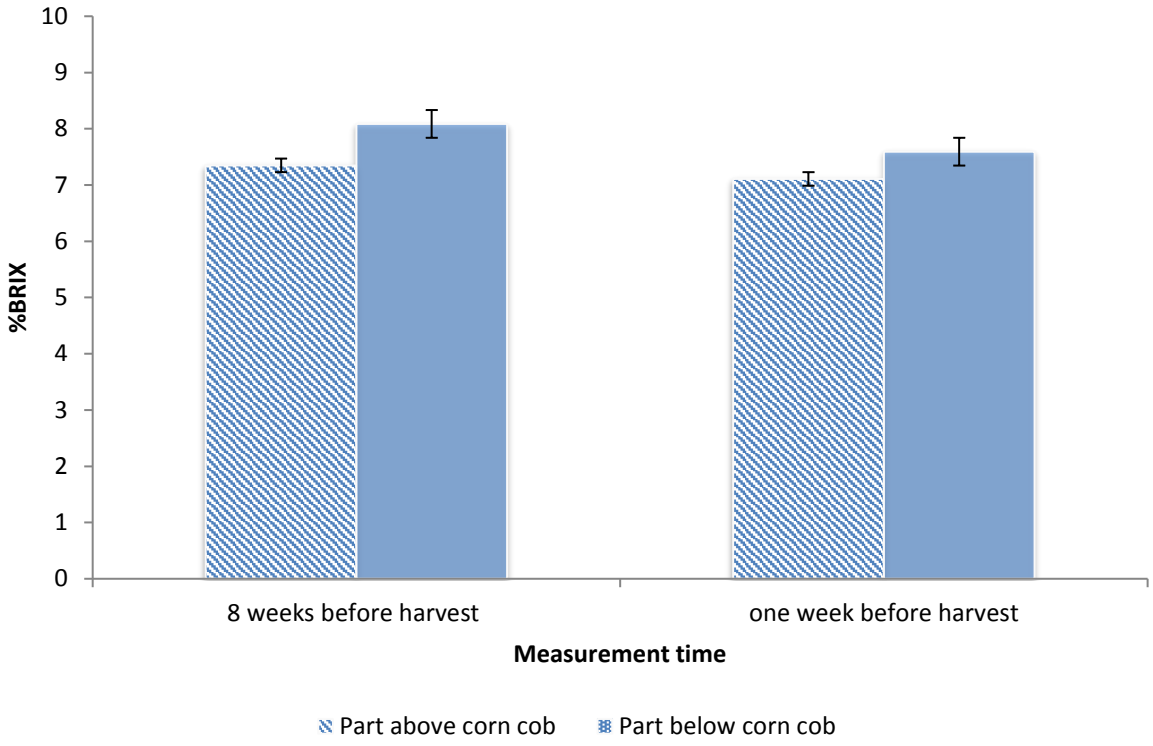

Figure II.31 Comparison between sucrose content of the factorial crosses at measuring time one (eight weeks before harvest) and measuring time two (one week before harvest) at different cut parts (above and below corn cob) in %BRIX (mean over two years)

The variation of genotypes was wide as Figure II.32 is showing. The figure is plotting % BRIX against grain dry matter content (%).The genotypes are variation form each other. The range of % BRIX was lying between 5 and 10.

The genotype with the highest %BRIX had the entry number 91 and showed a moderate grain dry matter content. While genotype 100 had the lowest % BRIX with a high grain dry matter content. Genotype 83 was showing a high grain dry matter content with a low to moderate % BRIX.

There was no correlation between % BRIX and grain dry matter content of the genotypes (-0.137/Table II.34).

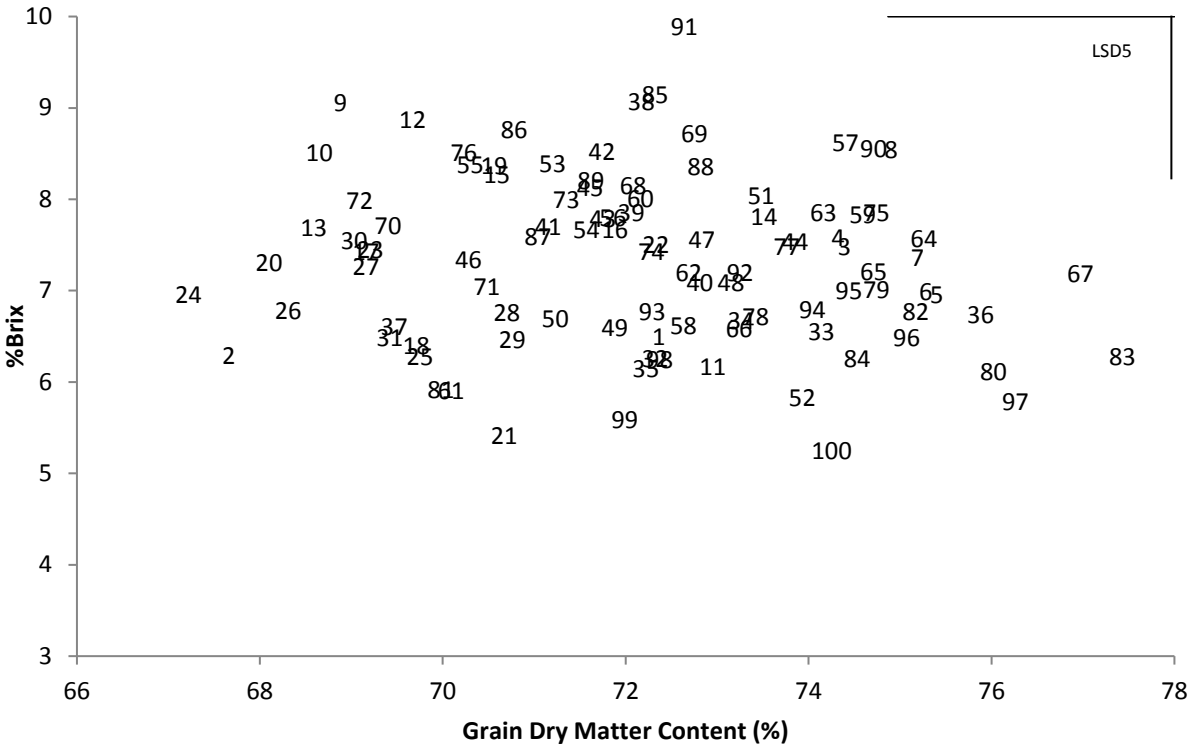

Figure II.32 % BRIX (one week before harvest [BRIX2]) plotted against Grain dry matter content (GDC) in % of the Factorial crosses, Numbers are the entry numbers of the testcrosses. Coefficient of determination R^2: 0.0177

The correlations of the different traits of interest are given in Table II.34. As already mentioned, there was no correlation between the grain dry matter content and the BRIX2. Beyond no correlation was shown between BRIX 2 and the stover dry matter content. BRIX 1 and BRIX 2 were correlated significantly (0.60**) with each other. BRIX 1 was highly significant correlated with BRIX 1 above corn cob and BRIX 1 below corn cob (0.94**/0.93**) as well as BRIX 2 with BRIX 2 above corn cob and BRIX 2 below corn cob (0.97**/0.98**). The correlation of the stover dry matter content was not correlated with the BRIX 1, BRIX 1 above corn cob and BRIX 1 below corn cob or with BRIX 2 above corn cob, BRIX 2 below corn cob and BRIX 2 (Table II.34).

Table II.34 Table of Correlation of the factorial crosses

	Grain dry matter content	Stover dry matter content	BRIX 1 above corn cob[a]	BRIX 1 below corn cob	BRIX 1	BRIX 2 above corn cob	BRIX 2 below corn cob
Stover dry matter content	0.43**						
BRIX 1 above corn cob	-0.33**	0.01					
BRIX 1 below corn cob	-0.35**	0.14	0.74**				
BRIX 1	-0.36**	0.08	0.94**	0.93**			
BRIX 2 above corn cob	-0.14	0.08	0.54**	0.49**	0.56**		
BRIX 2 below corn cob	-0.11	0.16	0.56**	0.57**	0.61**	0.89**	
BRIX 2	-0.13	0.13	0.57**	0.55**	0.60**	0.97**	0.98**

BRIX 1: eight weeks before harvest, BRIX 2: one week before harvest/ Significance level *p=0.05, **p=0.01; +p=0.1

Figure II.33 is showing % BRIX plotted against the stover dry matter content. The distribution of the genotypes was wide, and no correlation was found between the traits that are shown (R^2: 0.0165).

Genotype 91 was containing the highest % BRIX with a moderate stover dry matter content. On the other hand had genotype 40 the highest stover dry matter content with a moderate % BRIX.

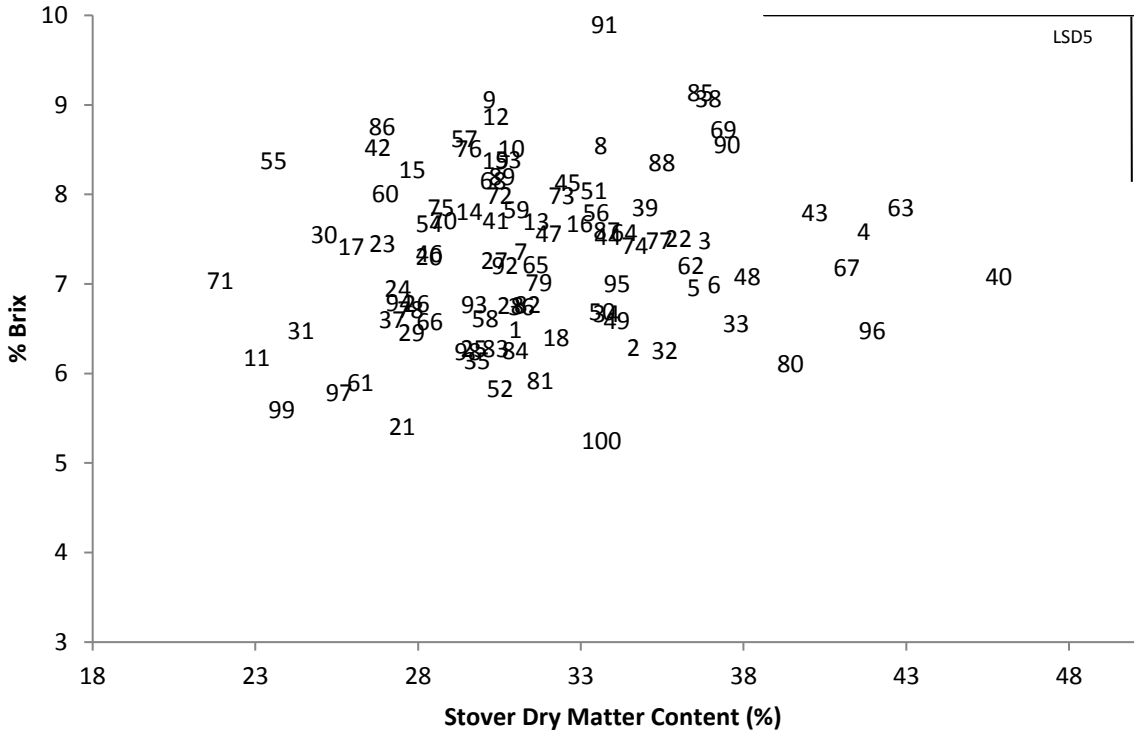

Figure II.33 % BRIX (one week before harvest [BRIX2]) plotted against Stover dry matter content (SDC) in % of the Factorial crosses, Numbers are the entry numbers of the testcrosses. Coefficient of determination R^2: 0.0165

The heritability of the trait BRIX at second measuring time was moderate with 58 %. The years were differing significantly from each other, while the locations were not significantly different. The interaction between years and locations was high significant. The highest variance component was given for the locations and the interaction of location and year, explained most of the variation. The genotypes were differing significantly from each other. The interaction between genotype, year and location was not significant, while the genotype-year interaction was significant (Table II.35).

Table II.35 Analysis of Variance for the trait BRIX total measuring time 2 (BRIX2) in % BRIX of the factorial crosses

Source	DF	MS	Var.cp	F-value	LSD5
Year	1	467.9650	2.3361	629.96**	0.17
Location	1	123.4654	-0.5991	0.51	19.82
Location-Year	1	243.2820	2.4254	327.50**	0.24
Genotype	99	3.4073	0.4962	2.40**	1.67
Genotype-Location	99	0.9327	0.0949	1.26	1.71
Genotype-Year	99	1.4225	0.3398	1.91**	1.71
Genotype-Location-Year	99	0.7429	-0.0847	0.90	2.53
Error	341	0.8276	0.8276		
Heritability 58 %					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

3.5 SPAD measurement

The SPAD was measured to analyze the chlorophyll content of the leaves and characterize the stay-green behavior of the different genotypes.

The SPAD measurement took place several times in the year, starting in the middle of August until the end of the season and the harvest of the plants. The Dent and Flint testcrosses have been measured during three years (2014/2015/2016) at two locations (Göttingen and Einbeck). The factorial crosses have been measured during two years (2015/2016) at the same locations. Because of a storm event in 2016 at the location Göttingen in late August that heavily damage the plants, the measurement was finished and the location was not taken into account.

3.5.1 Dent testcrosses

Figure II.34 shows the behavior of the plants during the season. The SPAD was decreasing during the measuring period, implying that the plants were riping and the leaves were turning form green to brown.

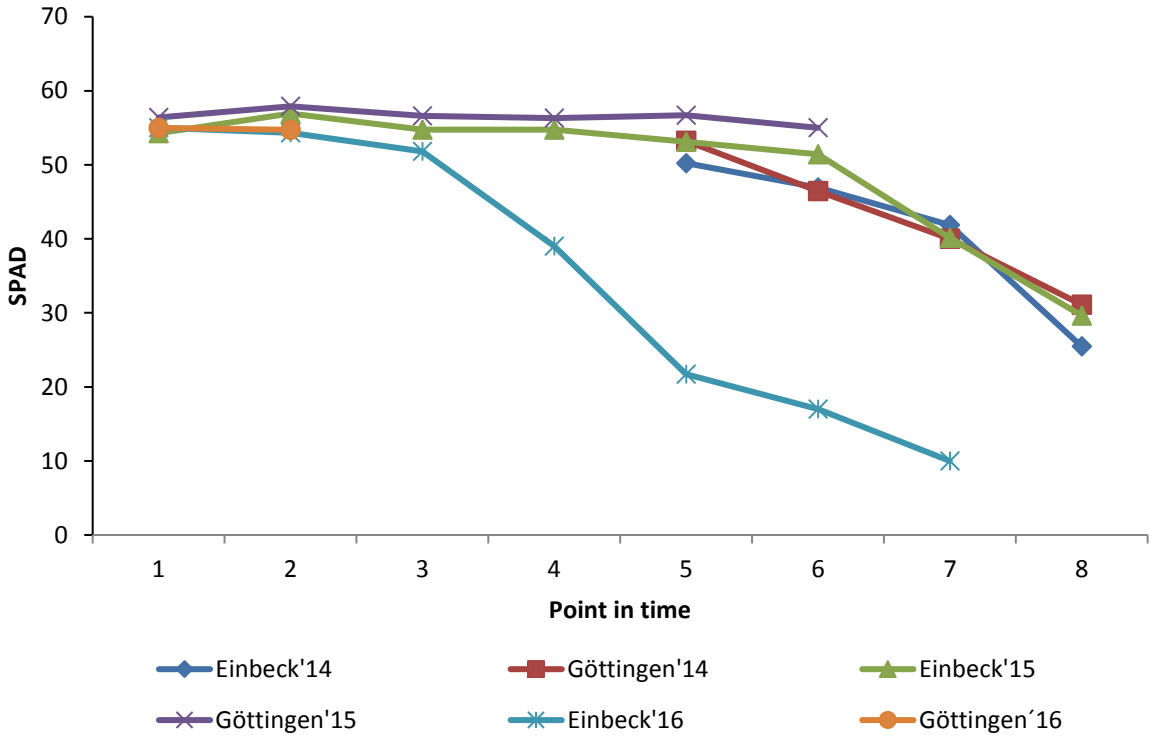

Figure II.34 SPAD behavior during the season of the Dent testcrosses. The point in time gives the date of measurement, starting in the middle of August until harvest. The colored lines are showing the two locations in the different years. Mean coefficient of determination R^2: 0.8116

Comparing the first time of measurement with the last time, it was shown, that not all plants with a high SPAD-value during the first time have a high SPAD at the last measuring (Figure II.35). Like Genotype 9, 7 and 64 were having a high SPAD early and late in the season. On the other hand was genotype 11 and 2 showing a low SPAD at the first measurement time and also a low SPAD at the last measurement. Genotype 29 and 83 had a low SPAD at the first measurement but still showed a moderate SPAD at the last measurement. Table II.36 shows a significant low correlation (0.315**) between the traits.

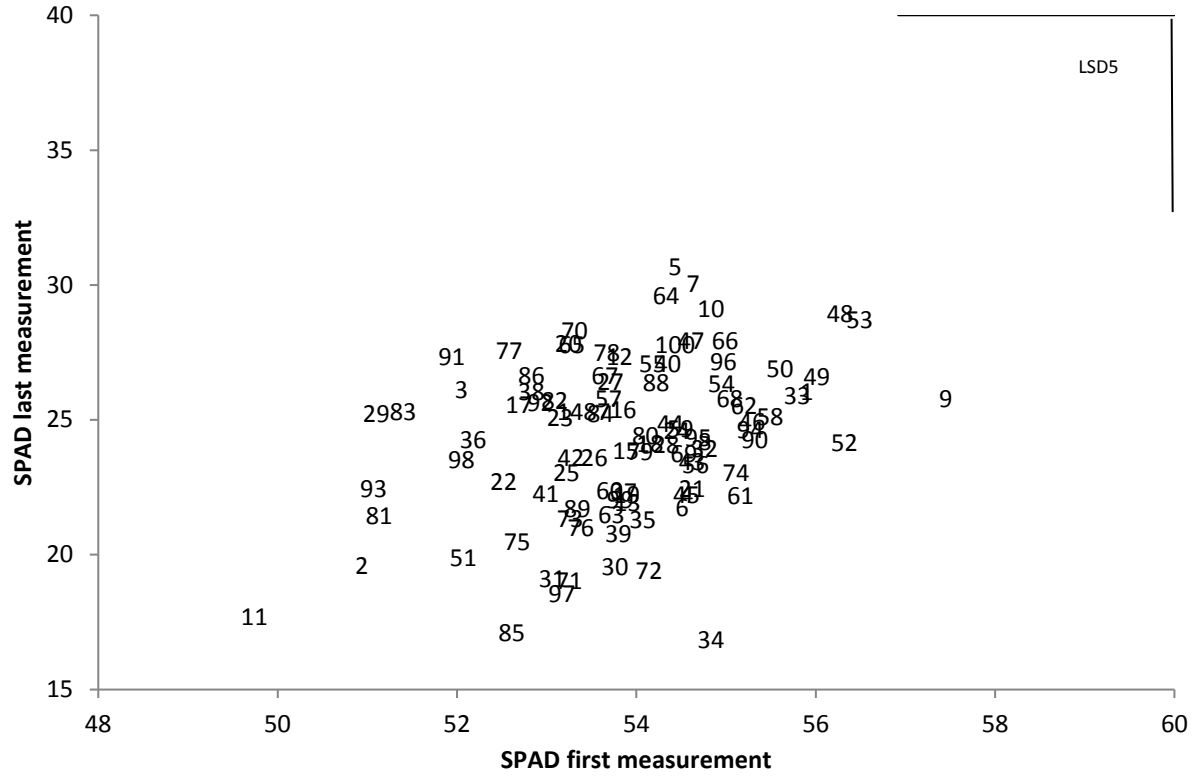

Figure II.35 Comparing SPAD first measurement with SPAD last measurement for Dent testcrosses . Numbers are giving the entry numbers, Coefficient of determination R^2: 0.1099

Figure II.36 is plotting the SPAD last measurement against the grain dry matter content. The traits grain dry matter content and SPAD at the last measurement were negative correlated with each other (-0.32**/Table II.36).

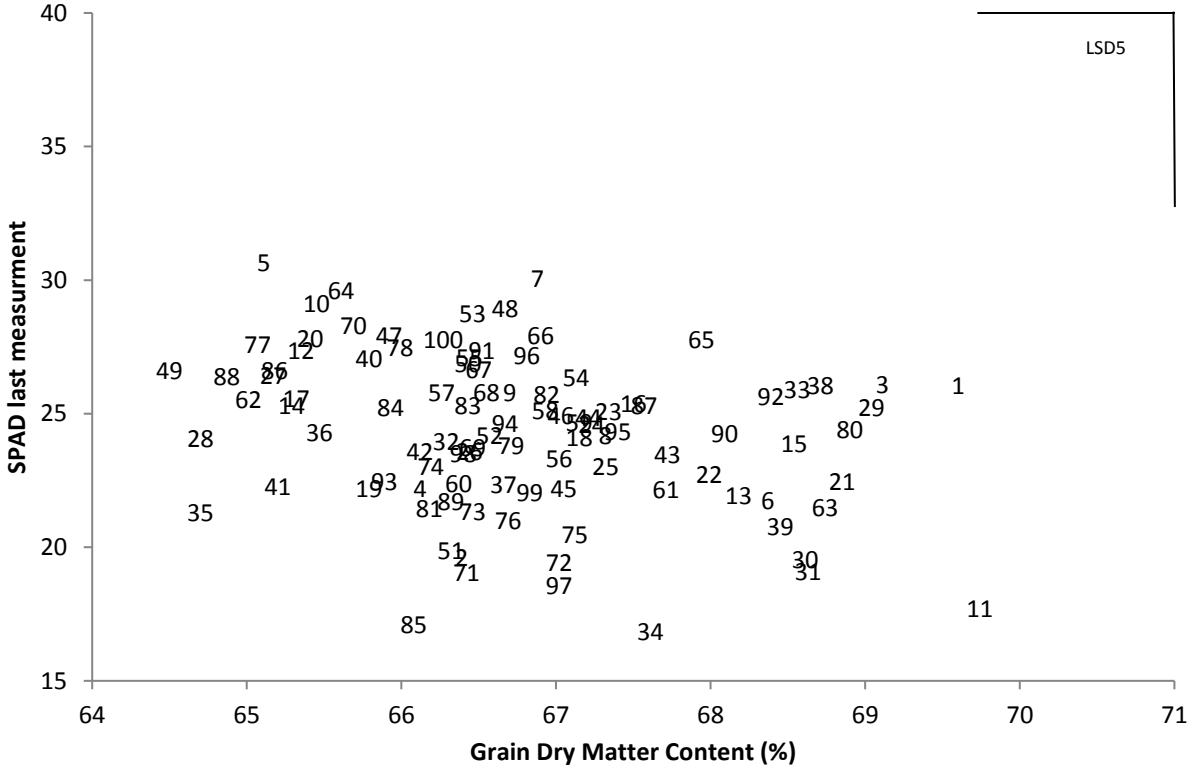

Figure II.36 SPAD last measurement plotted against Grain dry matter content (GDC) in % for Dent testcrosses. Numbers are giving the entry numbers, Coefficient of determination R^2: 0.0839

The variation of the genotypes was wide. Genotype 49 was showing a low grain dry matter content with a high SPAD at the last measurement. While genotype 11 had a high grain dry matter content and a low SPAD at the last measurement.

Table II.36 is showing that there is no correlation between grain dry matter content and SPAD at the first measurement (-0.09).

Table II.36 Table of Correlation for the Dent testcrosses

Stover dry matter content	0.53**		
SPAD 1	-0.09	-0.06	
SPAD 2	-0.32**	-0.19	0.32**
	Grain dry matter content	**Stover dry matter content**	**SPAD 1**

SPAD1: SPAD first measurement / SPAD2: SPAD last measurement /significance level *p=0.05, **p=0.01; +p=0.1

The stover dry matter content and the SPAD last measurement is plotted in Figure II.37. Genotype 82 was showing a low stover dry matter content while its SPAD is moderate high. On the other hand was genotype 11 containing a high stover dry matter content but having a low SPAD at the last measurement.

No correlation was showed between SPAD last measurement and stover dry matter content (Table II.36). Besides no correlation was found for the stover dry matter content and SPAD at the first measurement (-0.06).

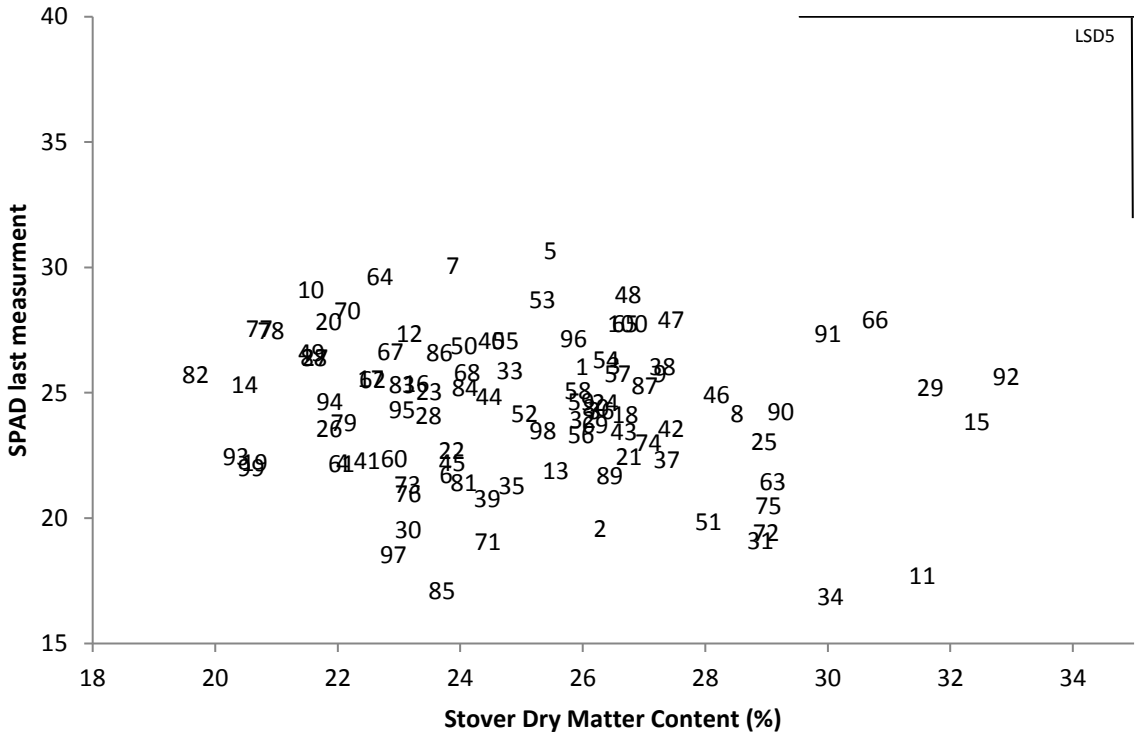

Figure II.37 SPAD last measurement plotted against Stover dry matter content (SDC) in % for Dent testcrosses. Numbers are giving the entry numbers, Coefficient of determination R^2: 0.0233

The analysis of variance for the trait SPAD first measurement is shown in Table II.37 The environments differed significantly from each other. Also the genotypes showed significant differences. The interaction between genotype and environments was significant and was explaining

most of the variation with the highest variance component. The heritability of the trait SPAD at the first measurement ws moderate (27 %).

Table II.37 Analysis of Variance for the trait SPAD first measurement (SPAD1) of the Dent testcrosses

Source	DF	MS	Var.cp	F-value	LSD5
Environment	3	83.4812	0.7859	17.04**	0.62
Genotype	99	664.6320	0.4531	1.37*	3.08
Genotype-Environment	297	1455.5710	2.5337	2.06**	4.29
Error	334	793.9766	2.3772		
Heritability 27 %					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

The heritability of the trait SPAD last measurement was low with 23 %. As Table II.38 shows differed the environments significantly from each other with a highst variance component and explained most of the variation. The interaction between genotype and environment was significant and had the second highest variance component. While the genotypes itself just showed a significance at a level of 10 %.

Table II.38 Analysis of Variance for the trait SPAD last measurement (SPAD2) of the Dent testcrosses

Source	DF	MS	Var.cp	F-value	LSD5
Environment	3	9645.5681	96.1919	365.70**	1.43
Genotype	99	34.1724	1.9492	1.30+	7.15
Genotype-Environment	297	26.3758	13.3246	2.02**	10.05
Error	320	13.0511	13.0511		
Heritability 23 %					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

3.5.2 Flint testcrosses

Figure II.38 shows the behavior of the plants responding to the SPAD measurement during the season. The SPAD was decreasing during the measuring period, implying that the plants were riping and the leaves were turning form green to brown.

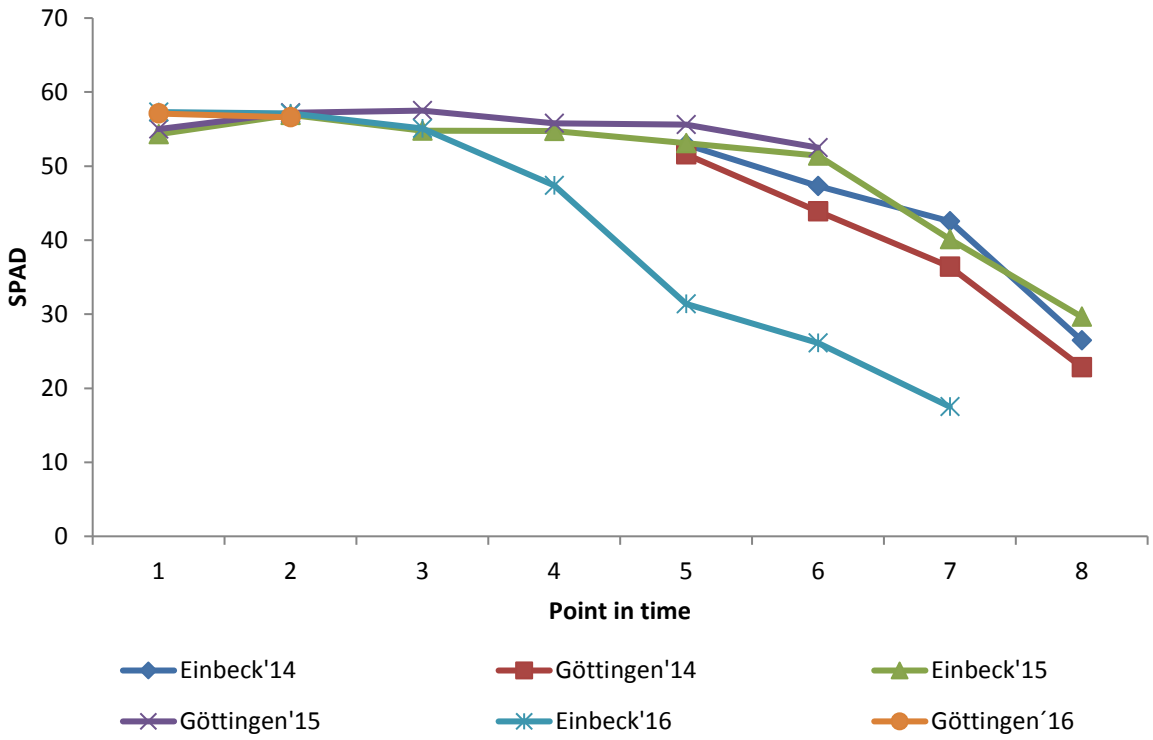

Figure II.38 SPAD behavior during the season of the Flint testcrosses. The point in time gives the date of measurement, starting in the middle of August until harvest. The colored lines are showing the two locations. Mean coefficient of determination R^2: 0.8008

Figure II.39 compares the SPAD of the genotypes for the first and the last measurement. It was shown that genotypes with a high SPAD at the first measurement did not necessarily had a high SPAD for the last measurement. There were genotypes showing a high SPAD at the first and the last measurement, like genotype 77, 58 and 40. On the other hand were genotype 2 and 89 showing a low SPAD at the first measurement time and a low SPAD at the last measurement. Genotype 91 and 20 had a low SPAD at the first measurement but still showed a moderate to high SPAD at the last measurement.

There was a moderate significant correlation between the first and the last SPAD measurement visible (Figure II.39/Table II.39).

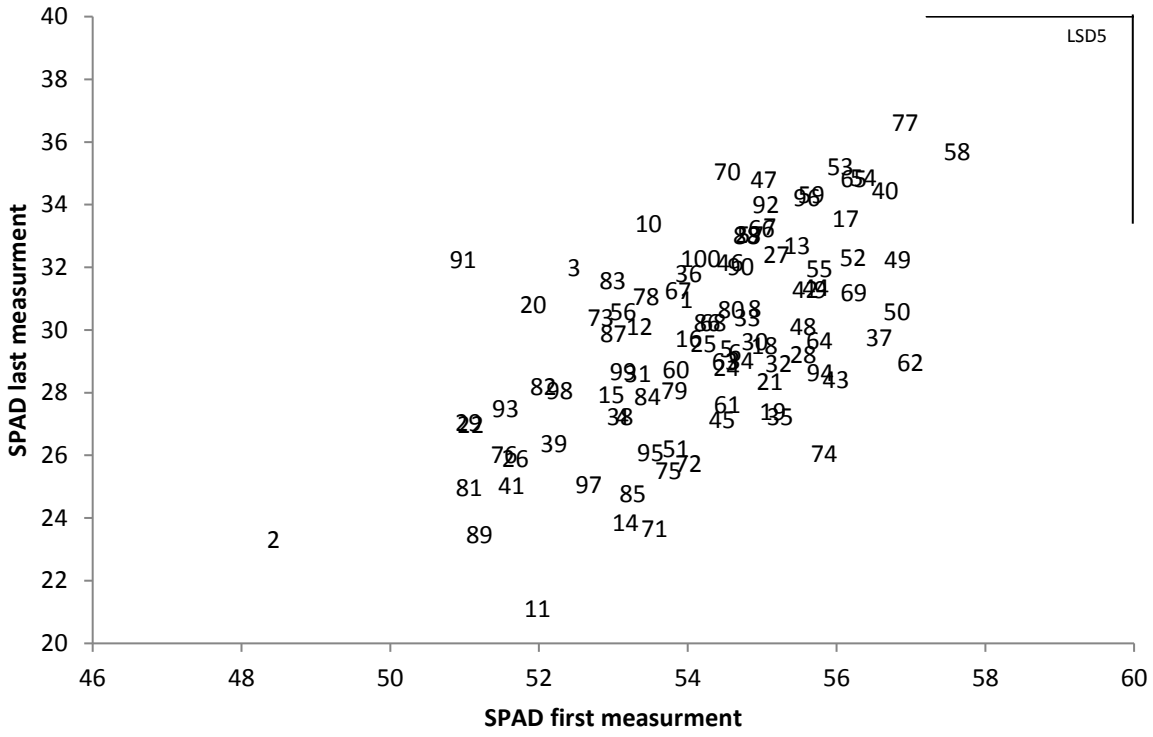

Figure II.39 Comparing SPAD first measurement with SPAD last measurement for Flint testcrosses . Numbers are giving the entry numbers, Coefficient of determination R^2: 0.3644

The grain dry matter content and the SPAD last measurement is plotted in Figure II.40. The correlation between grain dry matter content and SPAD last measurement was very low (Table II.39).

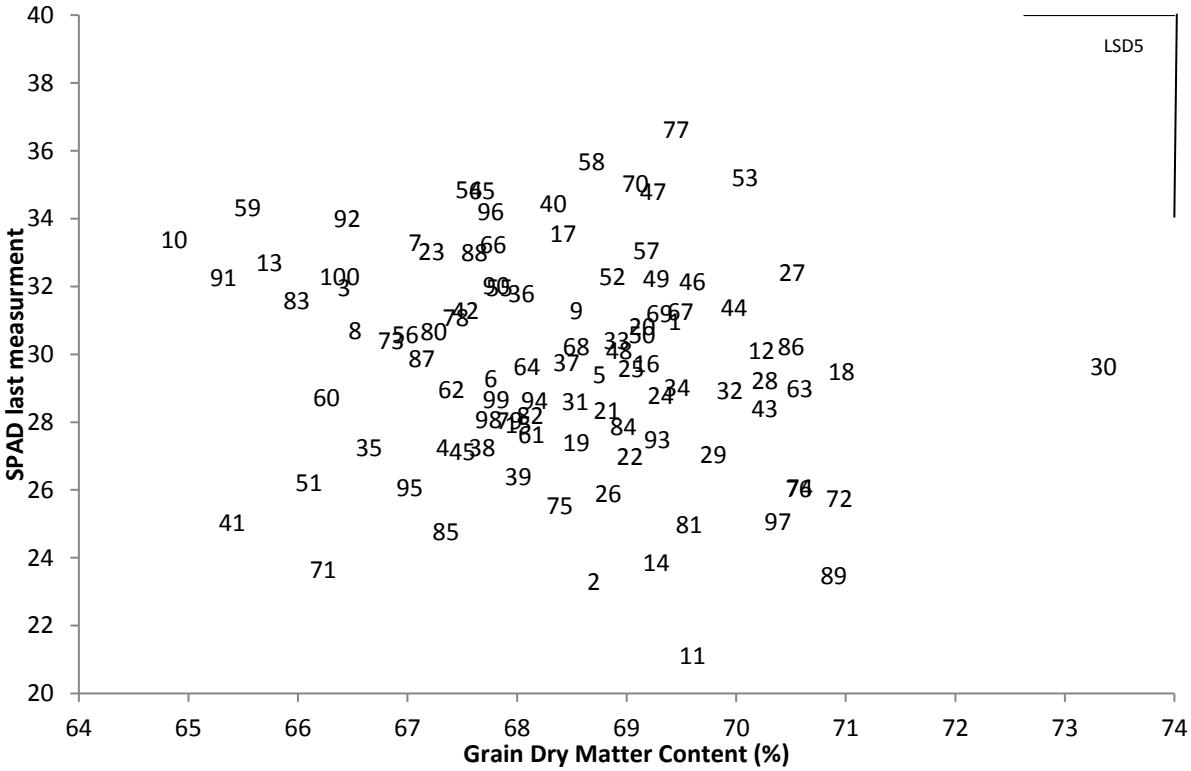

Figure II.40 SPAD last measurement plotted against Grain dry matter content (GDC) in % for Flint testcrosses. Numbers are giving the entry numbers, Coefficient of determination R^2: 0.0274

Genotype 30 was an outlier, had a moderate SPAD value and a high grain dry matter content. Genotype 77 was showing the highest SPAD while the grain dry matter content was high as well. Genotype 10 was showing a low grain dry matter yield and a high SPAD at the last measurement time. Genotype 71 was showing a low SPAD and a low grain dry matter yield (Figure II.40).

Table II.39 is showing the correlations between SPAD and stover dry matter content and grain dry matter content. There was no correlation between SPAD at the first measurement time and stover dry matter content, while the correlation between SPAD last measurement time and stover dry matter content was low, but negative and significant (-0.20**).

Table II.39 Table of Correlation for the Flint testcrosses

Stover dry matter content	-0.16		
SPAD 1	0.07	0.00	
SPAD 2	-0.17	-0.20**	0.60**
	Grain dry matter content	**Stover dry matter content**	**SPAD 1**

SPAD1: SPAD first measurement, SPAD2: SPAD last measurement /significance level *p=0.05, **p=0.01; +p=0.1

Figure II.41 is showing the stover dry matter content and the SPAD last measurement. Genotype 58 was showing a low stover dry matter content while its SPAD was high. On the other hand genotype 11 containeda moderate stover dry matter content but had a low SPAD at the last measurement. Table II.39 was showing no correlation for the traits (-0.20). Besides no correlation was found for the stover dry matter content and SPAD at the first measurement (-0.13).

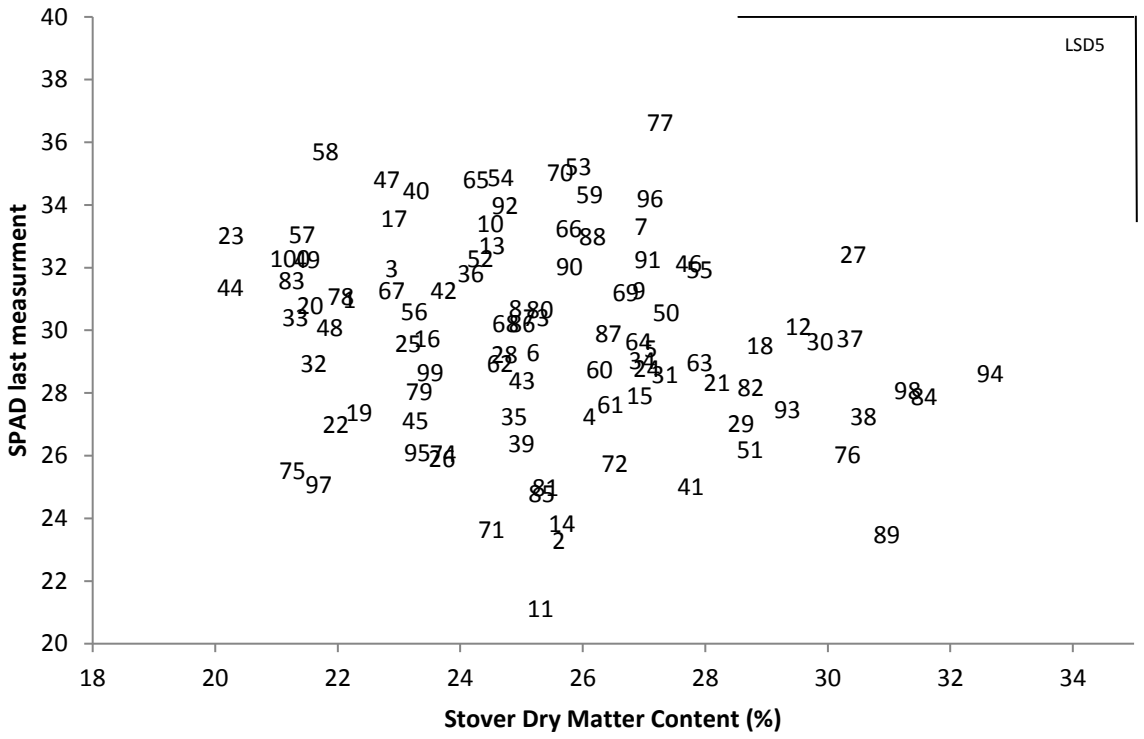

Figure II.41 SPAD last measurement plotted against Stover dry matter content (SDC) in % for Flint testcrosses. Numbers are giving the entry numbers, Coefficient of determination R^2: 0.0394

The heritability of the trait SPAD first measurement was high with 63 %. Table II.40 shows the analysis of variance. The environments were differing significantly from each other with the highest variance component, explained most of the variation. The interaction between genotype and environments was significant, while the genotypes itself did show significance as well.

Table II.40 Analysis of Variance for the trait SPAD first measurement (SPAD1) of the Flint testcrosses

Source	DF	MS	Var.cp	F-value	LSD5
Environment	4	427.4595	4.2241	84.71**	0.62
Genotype	99	13.7791	1.7466	2.73**	2.79
Genotype-Environment	396	5.0461	1.9888	1.65**	4.86
Error	436	3.0573	3.0573		
Heritability 63 %					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

The analysis of variance for the trait SPAD last measurement is shown in Table II.41. The environments differed significantly from each other with the highst variance component, explainedmost of the variation. Also the genotypes showed significant differences. The interaction between genotype and environment was significant as well, showed the second highest variance component. The heritability of the trait SPAD at the first measurement was moderate (52 %).

Table II.41 Analysis of Variance for the trait SPAD last measurement (SPAD2) of the Flint testcrosses

Source	DF	MS	Var.cp	F-value	LSD5
Environment	4	18142.5051	181.1872	762.73**	1.36
Genotype	99	49.9824	5.2392	2.10**	6.06
Genotype-Environment	396	23.7862	12.9991	2.21**	9.13
Error	403	10.7870	10.7870		
Heritability 52 %					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

3.5.3 Factorial crosses

Figure II.42 shows the SPAD measurement during the season. The SPAD ws decreasing during the time for different genotypes.

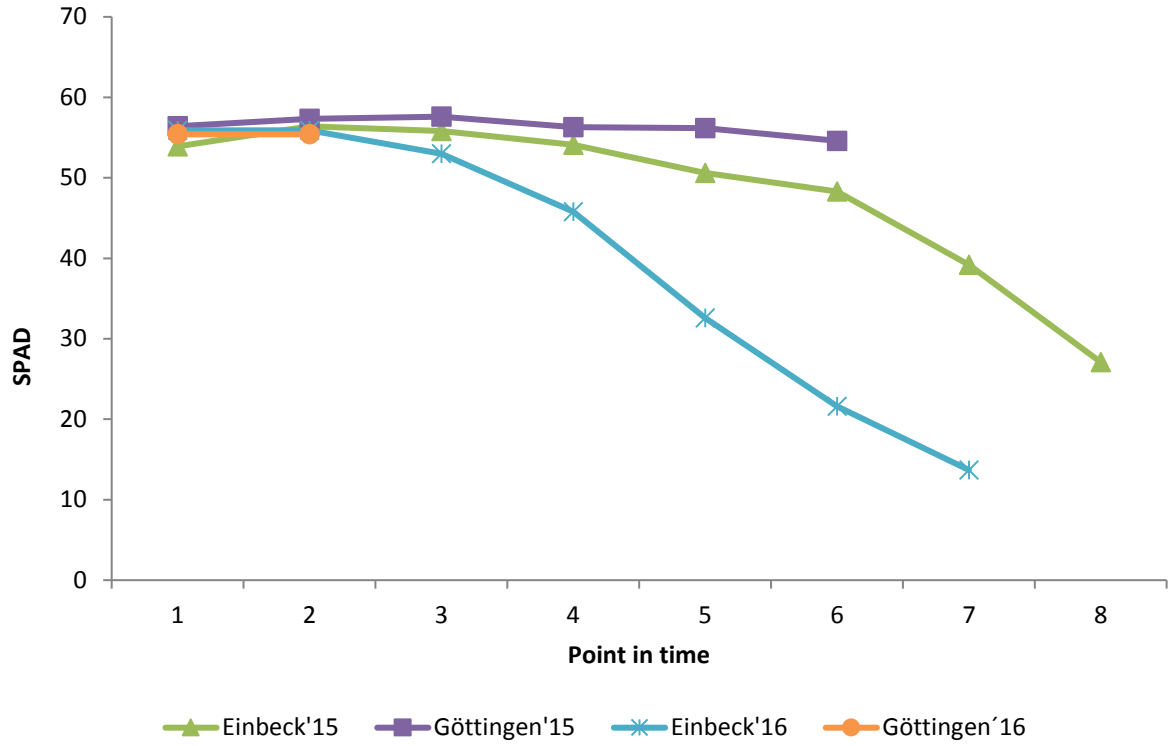

Figure II.42 SPAD behavior during the season of the factorial crosses. The point in time gives the date of measurement, starting in the middle of August until harvest. The colored lines are showing the two locations. Mean coefficient of determination R^2: 0.7881

Comparing the SPAD at the last point in time and the first SPAD measured, it was shown that not all genotypes are had a high first SPAD also had a high second SPAD. Figure II.43 is plotting the two traits of SPAD against each other. Genotype 97 was showing a high SPAD at the first measurement while the SPAD at the last measurement belonged to the lowest values. On the other hand were genotype 86, 85, 63 and 88 showing a high SPAD at both measuring times. Genotype 71 and 2 were showing a low SPAD at the first time of measuring and it was still belonging to the lower SPAD values at the last measurement.

There was a moderate significant correlation between the first and the last SPAD measurement visible (Table II.42).

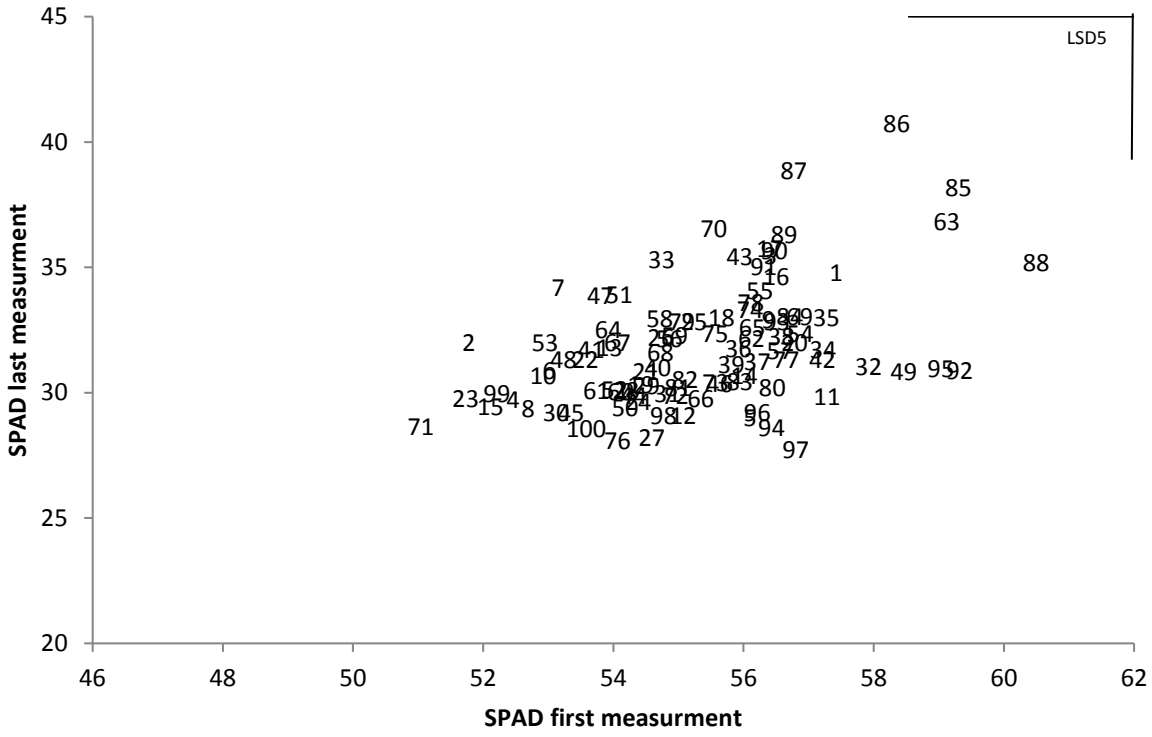

Figure II.43 Comparing SPAD first measurement with SPAD last measurement for factorial crosses. Numbers are giving the entry numbers, Coefficient of determination R^2: 0.2062

Figure II.44 is showing the grain dry matter content and the SPAD last measurement. There was no correlation between grain dry matter content and SPAD last measurement (Table II.42).

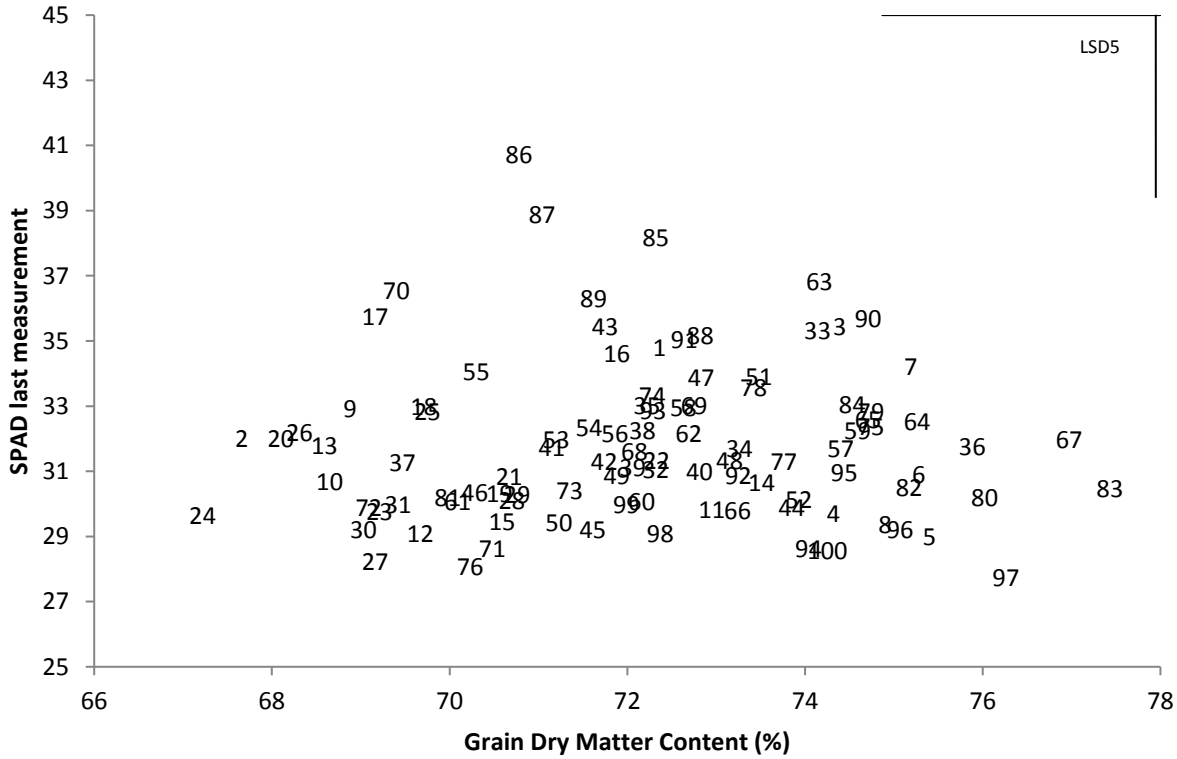

Figure II.44 SPAD last measurement plotted against Grain dry matter content (GDC) in % for factorial crosses. Numbers are giving the entry numbers, Coefficient of determination R^2: 0.0001

Genotype 86 was showing a moderate grain dry matter content, while its SPAD was the highest of the group. Genotype 87 also showed a moderate grain dry matter content and high SPAD. Genotype 97 and 83 were showing a high grain dry matter content, while the SPAD at the last measurement was comparable low. Genotype 24 had a low SPAD and a low grain dry matter content (Figure II.44).

Table II.42 shows the correlation between SPAD and grain dry matter content and stover dry matter content. There was a small significant correlation between SPAD at the first measurement time and stover dry matter content (0.205**).

Table II.42 Table of Correlation for the factorial crosses

Stover dry matter content	0.43**		
SPAD 1	0.20**	0.15	
SPAD 2	0.01	0.21**	0.45**
	Grain dry matter content	**Stover dry matter content**	**SPAD 1**

SPAD1: SPAD first measurement, SPAD2: SPAD last measurement/ significance level *p=0.05, **p=0.01; +p=0.1

The stover dry matter content and the SPAD at the last measurement is plotted in Figure II.45. Genotype 86 was showing a high SPAD. Its stover dry matter content on the other hand was low. Genotype 40 was showing a high Stover dry matter content, while its SPAD was low. Moreover were the genotypes 87, 85 and 63 showing high SPAD with moderate to high stover dry matter contents.

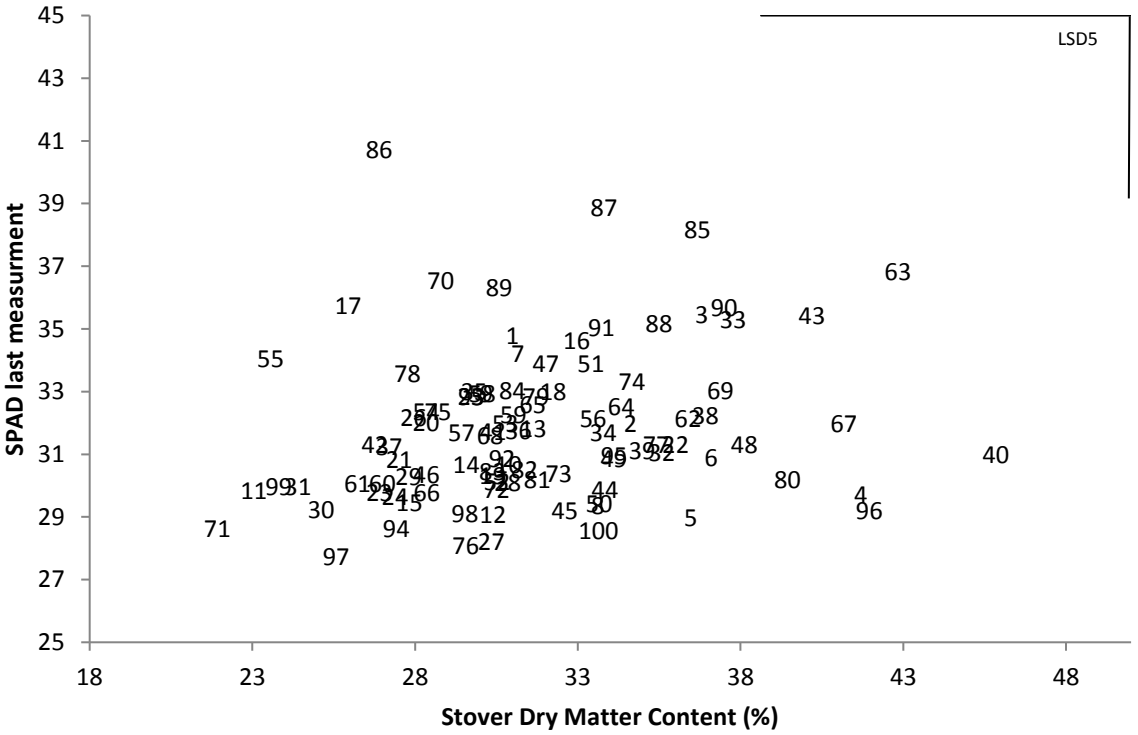

Figure II.45 SPAD last measurement plotted against Grain dry matter content (GDC) in % for factorial crosses. Numbers are giving the entry numbers, Coefficient of determination R^2: 0.042

Low significant correlation was found between stover dry matter content and SPAD last measurement (Table II.42). Besides no correlation was found for the stover dry matter content and SPAD at the first measurement (-0.147).

The trait SPAD first measurement had a moderate heritability of 52 %. As Table II.43 shows differed the environments significantly from each other. The genotypes itself did show also significance.

Locations and genotypes explained most of the variation, by having the highest variance components. The interaction between genotype and environment was significant, with the lowest variance component.

Table II.43 Analysis of Variance for the trait SPAD first measurement (SPAD1) of the factorial crosses

Source	DF	MS	Var.cp	F-value	LSD5
Environment	2	169.2885	1.6448	36.71**	0.60
Genotype	99	9.6724	1.6868	2.10**	3.46
Genotype-Environment	197	4.6119	1.5718	1.52**	4.86
Error	241	3.0402	3.0402		
Heritability 52 %					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

The analysis of variance for the trait SPAD last measurement is shown in Table II.44. The environments differed significantly from each other with the highest variance component. Also the genotypes showed significant differences. The interaction between genotype and environment was significant as well. The heritability of the trait SPAD at the first measurement was moderate (40 %).

Table II.44 Analysis of Variance for the trait SPAD last measurement (SPAD2) of the factorial crosses

Source	DF	MS	Var.cp	F-value	LSD5
Environment	2	43264.7485	432.5400	4026.97**	0.91
Genotype	99	17.9810	2.4124	1.67**	5.28
Genotype-Environment	197	10.7437	3.7894	1.54**	7.34
Error	255	6.9543	6.9543		
Heritability 40 %					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

3.6 Comparing SPAD and BRIX

3.6.1 Dent testcrosses

Figure II.46 is comparing SPAD last measurement and % BRIX one week before harvest the Dent testcrosses. The variation was around an average of 6.5 % BRIX. Genotype 51 was showing a low % BRIX with a low SPAD at the last measurement, while genotype 85 was showing a high % BRIX and also a low SPAD at the last measurement. On the other hand were genotype 77 and 10 showing a high % BRIX and a high SPAD at the last measurement as well.

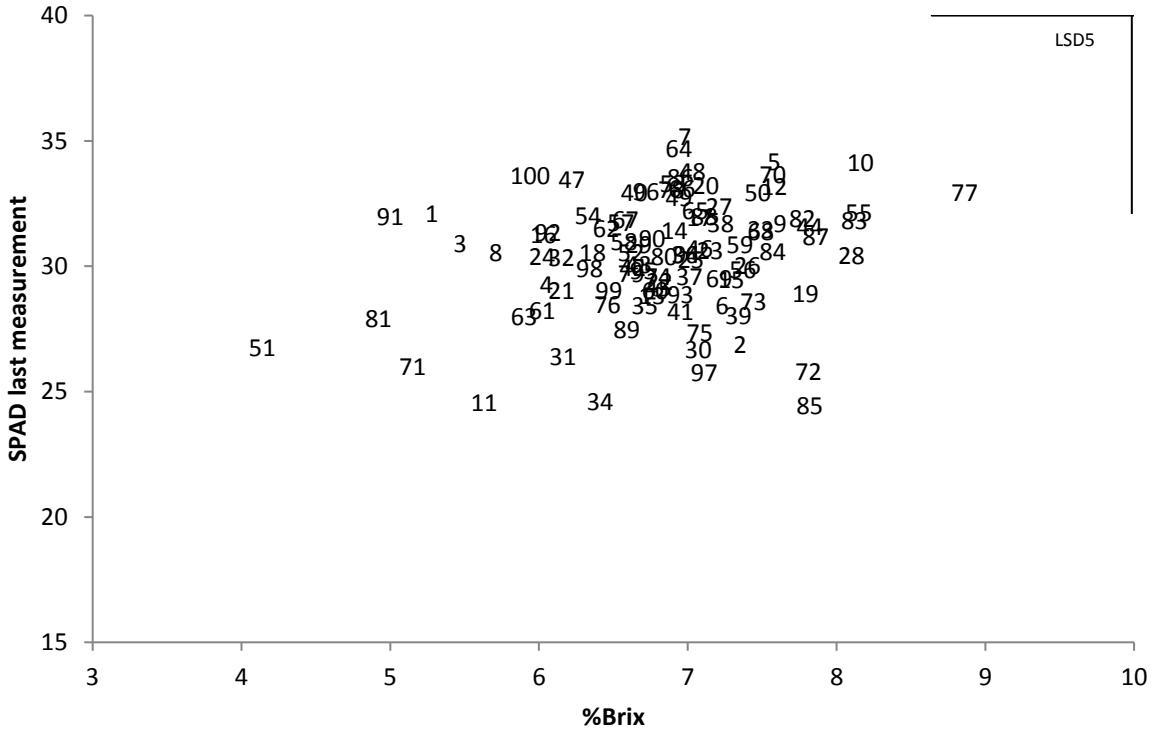

Figure II.46 Comparing % BRIX with SPAD last measurement (SPAD2) of the Dent testcrosses. Numbers are giving the entry numbers. Coefficient of determination R^2: 0.0574

Table II.45 is showing the low significant correlation between SPAD last measurement and BRIX second measurement (0.24*). BRIX at the first measurement was low significant correlated with SPAD of the last measurement (0.22*), while BRIX of the second measurement was showing no correlation with SPAD of the first measurement (0.18).

Table II.45 Table of Correlation for the Dent testcrosses

BRIX below corn cob 1[a]	0.76**						
BRIX 1[a]	0.93**	0.94**					
BRIX above corn cob 2[a]	0.40**	0.40**	0.43**				
BRIX below corn cob 2[a]	0.35**	0.31**	0.36**	0.87**			
BRIX 2[a]	0.39**	0.37**	0.41**	0.96**	0.97**		
SPAD 1[a]	0.00	0.00	0.00	0.18	0.17	0.18	
SPAD 2[a]	0.22*	0.20*	0.22*	0.16	0.30**	0.24*	0.32**
	BRIX above corn cob 1[a]	**BRIX below corn cob 1[a]**	**BRIX 1[a]**	**BRIX above corn cob 2[a]**	**BRIX below corn cob 2[a]**	**BRIX 2[a]**	**SPAD 1[a]**

[a]Number are indicating time of measurement / Significance level: **p=0.01, *p=0.05

3.6.2 Flint testcrosses

SPAD of the last measurement and % BRIX for the Flint testcrosses is plotted against each other in Figure II.47. The figure showed a variation of genotypes around an average of 6.5 % BRIX. Genotype 81 and 51 were containing a low % BRIX with an low SPAD at the last measurement, while genotype 85was showing a high % BRIX with a low SPAD at the last measurement. On the other hand were genotype 77 and 58 showing a high % BRIX and also a high SPAD at the last measurement.

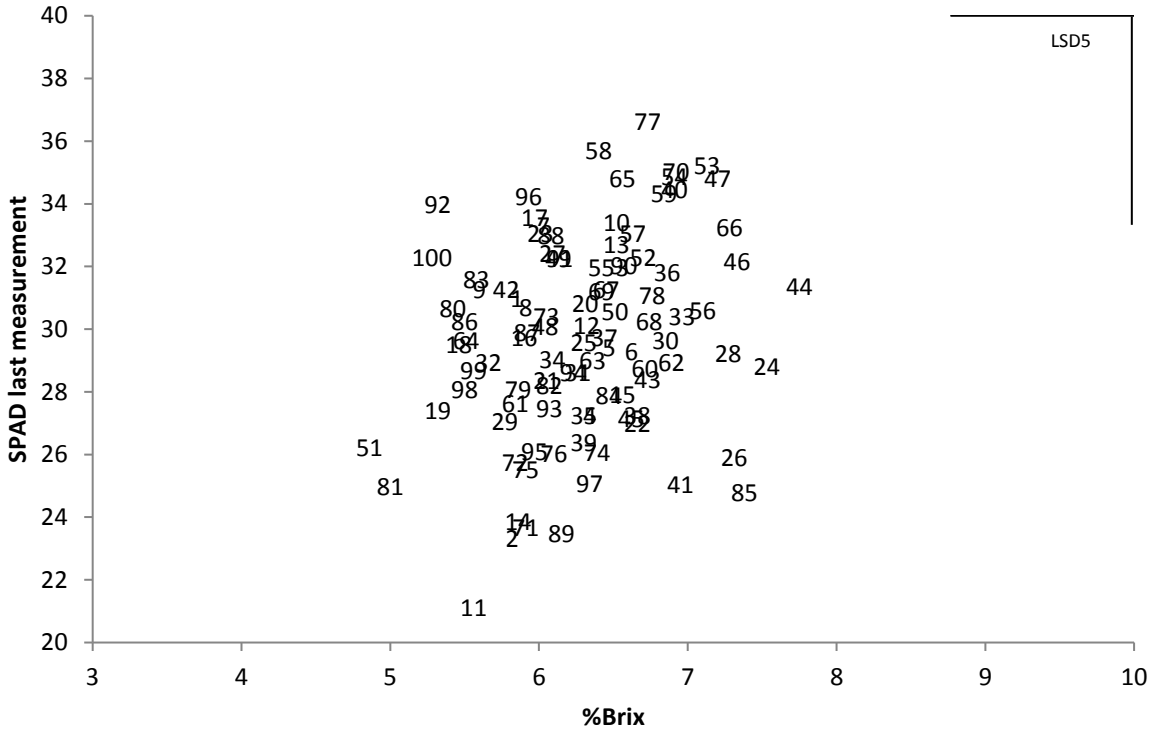

Figure II.47 Comparing % BRIX with SPAD last measurement (SPAD2) of the Flint testcrosses. Numbers are giving the entry numbers. Coefficient of determination R^2: 0.0642

Table II.46 showed a low significant correlation between SPAD last measurement and BRIX second measurement (0.253*). Comparing the SPAD and BRIX of the first measurement, no correlation was found (0.05). BRIX at the first measurement was low correlated with SPAD of the last measurement (0.148), while BRIX of the second measurement was showing a low significant correlation with SPAD of the first measurement (0.20*).

Table II.46 Table of Correlation for the Flint testcrosses

BRIX below corn cob 1[a]	0.78**						
BRIX 1[a]	0.94**	0.94**					
BRIX above corn cob 2[a]	0.47**	0.32**	0.42**				
BRIX below corn cob 2[a]	0.49**	0.41**	0.48**	0.79**			
BRIX 2[a]	0.45**	0.39**	0.47**	0.94**	0.95**		
SPAD 1[a]	0.00	0.06	0.05	0.18	0.21*	0.20*	
SPAD 2[a]	0.08	0.19	0.15	0.19	0.29**	0.28*	0.60**
	BRIX above corn cob 1[a]	**BRIX below corn cob 1[a]**	**BRIX 1[a]**	**BRIX above corn cob 2[a]**	**BRIX below corn cob 2[a]**	**BRIX 2[a]**	**SPAD 1[a]**

[a]Number are indicating time of measurement / Significance level: **p=0.01, *p=0.05

3.6.3 Factorial crosses

In Figure II.48 is SPAD of the last measurement and % BRIX for the factorial crosses plotted against each other. The figure showed a variation of genotypes around an average of 7.5 % BRIX. Genotype 21, 100 and 97 were showing a low % BRIX with an low SPAD at the last measurement, while genotype 76 was showing a high %BRIX and a low SPAD at the last measurement. On the other hand was genotype 86, 85 and 91 showing a high % BRIX and also a high SPAD at the last measurement.

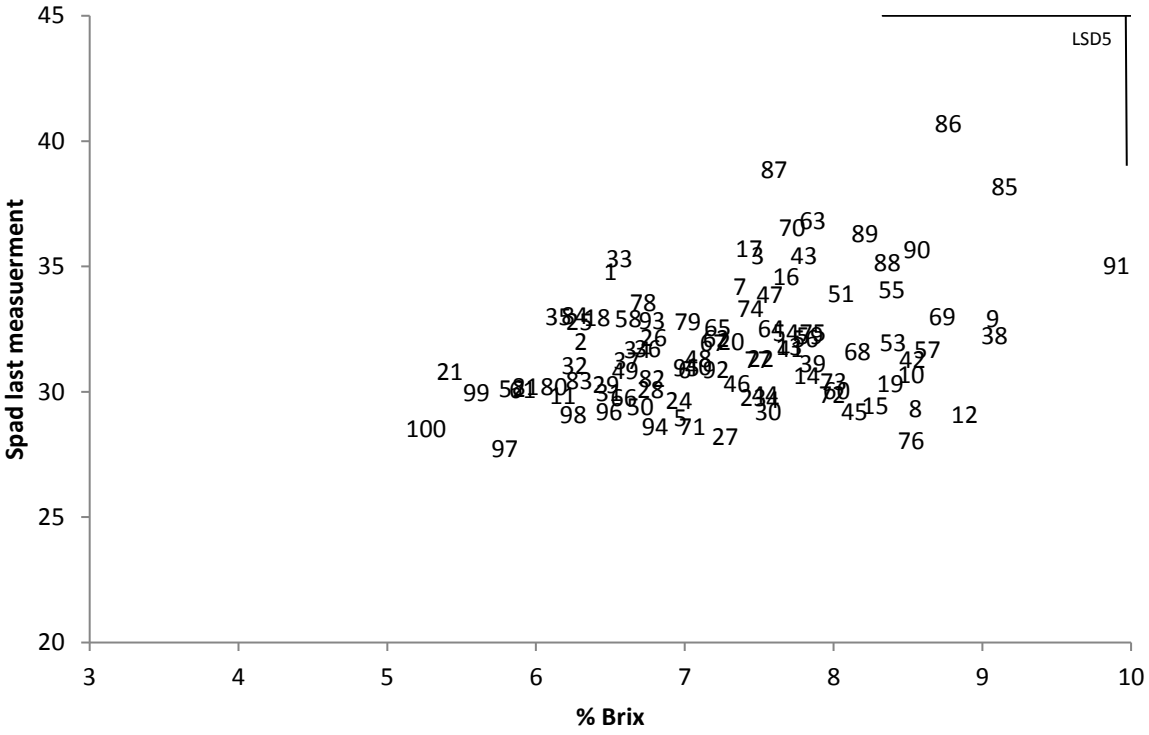

Figure II. 48 Comparing % BRIX with SPAD last measurement (SPAD2) of the factorial crosses. Numbers are giving the entry numbers. Coefficient of determination R^2: 0.1182

The correlation of the different BRIX and SPAD traits for the factorial crosses is shown in Table II.47. There was a low significant correlation between the last measurement of SPAD and the second measurement of BRIX (0.344**). Comparing the SPAD and BRIX of the first measurement, no correlation was found (-0.03). BRIX at the first measurement was low correlated with SPAD of the last measurement (0.22), while BRIX of the second measurement was showing a low correlation with SPAD of the first measurement (0.11*).

Table II.47 Table of Correlation for the factorial crosses

BRIX below corn cob 1[a]	0.74**						
BRIX 1[a]	0.94**	0.93**					
BRIX above corn cob 2[a]	0.54**	0.49**	0.56**				
BRIX below corn cob 2[a]	0.57**	0.57**	0.61**	0.89**			
BRIX 2[a]	0.57**	0.55**	0.60**	0.97**	0.98**		
SPAD 1[a]	0.06	-0.12	-0.03	0.00	0.00	0.00	
SPAD 2[a]	0.22**	0.19	0.22**	0.31**	0.36**	0.34**	0.45**
	BRIX above corn cob 1[a]	**BRIX below corn cob 1[a]**	**BRIX 1[a]**	**BRIX above corn cob 2[a]**	**BRIX below corn cob 2[a]**	**BRIX 2[a]**	**SPAD 1[a]**

[a]Number are indicating time of measurement / Significance level: **p=0.01, *p=0.05

3.7. Selection of parental lines

After the first season in the field in 2014 a selection of the parental lines took place. Caused by the fact, that several traits are of the same great interest, no selection index or selection line was used.

The traits, total dry matter yield (dual use maize harvest), grain dry matter yield, and water content of the stover, have been plotted against the grain dry matter content and the total dry matter content (dual use maize). Furthermore was the stay-green behavior and the sugar content of the stover analyzed and important requirements.

At first, genotypes showing a good stay-green behavior (SPAD) have been selected in a pre-selection step during the field season. Then the two kinds of yields, total dry matter yield and grain dry matter yield (dual use maize) have been plotted against the grain dry matter content and the total dry matter content (dual use maize). Furthermore was the water content of the stover plotted against the grain dry matter content. The pre-selected genotypes have been checked for their yield performance and water contents in the stover. Finally the sugar content of the stover (BRIX) of the pre-selected genotypes was checked, if high enough to be silage. When all requirements have been fulfilled, as last check was done by KWS SAAT SE and the genotype was selected.

The selection was based on the dual use maize harvest. The same genotypes have been tested for their silage maize performance. The selected genotypes have been identified for their yield performance as silage maize. Most genotypes are showing a moderate to high yield as silage maize as well. The priority of selection was based on the dual use maize harvest.

The selection was based on the testcrosses of the Dent and Flint lines. But further crosses have been conducted with the lines of the testcrosses. In total 13 Flint lines and 7 Dent lines have been selected (Table II.48). The entry numbers, showing double names (e.g. 89+96) are the same lines, but tested twice in the year under different entry numbers.

Table II.48 Summary of the selected testcrosses per genepool in 2014

Genepool	Entry number of selected testcrosses													Total
Dent	7	89+96	33	45+97	80	82	94+95							7
Flint	86	3+23	29	85	100	79	94	78	90	77	40	53	97	13

The selected lines have been crosses with each other,resulting in 88 factorial crosses, which have been tested for their performance during the experimental year 2015.

3.8 Response to direct selection and indirect selection

The performance test dual use maize and the performance test silage maize are expensive. Therefore it would be cheaper, if just one performance test could be made, instead of two. With direct selection the total dry matter yield, the total fresh matter and the total dry matter content of the dual use maize are defined as wanted traits. For the indirect selection, the total dry matter yield, total fresh matter and total dry matter content of the silage maize are used as assistant traits. The response of selection for indirect selection, with the assistant traits is compared to the response of direct selection with the wanted traits. The response to direct and indirect selection is calculated for the Dent testcrosses and the Flint testcrosses, as well as for the Factorial crosses.

3.8.1 Dent testcrosses

Table II.49 is showing the genotypic and phenotypic correlation of the traits of interest of the Dent testcrosses. The traits total dry matter yield (silage maize) and total dry matter yield (dual use maize) were genotypic significantly correlated with each other (0.76++). A higher genotypic correlation (1.01++) with each other were having the two traits of the total fresh matter (silage maize and dual use maize). The total dry matter content of silage maize was genotypic highly significant correlated with the total dry matter content of dual use maize (0.93++).

Table II.49 Genetic and Phenotypic Correlation of the Dent testcrosses

	Total fresh matter[a]	Total dry matter content[a]	Total dry matter yield[a]	Grain fresh matter[a]	Grain dry matter content[a]	Grain dry matter yield[a]	Stover dry matter yield	Stover fresh matter	Water content of stover	Total dry matter content[b]	Total fresh matter[b]	Total dry matter yield
Total fresh matter[a]		-0.81**	0.51**	0.43**	-0.59**	0.25**	0.46**	0.97**	0.61**	-0.72**	0.69**	0.19**
Total dry matter content[a]	-0.85++		0.08	-0.08	0.62**	0.13	-0.05	-0.86**	-0.88**	0.78**	-0.54**	0.06
Total dry matter yield[a]	0.40++	0.14		0.64**	-0.13	0.63**	0.71**	0.37**	-0.23*	-0.05	0.38**	0.46**
Grain fresh matter[a]	0.38++	0.11	0.91++		-0.30**	0.95**	-0.03	0.18	0.25*	-0.10	0.41**	0.46**
Grain dry matter content[a]	-0.63++	0.69++	0.00	-0.21+		0.02	-0.20	-0.57**	-0.45**	0.68**	-0.46**	0.06
Grain dry matter yield[a]	0.15	0.37++	0.91++	0.93++	0.16+		-0.09	0.00	0.10	0.14	0.26*	0.50**
Stover dry matter yield	0.72++	-0.41+	0.66++	0.40	-0.28+	0.28		0.50**	-0.37**	-0.21*	0.26**	0.13
Stover fresh matter	0.97++	-0.94++	0.18	0.14	-0.62++	-0.10	0.67++		0.60**	-0.74**	0.62**	0.08
Water content of stover	0.86++	-1.02++	-0.16	-0.10	0.69++	-0.35+	0.38	0.95++		-0.61**	0.44**	-0.02
Total dry matter content [b]	-0.99++	0.93++	-0.10	-0.10	0.77++	0.26++	-0.54+	-0.97++	-0.98++		-0.66**	0.13
Total fresh matter[b]	1.01++	-0.74++	0.62++	0.62++	-0.52++	0.34++	0.59+	0.89++	0.87++	-0.73++		0.66**
Total dry matter yield[b]	0.32+	0.00	0.76++	0.83++	0.13	0.84++	0.15	0.15	0.16	0.11	0.61++	

[a] traits are taken during dual use maize harvest/ [b] traits are taken during silage maize harvest

significance level phenotypic correlation: *p=0.05, **p=0.01/++: genotypic correlation is higher or has the same value than double of the error of the correlation

At first the trait total dry matter yield was compared for direct and indirect response to selection. The genotypic correlation between the two traits was 0.76 but its heritabilities were different (41 % vs. 64 %). The response to selection by direct selection was R: 12.19 dt/ha and thus higher, as if the trait would be selected indirectly (11.45 dt/ha). The second trait was the trait total fresh matter. The genotypic correlation was high and the heritabilities (77 %) are the same for both wanted trait and

assistant trait. The response to direct selection and the response to indirect selection were nearly the same (R: 5.37 kg/9m^2 vs. 5.68kg/9m^2). The last trait that was analyzed for its response to selection was the trait total dry matter content. The traits were genotypic high correlated and their heritabilities (86 % vs. 83 %) are nearly the same. The response to direct selection was a higher (R: 5.08 %) then the response to indirect selection (R: 4.88 %) (Table II.50).

For practical reason is dual use maize harvest work intensive and expensive. If the indirect selection during the silage maize harvest was as good as the selection during dual use maize harvest, only a silage maize harvest can be done. Then the double amount of testcrosses (200 instead of 100) can be tested and used for selection. The selection would be more intensive with 3.5 % instead of 7 %, if still seven testcrosses would be selected. This was leading to a changing selection intensity from 1.918 to 2.208.

The response to selection would be higher, because the selection would be more intensive, if still the same amount of genotypes would be selected (seven testcrosses). Comparing the three traits for their response to selection through indirect selection out of 100 or out of 200 respectively, the trait total dry matter yield was showing the highest response to selection with R: 8.50 dt/ha, if selected out of 200. Also the total fresh matter was responding higher to the indirect selection of 3.5 % (R: 5.65 kg/9m^2) compared to the indirect selection with 7 % (R: 4.91 kg/9m^2). The total dry matter content was giving a similar response to selection (3.5 %) with R: 5.15 % compared to the response to selection if selected out of 100 (R: 5.15 %) (Table II.50).

Table II.50 is comparing direct and indirect selection and their response to selection of the Dent testcrosses for the three named traits, depending on the selection method and the intensity.

Table II.50 Comparison between direct and indirect selection of the Dent testcrosses

Selected trait/ Assistant trait	Selection method	Response to selection (R)		Intensity of Selection (i)	Square root of heritability (h)	Genetical standard deviation (d)		Genetic correlation
Total dry matter yield	Direct$_{7\%}$	7.90	dt/ha	1.918	0.65	6.33	dt/ha	
Total dry matter yield[a]	Indirect$_{7\%}$	7.38	dt/ha	1.918	0.80	6.33	dt/ha	0.76
Total dry matter yield[a]	Indirect$_{3.5\%}$	8.50	dt/ha	2.208	0.80	6.33	dt/ha	0.76
Total fresh matter	Direct$_{7\%}$	4.64	kg/9m^2	1.918	0.84	2.88	kg/9m^2	
Total fresh matter[a]	Indirect$_{7\%}$	4.91	kg/9m^2	1.918	0.88	2.88	kg/9m^2	1.00[b]
Total fresh matter[a]	Indirect$_{3.5\%}$	5.65	kg/9m^2	2.208	0.88	2.88	kg/9m^2	1.00[b]
Total dry matter content	Direct$_{7\%}$	4.66	%	1.918	0.92	2.64	%	
Total dry matter content[a]	Indirect$_{7\%}$	4.47	%	1.918	0.95	2.64	%	0.93
Total dry matter content[a]	Indirect$_{3.5\%}$	5.15	%	2.208	0.95	2.64	%	0.93

[a]traits are taken during silage maize harvest/ [b] Genetical correlation is transformed to 1.00 instead of 1.01 as given in table II.49

3.8.2 Flint testcrosses

Table II.51 is showing the genotypic and phenotypic correlation of the traits of interest of the Flint testcrosses. The traits total dry matter yield (silage maize) and total dry matter yield (dual use maize) were genotypic significantly correlated with each other (1.50++). The two traits of the total fresh matter (silage maize and dual use maize) were genotypic high significant correlated as well (0.95++). The total dry matter content (silage maize) was genotypic highly significant correlated with the total dry matter content (dual use maize) with 0.85++.

Table II.51 Genetic and Phenotypic Correlation of the Flint testcrosses

	Total fresh matter[a]	Total dry matter content[a]	Total dry matter yield[a]	Grain fresh matter[a]	Grain dry matter content[a]	Grain dry matter yield[a]	Stover dry matter yield	Stover fresh matter	Water content of stover	Total dry matter content[b]	Total fresh matter[b]	Total dry matter yield
Total fresh matter[a]		-0.76**	0.82**	0.64**	-0.52**	0.58**	0.63**	0.97**	0.29**	-0.65**	0.68**	0.36**
Total dry matter content[a]	-0.98++		-0.26**	-0.39**	0.40**	-0.32**	-0.11	-0.77**	-0.77**	0.73**	-0.54**	-0.11
Total dry matter yield[a]	0.97++	-0.89++		0.61**	-0.42**	0.58**	0.85**	0.77**	-0.24*	-0.33**	0.53**	0.45**
Grain fresh matter[a]	0.71++	-0.52++	0.96++		-0.73**	0.95**	0.13	0.43**	0.32**	-0.40**	0.51**	0.35**
Grain dry matter content[a]	-0.64++	0.43++	-0.86++	-0.96++		-0.48**	-0.21*	-0.38**	-0.16	0.45**	-0.50**	-0.29**
Grain dry matter yield[a]	0.72++	-0.56++	0.84++	0.98++	-0.88++		0.07	0.38**	0.35**	-0.32**	0.42**	0.28**
Stover dry matter yield	0.88++	-0.78+	0.91++	0.54+	-0.62+	0.52		0.70**	-0.52**	-0.20*	0.34**	0.30**
Stover fresh matter	0.98++	-1.00++	0.87++	0.54++	-0.47++	0.57++	0.88++		0.24*	-0.63**	0.63**	0.32**
Water content of stover	0.79++	-0.91++	0.50	0.38+	-0.21+	0.46+	0.44	0.82++		-0.47**	0.25*	-0.07
Total dry matter content [b]	-0.82++	0.85++	-0.74++	-0.59++	0.52++	-0.64++	-0.52+	-0.80++	-0.82++		-0.70**	-0.11
Total fresh matter[b]	0.95++	-0.68++	1.36++	0.74++	-0.61++	0.75++	1.08++	0.91++	0.41+	-0.81++		0.78**
Total dry matter yield[b]	0.67++	-0.14	1.50++	0.54++	-0.41++	0.45+	1.23+	0.64++	-0.32+	-0.24+	0.78++	

[a] traits are taken during dual use maize harvest/ [b] traits are taken during silage maize harvest

significance level phenotypic correlation: *p=0.05, **p=0.01/++: genotypic correlation is higher or has the same value than double of the error of the correlation

The selected trait total dry matter yield had very low heritability (22 %) compared to the assistant trait (68 %). The response to selection was higher if selected indirect (23.62 dt/ha) then if selected direct (9.17 dt/ha). The second trait was the trait total fresh matter. The genotypic correlation was high and the heritability of the assistant trait (81 %) was higher than of the selected trait (65 %). The response to direct selection and the response to indirect selection were nearly the same (R: 4.55kg/9m^2 vs. 4.80kg/9m^2). The last trait that was analyzed for its response to selection was the trait total dry matter content. Their heritabilities are similar (72 % vs. 88 %) and the traits are genotypic high correlated. The response to direct selection was a higher (R: 3.51 %) then the response to indirect selection (R: 3.30 %) Table (Table II.52).

For practical reason was the dual use maize harvest work intensive and expensive. If the indirect selection during the performance test silage maize harvest was as good as the selection during the dual use maize harvest, only silage maize harvest can be done. Then the double amount of testcrosses (200 instead of 100) can be tested and used for selection. The selection intensity would change from 1.627 to 1.951 if still thirteen genotypes would be selected (13 % vs. 6.5 %).

The response to selection would be higher, because the selection would be more intensive, if still the same amount of genotypes would be selected (thirteen testcrosses). Comparing the three traits for their response to selection through indirect selection out of 100 or out of 200 respectively, the trait total dry matter yield was showing the highest a response to selection with R: 9.02 dt/ha, if selected out of 200. Also the total fresh matter showed nearly the same response to selection if selected more sharp with 6.5 % (R: 4.67 kg/9m^2) compared to the indirect selection with 13 % (R: 3.90 kg/9m^2). The total dry matter content was giving a response to selection (6.5 %) with R: 3.37 % which was a higher compared to the response to selection if selected out of 100 (R: 2.81 %) (Table II.52)

Table II.52 is comparing direct and indirect selection and their response to selection of the Flint testcrosses for the three named traits, depending on the selection method and the intensity..

Table II.52 Comparison between direct and indirect selection of the Flint testcrosses

Selected trait/ Assistant trait	Selection method	Response to selection (R)		Intensity of Selection (i)	Square root of heritability (h)	Genetical standard deviation (d)		Genetic correlation
Total dry matter yield	$Direct_{13\%}$	4.35	dt/ha	1.627	0.48	5.57	dt/ha	
Total dry matter yield[a]	$Indirect_{13\%}$	7.52	dt/ha	1.627	0.83	5.57	dt/ha	1.00[b]
Total dry matter yield[a]	$Indirect_{6.5\%}$	9.02	dt/ha	1.951	0.83	5.57	dt/ha	1.00[b]
Total fresh matter	$Direct_{13\%}$	3.69	kg/9m^2	1.627	0.81	2.80	kg/9m^2	
Total fresh matter[a]	$Indirect_{13\%}$	3.90	kg/9m^2	1.627	0.90	2.80	kg/9m^2	0.95
Total fresh matter[a]	$Indirect_{6.5\%}$	4.67	kg/9m^2	1.951	0.90	2.80	kg/9m^2	0.95
Total dry matter content	$Direct_{13\%}$	2.99	%	1.627	0.85	2.16	%	
Total dry matter content[a]	$Indirect_{13\%}$	2.81	%	1.627	0.94	2.16	%	0.85
Total dry matter content[a]	$Indirect_{6.5\%}$	3.37	%	1.951	0.94	2.16	%	0.85

[a]traits are taken during silage maize harvest/ [b] Genetical correlation is transformed to 1.00 instead of 1.50 as given in table II.51

3.8.3 Factorial crosses

The factorial crosses have not been selected or crossed further. Therefore the selection intensity is set to 10 % (i: 1.755). Selecting 10 genotypes out of 100 is a common method.

The genotypic and phenotypic correlation of the traits of interest for the factorial crosses are shown in Table II.53. The traits total dry matter yield (silage maize) and total dry matter yield (dual use maize) were genotypic significantly correlated with each other (1.01++). The two traits of the total fresh matter (silage maize and dual use maize) were genotypic high significant correlated as well (0.94++). The total dry matter content (silage maize) was genotypic highly significant correlated with the total dry matter content (dual use maize) with 0.32++.

Table II.53 Genetic and Phenotypic Correlation of the Factorial crosses

	Total fresh matter[a]	Total dry matter content[a]	Total dry matter yield[a]	Grain fresh matter[a]	Grain dry matter content[a]	Grain dry matter yield[a]	Stover dry matter yield	Stover fresh matter	Water content of stover	Total dry matter content[b]	Total fresh matter[b]	Total dry matter yield
Total fresh matter[a]		-0.80**	0.81**	0.58*	-0.65**	0.47**	0.67**	0.97**	0.54**	-0.10**	0.75**	0.37**
Total dry matter content[a]	-0.96++		-0.34**	-0.38**	0.66**	-0.24*	-0.28**	-0.80**	-0.86**	0.24**	-0.59**	-0.02
Total dry matter yield[a]	0.86++	-0.65++		0.60**	-0.42**	0.56**	0.79**	0.75**	0.08	0.10	0.63**	0.57**
Grain fresh matter[a]	0.48++	-0.38++	0.60++		-0.63**	0.97**	0.03	0.36**	0.51**	0.04	0.45**	0.39**
Grain dry matter content[a]	-0.72++	0.71++	-0.62++	-0.72++		-0.41*	-0.25*	-0.56**	-0.52**	0.13	-0.63**	-0.21*
Grain dry matter yield[a]	0.30+	-0.17	0.48++	0.95++	-0.47++		-0.05	0.24*	0.44**	0.10	0.32**	0.37**
Stover dry matter yield	0.80++	-0.68++	0.79++	0.02	-0.42++	-0.17		0.76**	-0.19	0.07	0.54**	0.40**
Stover fresh matter	0.97++	-0.95++	0.79++	0.27+	-0.60++	0.07	0.87++		0.46**	-0.13	0.71**	0.30
Water content of stover	0.82++	-0.90++	0.46+	0.59++	-0.67++	.045+	0.29	0.75++		-0.24*	0.36**	-0.09
Total dry matter content [b]	-0.18+	0.32++	0.07	0.01	0.14+	0.12	0.07	-0.20+	-0.51++		0.15	0.33**
Total fresh matter[b]	0.94++	-0.83++	0.92++	0.49++	-0.86++	0.26+	0.86++	0.89++	-0.62++	0.62++		0.73**
Total dry matter yield[b]	0.42++	0.10	1.01++	0.49++	-0.39+	0.44+	0.80++	0.33+	-0.56+	0.51++	0.59++	

[a] traits are taken during dual use maize harvest/ [b] traits are taken during silage maize harvest

significance level phenotypic correlation: *p=0.05, **p=0.01/++: genotypic correlation is higher or has the same value than double of the error of the correlation

The heritability of the selected trait total dry matter yield was moderate 44 %. Compared to the assistant trait (33 %) the heritability was higher in the selected trait. Selecting directly the response to selection was higher (10.23 dt/ha), compared to indirect selection (9.07%) with the same selection intensity. As second trait was the total fresh matter with a heritability of 46 % used. Its assistant trait had a heritability of 58 %. The response to selection was the same with direct and indirect selection (3.90kg/9m^2 vs. 3.93kg/9m^2). The last trait analyzed was the total dry matter content. The heritabilities were very different to each other. The selected trait total dry matter content had a heritability of 23 %, while the assistant trait had a heritability of 76 %. On the other hand the response to selection for direct selection was higher (2.67 %) compared to the response of indirect selection with the same selection intensity (1.55 %). In Table II.54 the results of indirect and direct selection are compared.

For practical reasons the performance test dual use maize harvest work was intensive and expensive. If the indirect selection during the silage maize harvest was as good as the selection during the dual use maize harvest, only a silage maize harvest could be done. Then probably the double amount of testcrosses (200 instead of 100) could be tested and used for selection. The selection intensity would change from 1.755 to 2.063, if still ten genotypes would be selected (10 % vs. 5 %).

The response to selection would be higher, because the selection would be more intensive, if still the same amount of genotypes would be selected (ten testcrosses). Comparing the three traits for their response to selection through indirect selection out of 100 or out of 200 respectively, the trait total dry matter yield was showing the highest a response to selection with R: 10.66 dt/ha, if selected out of 200. The trait total fresh matter was showing also a slightly higher response to selection if selected more sharply with 5 % (R: 4.61 kg/9m^2) compared to the indirect selection with 10 % (R: 3.93 kg/9m^2). The total dry matter content was giving a response to selection (5 %) with R: 1.82 %. This was still lower then the response to selection under direct selection. Even though the response to selection with an intensity of 5 % was higher compared to the response of selection with an selection intensity of 10 % (Table II.54).

Table II.54 is comparing direct and indirect selection and their response to selection of the factorial crosses for the three named traits, depending on the selection method and the intensity. Also the needed factors for calculation are given for the selected and assistant trait.

Table II.54 Comparison between direct and indirect selection of the factorial crosses

Selected trait/ Assistant trait	Selection method	Response to selection (R)	Intensity of Selection (i)	Square root of heritability (h)	Genetical standard deviation (d)	Genetic correlation
Total dry matter yield	$Direct_{10\%}$	10.23 dt/ha	1.755	0.67	8.76 dt/ha	
Total dry matter yield[a]	$Indirect_{10\%}$	9.087 dt/ha	1.755	0.59	8.76 dt/ha	1.00[b]
Total dry matter yield[a]	$Indirect_{5\%}$	10.66 dt/ha	2.063	0.59	8.76 dt/ha	1.00[b]
Total fresh matter	$Direct_{10\%}$	3.90 kg/9m^2	1.755	0.68	3.27 kg/9m^2	
Total fresh matter[a]	$Indirect_{10\%}$	3.93 kg/9m^2	1.755	0.76	3.27 kg/9m^2	0.94
Total fresh matter[a]	$Indirect_{5\%}$	4.61 kg/9m^2	2.063	0.76	3.27 kg/9m^2	0.94
Total dry matter content	$Direct_{10\%}$	2.67 %	1.755	0.48	3.17 %	
Total dry matter content[a]	$Indirect_{10\%}$	1.55 %	1.755	0.87	3.17 %	0.32
Total dry matter content[a]	$Indirect_{5\%}$	1.82 %	2.063	0.87	3.17 %	0.32

[a]traits are taken during silage maize harvest/ [b] Genetical correlation is transformed to 1.00 instead of 1.01 as given in table II.53

4. Discussion

For the usage of dual use maize different requirements are given. High grain dry matter yield, usable as feed and high stover dry matter yield, usable as substrate to reach high methane yield, are the most important requirements for a dual use maize variety. Most important for successful cultivation is the total usage of the vegetation period (Amon et al. 2003, Oechsner et al. 2003). Actually the region where the variety is cultivated is important to be decided between early and late varieties. For a benefical use of dual use maize, the grain yield and the stover yield should be high. Some of the genotypes that have been tested during the study are supporting the findings of Amon et al. (2003) and Oechsner et al. (2003), like entry number 4. During silage maize harvest the genotype was showing a moderate yield, while the stover yield of the genotype during dual use maize harvest was the highest.

No phenotypic or genetic correlation between the two traits grain dry matter yield and stover dry matter yield is shown. In the Dent testcrosses especially genotype 88 and 5 and for the Flint testcrosses, like 10 and 91, are having a high yield in grain dry matter and stover dry matter. The yields for the factorial crosses are more important, because those should later on become dual use varities. General and specific combining ability of the lines can lead to a higher range in yield for the factorial crosses. Genotypes, like 10 and 84, are high yielding in grain dry matter and stover dry matter. The Dent lines have always been the mother line, while the Flint lines are always used as father for the factorial crosses. Especially Dent line 80 is showing a low general combining ability, while particularly the Flint line 3+23 is showing a high general combining ability.

Oechsner et al. (2003) supposed that the breeding for silage maize, used as substrate for energy production, and silage maize used for feed, should be different. The methane yield as an important trait for the usage of maize as energy source, is highly depending on the yield by hectare of the dry matter yield (Oechsner et al. 2003). Dual use maize is a mixture of feed and substrate for energy production. The correlation between grain dry matter yield and total dry matter yield during silage maize harvest is moderate and significant (Dent: 0.47** / Flint: 0.30** / factorial crosses: 0.21*). The stover dry matter yield of the Flint testcrosses and the factorial crosses is also moderate significant correlated with the total dry matter yield during performance test silage maize harvest (Flint: 0.41** / factorial crosses: 0.30**). The Dent testcrosses are showing no correlation (0.20). The correlation between grain dry matter yield and stover dry matter yield is very low and not significant (Dent: -0.04 / Flint: 0.14 / factorial crosses: -0.16).

For breeding a dual use variety not only yield is an important trait. Furthermore traits like water content of the stover and dry matter content leading to a stable biogas production, are important, too. A negative correlation between dry matter content and methane yield is found in several studies (Weiland 2003, Li et al. 2011). Thus, the wetness of the stover is also an important trait to guarantee a stable and environmentally friendly as well as a cheap silage process. The fermentation inside the biogas plant depends on the substrate and the fermentation process. With a dry matter content of 10 % to 13 % wet fermentation can be processed (Weissbach 2000, Weiland 2003, Fernández et al. 2008). Wet fermentation is most commonly used in Germany (mifratis.de 2016) for feedstocks that cannot percolated well because of their low solids content (GICON 2017). The water content of the stover confirmed in every experiment seemed to be high (Dent: 67 %-80 % / Flint: 67 %-81 % / factorial crosses: 54 %-78 %). The heritability of the trait water content in the stover is moderate (Dent: 54 % / Flint: 31 % / factorial crosses: 20 %). With those results it can be stated that the water

content is high enough to guarantee a wet fermentation process. Weiland (2003), Kaiser (2007) and Li et al. (2011) are showing that a low dry matter content is leading to an effecitive use of biogas production with maize stover as energy source, aiming at a dry matter content between 28 %-35 % (Weiland 2003, Kaiser 2007, Fernández et al. 2008, Li et al. 2011). On the other hand Baserga (2000) is showing that a dry matter content of 86 % of the stover is giving an methane yield as high as for grass silage or clover (Baserga 2000). Therefore the high water content can be a problem because the methane yield is too low. By taking long drought stress into account the dry matter content of the stover is increasing. But as the experimental years 2014 and 2015 have shown, the water content of the stover is still high enough for biogas production. Aiming at a stable and high yielding biogas process, the found water contents in the stover are fitting well the recommondations (Herrmann and Rath 2012, Neumann 2015).

The dual use maize harvest is a combination of grain maize harvest and whole plant harvest, which very time consuming (Schmidt et al. 2016). The grain dry matter yield and the stover dry matter yield are showing constant heritabilities leading to the idea, that only a grain harvest and a silage maize harvest, earlier in the season, could be enough to breed for a dual use maize variety. If the dual use maize harvest is done in two steps, grain maize harvest and whole plant harvest, a lot of space is needed, because both harvests take place at the same time. By doing just two separate harvests, cost and time would be reduced. Therefore more genotypes could be tested or it could be bred for another use. Because of the low correlation an indirect selection based on the total dry matter yield for silage maize for stover dry matter yield is not possible. False positive selection is also possible because of the low correlations between total dry matter yield and grain dry matter yield as well as stover dry matter yield and total dry matter yield. The grain dry matter yield is also not helping to select for high stover dry matter yield because no correlation between the traits is found. Moreover is the calculation of the response to selection showing, that the difference by selecting with the same selection intensity for direct and indirect response to selection is small. When, increasing the selection intensity, changes in the response of selection are neglectable. Traits, usable for indirect selection during silage maize harvest, are total dry matter yield, total fresh matter and total dry matter content. Those traits are showing in the dual use maize harvest low correlation with the traits of interest like grain dry matter yield and stover dry matter yield, as well as water content of the stover. The total dry matter yield of silage maize is not correlated with the water content in the stover. Comparing the correlations, the two reverse traits water content of the stover and stover dry matter content are not as strong correlated as expected. Selection on one trait is leading to a contrary selection of the other trait, but still in the range need for stable wet fermentation in biogas plants. An indirect selection by grain dry matter yield is not advisable, because of the low correlations between grain dry matter yield and water content of the stover plant. A grain harvest and a silage maize harvest can be informative to get an idea about suitable genotypes. Especially if the part of corn cob is measured during silage maize, an idea aobut the amount of stover and grain can be given already during silage maize harvest. Nevertheless a dual use maize harvest is needed to identify genotypes usable as dual use maize and avoid false positive selection.

Likewise it is reported that the sugar content of the substrate has to be high, otherwise the fermentation would not run in the desired way but unwanted activity of bacteria was observed (Gross and Riebe 1974). Therefore the sugar content of the stover was measured with the destructive BRIX method. In 1998 Van Waes et al. (1998) showed that the BRIX value gives a good estimation for the total sugar content in the plant (van Waes et al. 1998). Comparision of the sugar contents measured with the BRIX method and with the common HPLC-anylsis for sugar content, are

supporting this findings (e.g.: check 1: BRIX method: 3.36 % / HPLC: 3.56 % , check 41: BRIX method: 5.16 /HPLC:5.05). As the HPLC is showing, sucrose, measured also mainly with the BRIX method, is found in higher amounts, compared to fructose and glucose (nährwertrechner.de 2017). In earlier studies the measurement of BRIX was not done to validate the usability of the maize stover for stable biomethane production but used as a quality criteria to analyze especially the sugar content in sweet corn. Here the BRIX-values contains a range of 14 % -22 % (van Waes et al. 1998, Mok et al. 2014) which is probably higher than expected in silage maize or grain maize used for biogas production and animal feeding.

The BRIX values are changing over time, giving a non linear line decreasing from the beginning of the season until harvest. The stover would be harvested during grain maturation and therefore the second measurement shortly before harvest is of greater interest. The measurements at two different locations over three years showed that there is strong environmental effect on the % BRIX (Dent: 242.59**/ Flint: 174.26** / Factorial crosses: 327.50**), while the genotype interaction with year and location is still significant but less strong (Dent: 1.63** / Flint: 1.37**/ Factorial crosses: 0.90). On the other hand the heritability for the trait BRIX is moderate (Dent: 58 % / Flint: 41 % / Factorial crosses: 58 %). Facing those results they are leading to the idea that there is genetic background for the stover sugar content, which is influenced highly by the environment. First, the sugar content of sweet sorghum was measured because of bioenergy reason. Therefore Murray et al. (2009) found two chromosomes (1 and 3) that are encoding for the sugar content in sweet sorghum (Murray et al. 2008a, Murray et al. 2008b, Murray et al. 2009). Sugar content in maize stover was analyzed by Bian et al. (2015). They found a candidate QTL on chromosome 2 (Bian et al. 2015). Also Bian et al. (2014/2015) stated that the sugar content of the stover is coded by different QTLs chaning during the season, as the sugar content (Bian et al. 2014, Bian et al. 2015).

The sugar content of the stover is not related to the grain dry matter content (Dent: 0.23**/ Flint: 0.09 / factorial crosses: 0.13) and the stover dry matter content (Dent: 0.30**/ Flint: -0.07/ factorial crosses: 0.13). Only the Dent testcrosses are showing a low significant correlation. Using the dry matter contents as an indirect selection tool is thus not possible.

Another trait which was analyzed during the study is the stay-green behavior of maize. The idea behind this for analyzing, was getting a trait that can lead to indirect selection of plants containing a high sugar content and water content in the stover without any destructive method. The chlorophyll content of the plant is decreasing during the season. Stay-green behavior is correlated with a longer photosynthetic activiety, including a longer production of assimilates (Bekavac et al. 1998, Thomas and Howarth 2000, Bekavac et al. 2007). It is hypothesized that a maize genotype, which stays green also after grain filling is assimilating further, would lead to a higher amount of sugars in the stem, because it is then functioning as a sink (Rajendran et al. 2000, White et al. 2011).

The results show that the SPAD and BRIX values are significantly correlated, but on a low level (Dent: 0.24* / Flint: 0.25* / factorial crosses: 0.34**). On the other hand, getting an idea about the dry matter contents of the grain (Dent: -0.32** / Flint: -0.17 / factorial crosses: 0.01) or the stover (Dent: -0.19 /Flint: -0.20** /factorial crosses: 0.21**) with help of the SPAD is not possible because of a low correlation. Therefore the hypothesis is not valid for practical reason.

The economic value of dual use maize has to be taken into account as well. By selling the grains and the stover independently of each other, an increase of profit for the farmers is expected. But the grain maize price is varying during the year, depending on the month of supplementation. The expected price (in €/t) for grain maize depending on the time of supplyng the maize from now until the next years (Figure II.49). If the price for grain maize is high, the usage of dual use maize will become advantageous, because of a profit through selling the stover extra.

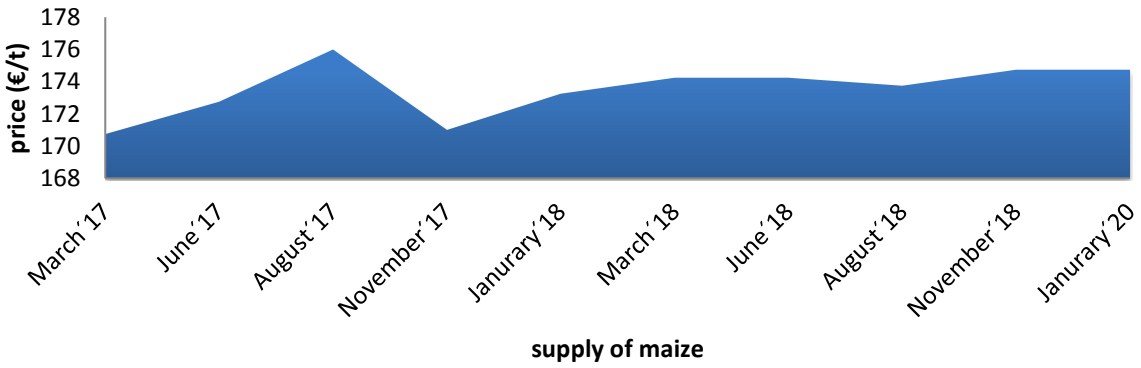

Figure II.49 Price (€/t) for grain maize depending on the month supplied (raiffeisen.com 2017)

Studies of KWS SAAT SE showed that a grain maize price of at least 23€/dt grain maize is needed for an economic profit (Schmidt et al. 2016). Comparing the experience of Austria and Bavaria, the payment for maize straw is high and leading for the biogas plant operator to a cheap substrate (Neumann 2015). If the grain maize price is below 23€/dt, it could be favorable to sell dual use maize as common grain maize or sell it as energy maize. Using dual use maize as silage maize for feeding is possible, because the used stover will reach the feed value of hay. But comparing this to the feed value of silage maize bred for feeding, it is too low for an efficient use. Therefore it would be favorable to use dual use maize as silage maize, if the grain maize price is so low (Schmidt et al. 2016).

By selling maize as expected, the biogas plant operator gets a cheap substrate, which leads to no increase of costs, if supplementation of trace elements is necessary. Lebhun et al. (2008) showed that the process of instability of biogas production from maize silage can be caused due to deficiency in trace elements (Lebuhn et al. 2008). An efficient methane production by maize silage for long-term is only possible, if essential trace elements are not missing in the substrate (Lebuhn et al. 2008). Those trace elements have not been studied here, but it is assumed that essential trace elements are found in the material. Otherwise a supplement of those elements is necessary. Another alternative for more stable production process is the usage of co-substrates, like chicken manure or kitchen waste (Li et al. 2013, Neumann 2015).

Dual use maize harvest is studied as well. Fleschhut, et al. (2016) showed that there are already different ways to harvest grain maize and afterwards the stover, with a rescue of around 50 % (Fleschhut 2015, Neumann 2015). For more economic profit, the resuce has to be increased. An occurring problem could be pollution by soil. If the pollution is as high as for energy maize, no problem will occur for the biogas production. Therefore the harvest has to be as clean as silage maize harvest (Neumann 2015, Holzhammer 2016). Another advantage coming up while harvesting the stover from the field is the indirect combat of corn moth (*Ostrinia nubialis*). Furthermore is it a good start for further soil treatment and the usage of own fertilizer (Neumann 2015).

The basic idea of the substrate used for bioenergy production was material, which is not used anymore for feeding or food production. Maize stover left on the field after grain maize harvest is such a material. By developing dual use maize cultivars, the basic idea of biogas plants is included again. The tested maize genotypes are showing the ability for dual use maize. Genotypes with a high grain dry matter yield while having a high stover dry matter yield as well have been found and selected. Water content and sugar content of the stover are high enough to garantuee stable and environmental biogas production. The stay-green behavior, which is not closely correlated with the sugar content of the stover, as expected is showing a correlation with dry matter contents of grain, stover and total plant. All important characteristics of dual use maize have been fullfild. The developed factorial crosses are showing promising genotypes, probably used as dual use maize varities later on. Also it is shown that an own breeding program has to be investigated for dual use maize.

III.

Genome-Wide Association mapping

1. Introduction

Genome-wide association mapping has made a dramatic increase during the last years. In medicine it was used as a powerful tool to identify human genes for common disease and complex traits (Li and Jiang 2005, Pearson and Manolio 2008, Yan et al. 2011, Wang et al. 2012b). Already in the late 90's QTL mapping with polymorphic markers started, finding genes coding for quantitative traits (Kearsey and Farquhar 1998). Usage of genome-wide association mapping nowadays is advantageous permitting interrogation of entire genomes. On the other hand is the high amount of statistical tests, made within the study, leading to an unpredictable number of false-positive results (Pearson and Manolio 2008). A careful selection of variants is important to reduce the disadvantages (Pearson and Manolio 2008) and still find as many significant features as possible in the genome (Storey and Tibshirani 2003). Even though genome-wide association mapping has a lower power to detect rare alleles, it is able to detect small effects on a large number loci, making the analysis of data more challenging (Yan et al. 2011).

By reducing the costs of markers and genome-wide association mappings, the analysis is now commonly used, for humans, animals and plant species (Zhu et al. 2008, Huang et al. 2010, Racedo et al. 2016). Genome-wide association studies are enabling researchers to study a broader germplasm and search for functional variation and natural diversity, compared to the human genome (Zhu et al. 2008, Yan et al. 2011). Identification of biochemical and regulatory pathways and the check behind by genes is of great interest (Peleman and van der Voort 2003, Riedelsheimer et al. 2013, Rippe and Angelopoulos 2013, Romay et al. 2013, Wallace et al. 2014) and association mapping is offering a great potential to enhance genetic improvement (Yan et al. 2011, Riedelsheimer et al. 2012) before identifiying candidate genes with QTL mapping. Using genome-wide association mapping and QTL-mapping the efficiency of plant breeding could be increased, due to a new approach for marker-assisted breeding.

The development of platforms for genome-wide association studies, like easyGWAS, is usable to compare results of different plant and animal species and their quantitative traits (Grimm et al. 2017). Even though mapping loci involved in relevant traits by using introgression line libraries is a powerful tool to determine a precise position for the loci (Peleman and van der Voort 2003). Breeding by design, based on the genetic background, by knowing the position of loci of all traits of interest, its allelic variation and the contribute to the phenotype, the breeder should be able to design a superior genotype containing all traits of interest, even though the exact position is still unknown (Kearsey and Farquhar 1998, Peleman and van der Voort 2003). The knowledge is helping to develop future breeding strategies and programs (Peleman and van der Voort 2003).

To start genome-wide association mapping a genotypic characterization of individuals with a sufficient number of polymorphic markers is necessary. The minimum amount of markers needed depends on the size of the genome and the rate of linkage disequilibrium (Peleman and van der Voort 2003, Yan et al. 2011, Pasam et al. 2012). Moreover it is important to take the population structure into account, to avoid highly significant associations between marker and phenotype even when the marker is not linked with a loci (Pritchard 2001). Population structure has a similar effect on all loci and can thus end up in a problem, if associations are found all over the genome for random marker loci (Pritchard 2001). On the other hand the population structure is giving an idea about the general combining ability of parental lines reflecting the performance of their progeny (Riedelsheimer et al. 2013).

The maize genome is complex with a high level of genetic diversity caused by constant flux (Romay et al. 2013). Especially the high genetic diversity and the rapid decay in linkage disequilibrium is making maize an ideal crop for association mapping by increasing the power of the study (Yan et al. 2011, Yang et al. 2011). Gene mapping in maize has already a long tradition. In the late 80's and 90's several maps based on restriction fragment polymorphism (RFLP) and simple sequence repeat (SSR) have been presented (Davis et al. 1999). Other grass species, like rice and sorghum, have been used for genome-wide association mapping as well (Xu et al. 2000, Huang et al. 2010, Biscarini et al. 2016). Comparing the results of the other grasses while analyzing the genome of maize could give an idea about possible associations and potential candidate genes (Buckler et al. 2009).

Due to the availability of the maize genome sequence and advance genotyping techniques insight is given into complex quantitative traits (Yang et al. 2011). Genome-wide association studies and QTL mapping have been done on several quantitative traits of maize like flowering time (Veldboom et al. 1994, Buckler et al. 2009, Wallace et al. 2014) and kernel starch, as well as for some quality traits, like forage quality and kernel oil (Cook et al. 2012). Also yield, maturation and response to biotic and abiotic stress have been analyzed (Melchinger et al. 1998) to identify variants that are associated (Melchinger et al. 1998, Cockram et al. 2007, Yan et al. 2011, Yang et al. 2011).

Genome-wide association mapping as a breeding tool is used in plant breeding (Peleman and van der Voort 2003). Breeding programs are depeding on the usage of maize, as grain maize, silage maize or energy maize, because of different demands are given (Oechsner et al. 2003). A further use of maize is dual use. Therefore maize grains are used for animal fed and maize stover is used as energy source for bioenergy production. Requirements for dual use maize are high stover yield, high water content of the stover and high grain dry matter yield. The leaf structure as well as the senescence of the leafs can strongly influence the grain yield and the quality of yield (Xu et al. 2000, Zheng et al. 2009, Wang et al. 2012a). Furthermore, species that expose a stay-green behavior are showing a higher resistance against diseases and have a higher quality for forages for animals as well as showing a better resistance against drought, which is a positive effect (Xu et al. 2000, Zheng et al. 2009, Wang et al. 2012a, Gregersen et al. 2013). Therefore stay-green behavior is also an important requirement in terms of dual use maize. The stay-green behavior is commonly analyzed with the SPAD method. With the SPAD method mainly the cholorphyll content is measured, which is highly correlated with the photosynthetic activitiy of the leaf (Konica Minolta Optics, Inc. 2009). Decoding the genetic background of the stay-green behavior has been of great interest during the last years (Bekavac et al. 2007, Zheng et al. 2009, Thomas and Ougham 2014). Identifying the stay-green characteristic in the classical breeding way is hard and time consuming. Depending on environmental factors like water deficiency and interactions with drought, but also the inability to evaluate stay-green until the plant has reached its physiological maturity, is leading to slow progress (Xu et al. 2000). Identifying the genetic background of stay-green, many researchers showed that stay-green is a large polygene-regulated quantitative trait (Xu et al. 2000, Zheng et al. 2009) and has been studied already for grass species like sorghum (Xu et al. 2000), rice, wheat and maize (Beavis et al. 1994, Zheng et al. 2009, Huang et al. 2010, Wang et al. 2012a, Kante et al. 2016).

To guarantuee a stable biogas production by using maize stover as bioenergy source, not only the water content is important, but also the sugar content of the stover has to be high, otherwise the fermentation would not run in the desired way but unwanted activity of bacteria occur (Gross and Riebe 1974). Besides the energy densitiy is increasing, while the costs are reduced (Seale et al. 1986, Murray et al. 2008b, Bian et al. 2014, Bian et al. 2015). In earlier studies, the sugar content of sweet

corn was measured, with the BRIX-method, giving the amount of sucrose in the % BRIX, as a quality criteria (van Waes et al. 1998, Mok et al. 2014). There is a lack in studies about sugar content found in maize stover, even though maize stover gets more interesting in terms of bioenergy (Bian et al. 2014, Bian et al. 2015). Bian et al. (2014) studied the sugar content in maize stover with the method BRIX in terms of sugar related traits, that can be modified with breeding to increase the sugar content in maize stover and make it more suitable for fermentation processes and silage (Bian et al. 2014, Bian et al. 2015). The sugar content in maize stover is variating during different growth stages and controlled by polygenes, which are selectively expressed (Bian et al. 2014, Bian et al. 2015). Other agronomic traits like grain yield should be taken into account while planning breeding strategies for higher sugar content in maize stover (Bian et al. 2014, Bian et al. 2015).

Identifiying the genetic background of the traits stay-green behavior and sugar content of the stover is helping while breeding for dual use maize. Identifiying significant associations between the traits SPAD and BRIX can help in further breeding programs as a pre-breeding tool. Especially the BRIX-method, used for measuring the sugar content, is destructive and time-consuming. Developing markers, especially for BRIX and identifiying QTLs with QTL mapping for the trait, can reduce cost in further breeding programs. The effort needed to analyze BRIX would be reduced, because already young plants and a higher amount of lines or testcrosses can be analyzed. Therefore the genome-wide association mapping in this study should make a first step by identifying associations between SNPs and the two traits sugar content of the stover (BRIX) and stay-green behavior (SPAD).

2. Material and Methods

2.1 Plant Material and Genotyping

The plant material are breeding lines from KWS SAAT SE, consisting different genotypes of the Dent-Genepool and Flint-Genepool. In total 81 Dent breeding lines and 84 breeding Flint lines have been selected for genotyping. The lines have been included in several studies before, and selected lines of those material are used in this study as parental lines for factorial crosses.

Four genotypes G14-156/8, G14-156/37, G14-156/98 and G14-155/10 are excluded from the further analysis. The genotypes G14-156/8, G14-156/98 and G14-155/10 are outliers from their belonging genepools. Genotypes G14-156/37 is showing a high frequency of heterozygous markers and is therefore excluded as well.

The genotyping of the 81 Dent lines and 84 Flint lines was done by KWS SAAT SE. Each line was genotyped with 8917 single nucleotide polymorphisms (SNPs) using the 12K KWS Illumina Chip. This Chip is part of the 50K public maize Chip of Illumina.

Quality check of the SNPs was performed according to Riedelsheimer et al. (2012), but with modifications (Riedelsheimer et al. 2012). SNPs with call rates below 100 %, as well as SNPs in full linkage disequilibrium and with a minor allele frequency below 0.05 were excluded from further analysis. All heterozygous detections have been handled as missing data.

2.2 Experimental Design and Phenotyping

Two traits, sugar content of the stover and the stay-green behavior of the plant, have been phenotyped during the study. Both traits have been recorded in field trails in Göttingen and Einbeck during three years (2014, 2015, 2016). Each genepool, Dent and Flint was represented in one experiment. The experiments were build up in a lattice design with two replications each and the plots are consisting of two rows and have been 6m long while the row distance was 75cm. The plant density was 10 plants per m^2. In 2016 a storm event damaged the experiments at the location Göttingen. The location was no longer used for data evaluation for SPAD and is not integrated in the analysis of chlorophyll content (SPAD) and the further genome-wide association mapping.

The phenotypic data is taken from testcrosses made between the lines as a mother and one line form the other genepool as father. Those testcrosses have been sown in the field, with two replications and filled up with check varieties of KWS SAAT SE.

2.2.1 Sugar Content in the Stover (BRIX-method)

The sugar content in the stem is measured with help of the BRIX method. With an electrical refractometer Pocket PAL 1 (ATAGO 2016) the BRIX-value in % BRIX is given, showing the sucrose content of the sample. Per plot three plants were cut into two parts.

The first part was taken directly above the fully formed corn cob, while the second part was taken from below the fully formed corn cob (Figure III.1). In total six samples per plot of around 10-15cm were taken.

Sample above corn cob **Sample below corn cob**

Figure III.1: Cutting the samples for the sugar (BRIX) measurement

The samples were put into a bench vise to squeeze out the maize sap. The sap was put into the electrical refractometer Pocket PAL 1 and analyzed (ATAGO 2016). The refractometer Pocket PAL 1 was calibrated with tap water. The values are averaged for each part of the plant (above and below corn cob). An overall average were calculated to get a value for the genotype of one plot. After measuring a sample the bench vise and the refractometer were cleaned with water before using it again.

The measurement was done twice each year for all plots and experiments. The first measurement took place around eight weeks before harvest, while the second measurement was done close to harvest (around one week before, until a few days earlier). After cutting the samples they were put into cooling boxes and squeezed during the following three days. The samples had been stored in a cooling chamber.

2.2.2 Stay-Green Characteristic (SPAD-method)

The stay-green characteristic means a high chlorophyll content of the leaves while the grain is already mature. The chlorophyll content is measured indirectly with Chlorophyllmeter SPAD 502 (Konica Minolta Optics, Inc. 2009). The SPAD-value is not directly the Chlorophyll content but is proportional to it (Konica Minolta Optics, Inc. 2009).

Ten plants were measured per plot, five per row. The leaf at the corn cob was taken, around 10 cm away from the connection between the leaf sheath and the leaf blade at the leaf blade (Figure III.2). An average was taken of all ten measured plants.

Figure III.2: Position of the Chlorophyllmeter SPAD 502 while measuring

The measurement was done several times during the season. In the middle of August the first measurement took place, around eight weeks before harvest. Weekly the SPAD-values were measured to see how the Chlorophyll content was changing during the season. The last measurement was done before harvest. Because of early frost, the last measurement in 2015 at the locations Einbeck and Göttingen was already in the middle of October. In 2016 the last measurement was already done at the beginning of October, because of a long drought stress in September and October at the location Einbeck. The location Göttingen was destroyed by a storm event in August 2016 and not usable for data collection anymore.

2.3 Population Structure and Linkage Disequilibrium

The analysis was done separately for each genepool, Dent and Flint. Based on the used SNPs for analysis, at first a distance matrix was calculated. The distance model was using a modified Euclidean distance (Atlassian Bitbucket 2014a), which is further used for a Principal coordinate Analysis (PCoA, multi-dimensional scaling). Furthermore a Kinship matrix (K) of proportion of shared SNP alleles was calculated between the lines for each genepool (Bradbury et al. 2007, Endelman and Jannink 2012, Strigens et al. 2013). Population structure was estimated with the principal coordinate analysis. Correction for population structure in the genome wide association mapping was done with help of the resulting principal coordinate matrix (Q) (Strigens et al. 2013).

The population structure can be influenced by extent and decay of linkage disequilibrium (Yan et al. 2011, Pasam et al. 2012, Strigens et al. 2013). The linkage disequilibrium was calculated as squared allele frequency correlation (r^2) between pairs of loci for each chromosome in each genepool separately. As threshold was a level of 0.1 of r^2 used. Below this level, the linkage disequilibrium was considered as non-significant (Zhu et al. 2008, Strigens et al. 2013)

2.4 Statistical Analysis

For the statistical analysis the software PlabStat (Plant Breeding Statistical program, Version 3A) was used (Utz 2011). The experiments have been analyzed at first as a lattice design for each year and each location separately, including all checkvarieties.

The standard error of the genetical correlation coefficients was calculated after Mode and Robinson 1959. Here the checkvarieties are not included. For the single environments the experimental error is calculated with help of the lattice analysis. Also the means of the different experiments are calculated with the lattice analysis. The experimental errors as well as the calculated means are taken for further ANOVA analysis.

To analyzed the sugar content of the stover (BRIX), the following statistical model was used:

$$x_{ijk} = \mu + y_k + l_i + yl_{ki} + g_j + lg_{ij} + gy_{jk} + gly_{jik} + m_{ijk}$$

x_{ijk} = observation value of genotype j in location i and year k
μ = general mean
y_k= effect of year k
l_i = effect of location i
yl_{ki}= interaction between year k and location i
g_j = effect of genotype j
lg_{ij} = interaction between location i and genotype j
gy_{jk} = interaction between genotype j and year k
gly_{jik} = interaction between genotype j, location I and year k
m_{ijk} = experimental error, estimated from lattice analysis of single locations

Equation III.1

For analyzing the chlorophyll content of leaves (SPAD) the following statistical model was used:

$$x_{ij} = \mu + e_i + g_j + eg_{ij} + m_{ij}$$

x_{ij} = observation value of the genotype j in environment i
μ = general mean
e_i= effect of environment i
g_j= effect of genotype j
eg_{ij}= interaction between environment i and genotype j
m_{ij}= experimental error, estimated from lattice analysis of single environments

Equation III.2

The heritability was calculated with the following equation (Falconer and Mackay 2009) for all traits:

$$h^2 = \frac{\sigma_g^2}{\sigma_P^2} = \frac{\sigma_g^2}{(\sigma_g^2 + \left(\frac{\sigma_{ge}^2}{e}\right) + \left(\frac{\sigma_m^2}{er}\right)}$$

h^2 = heritability
σ^2_g = genotypic variance of the average
σ^2_P=phenotypic variance of the average
σ^2_{ge}=variance of the genotype-environment interaction
σ^2_m=variance of error
e = number of environments
r = number of replications

Equation III.3

2.5 Genome-Wide Association Mapping

The genome-wide association mapping was performed for sugar content in the stover (BRIX) and chlorophyll content in the leaves (SPAD) with the calculated means across all environments, to identify significant associations between SNPs and calculated means across all environments. The two genepools have been analyzed separately from each other. For analysis of the population structure between the genepools, both have been used. Correction for population structure was done first. To get more pedigree information, a UPGMA Cladogram was conducted for Flint and Dent lines separately. The used population structure was based on the first five principal coordinates as fixed effects and to avoid disturbing associations. The first ten principal coordinates have been used because they explain most of the genetic variation and distances within the genepool and it avoids assigning genotypes to groups (Strigens et al. 2013). For the Dent genepool, the first ten principal coordinates explain 66 % of the genetic variation. For the Flint genepool the first ten principal coordinates explain 64 % of the genetic variation. Even though the principal coordinate analysis is a good way to identify outliers or subpopulations in the genetic material (Begum et al. 2015). The general linear model is corrected for population structure by the Q-Matrix analyzed by the principal coordinate analysis (PCoA) with multi-dimensional scaling (MDS). The PCoA was done with the software Tassel Version 5.0 (Trait Analysis by aSSociation, Evolution and Linkage) developed by Buckler Lab (Bradbury et al. 2007).

The kinship matrix, used for the mixed linear model, was considered as centered_IBS kinship. Therefore simple matching is used for a scaled matrix. The resulting kinship is giving a likelihood implementation for the genotypes (Atlassian Bitbucket 2016). The mixed linear model is also corrected population structure (Q-matrix) and kinship (K-matrix). The kinship was developed with the software Tassel Version 5.0 (Trait Analysis by aSSociation, Evolution and Linkage) developed by Buckler Lab (Bradbury et al. 2007).

To identify the genome-wide significant threshold two methods can be used. With the Bonferroni-method, the threshold is also at a significance level of 0.05. Caused by the fact, that the significance level is always the same, the power of the test is reduced (Bender et al. 2007). The second method is the so called false discovery rate (FDR) of Benjamini and Hochberg (1995) (Benjamini and Hochberg 1995). With the FDR the significance level is adjusted stepwise, depending on the p-values and number of total tests. In this study, a FDR of 0.2 was used to checkfor multiple testing and to identify significant marker-trait associations.

The FDR of the Dent lines for both traits was calculated with the following model:

$$FDR = \frac{(rank * 0.2)}{2672}$$

Rank= Number between 1 and 2672, depending on the p-value. Lowest p-value has rank 1, highest p-value gets rank 2672

Equation III.4

For the Flint lines, the following model was used:

$$FDR = \frac{(rank * 0.2)}{2629}$$

Rank= Number between 1 and 2629, depending on the p-value. Lowest p-value has rank 1, highest p-value gets rank 2629

Equation III.5

This is necessary because multiple tests are used (Bender et al. 2007). For the general linear model (GLM) and for the mixed linear model (MLM) the same FDR was used for both traits.

To evaluate the ability of the models, quantile-quantile (QQ)-plots have been used. This a standard methodology to assuming the uniform distribution of the p-values under the null-hypothesis and to indicate false positive signals of association. The observed p-values are plotted against the expected theoretical p-values (Riedelsheimer et al. 2012, Racedo et al. 2016). The analysis have been made with the software Tassel Version 5.0 (Trait Analysis by aSSociation, Evolution and Linkage) developed by Buckler Lab (Bradbury et al. 2007). The manhatten plots have been made with the software R Studio, while the QQplots are made with Tassel Version 5.0.

The genome-wide association mapping was done in TASSEL Version 5.0 (Trait Analysis by aSSociation, Evolution-and Linkage) developed in Bucker Lab (Bradbury et al. 2007). Two models have been performed, the general linear model and the mixed linear model. The general linear model is fixing the phenotypic and marker effects. It was controlling for population structure, with help of the generated Q-Matrix (Q_{10}) of the PCoA as a covariant, to avoid spurious associations. The mixed linear model (Q+K model) was also controlling for population structure with the Q_{10}-Matrix, as a covariant and used the generated kinship (K) as well to checkfor familial relatedness. The power of the mixed linear model is higher, compared the general linear model, because multiple levels of relatedness among individuals (Yu et al. 2006). Therefore significances identified with the general linear model are no longer observed in the mixed linear model. For the Dent genepool 2672 SNPs have been used for genome-wide association mapping, while for the Flint genepool 2629 SNPs have been used.

3. Results

3.1 Principal Coordinate Analysis of the two genepools

The principal coordinate analysis (PCoA) of the genotypic material was showing that there were two different genepools like expected, Flint and Dent. Figure III.3 is showing the PCoA of the two genepools.

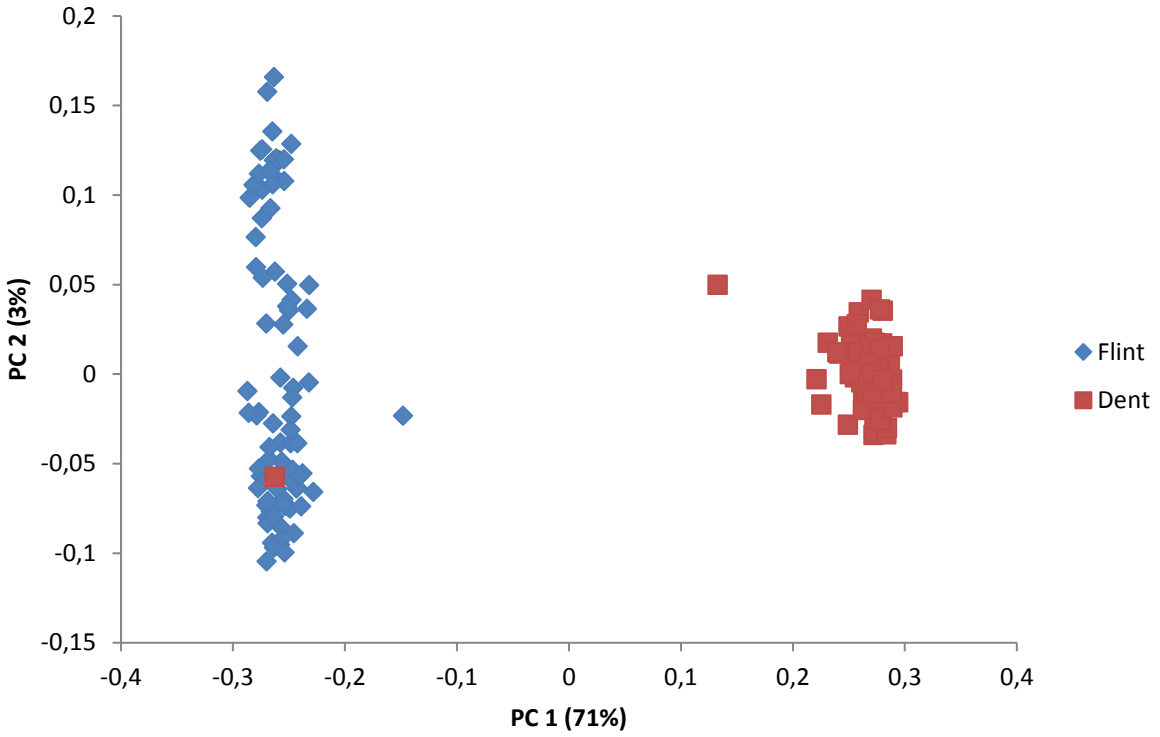

Figure III.3 Principal Coordinate Analysis within the two genepools, Dent and Flint. The first principal coordinate is explaining 71 % of the variation while the second principal coordinate is explaining 3 % of the variation

Comparing the two genepool, Flint was showing a greater spreading for the second principal component. Two, close related subpopulations were visible in the Flint genepool. The outlier was the genotype G14-155/10. The Dent genepool was much closer in its genetic distance and familial relatedness. It was containing outliers, G14-156/8 and G14-156/98 (Figure III.3). The outlier G14-156/98 was lying inside the Flint genepool. All outliers were exclude for the further analysis.

The first principal coordinate was explaining 71 %, and the second principal coordinate was explaining 3 % of the genetic variation and distance among the genepools. The first five principal coordinates were explaining 79 % of the total genetic variation and distance. This indicated that at least five principal coordinates are necessary to characterize the population structure (Table III.1).

Table III.1 Eigenvalues of the first five principal coordinates and its proportion of variance for both genepools

Principal coordinate	eigenvalue	Proportion of variance (%)
1	11.79	70.67
2	0.54	3.26
3	0.34	2.06
4	0.27	1.60
5	0.22	1.31

3.1.1 Dent Lines

The principal coordinate analysis (PCoA) of the Dent genepool was showing that the genetic variation and distance within the genepool was low (Figure III.4).

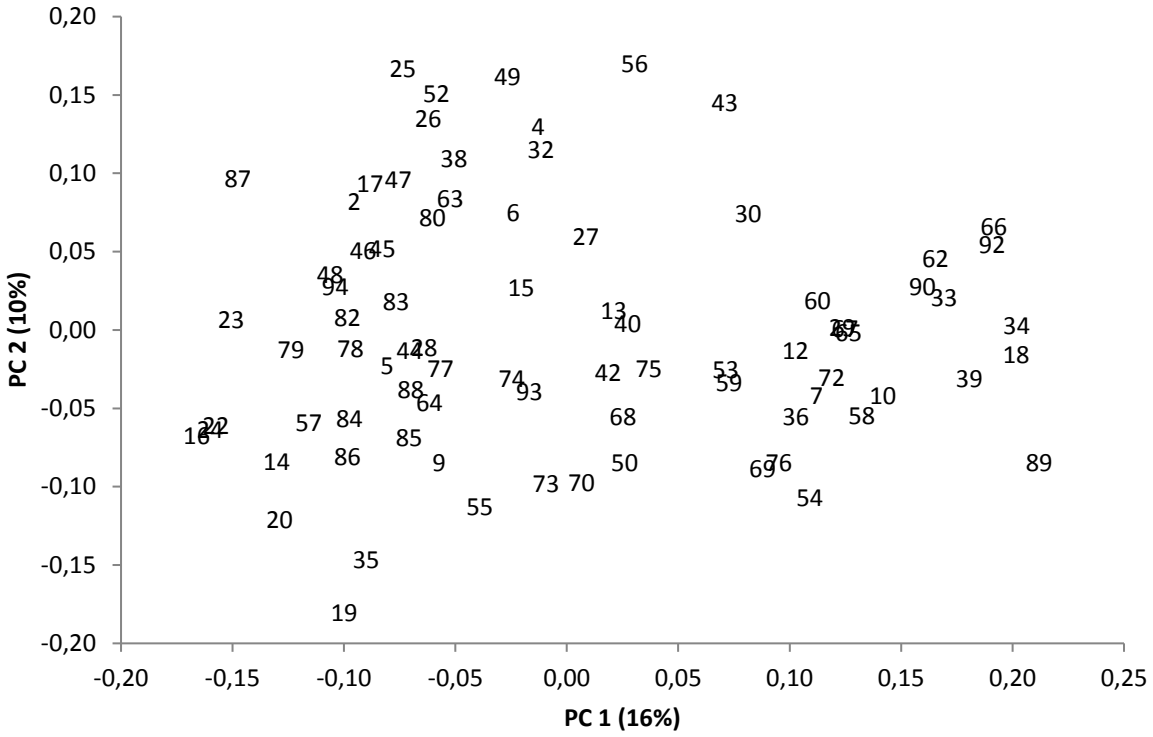

Figure III.4 Principal Coordinate Analysis for the Dent genepool. The first principal coordinate is explaining 18 % of the variation while the second principal coordinate is explaining 10 % of the genetic variation

The principal coordinates based on the Eucalien distance matrix were showing no separation of the genepool in several subpopulations. The first principal coordinate was explaining 18 % and the second principal coordinate explained 10 % of the genetic variation and distance within the genepool. In total the first ten principal coordinates explained 66 % of the genetic variation and distance. As Table III.2 showed are the first ten principal coordinates necessary to explain the population structure of the Dent genepool.

Table III.2 Eigenvalues of the first five principal coordinates and its proportion of variance of the Dent genepool

Principal coordinate	eigenvalue	Proportion of variance (%)
1	0.82	16.77
2	0.49	10.00
3	0.39	8.00
4	0.32	6.48
5	0.27	5.60
6	0.23	4.66
7	0.20	4.08
8	0.19	3.86
9	0.17	3.52
10	0.16	3.21

3.1.2 Flint Lines

The principal coordinate analysis (PCoA) of the Flint genepool was showing genetic variation and different parts of familial relatedness within the genepool. There were two subpopulations visible, that were closer related to each other, compared among the subpopulations. Several genotypes were outside the populations (Figure III.5), showing that their genetic distance was closer to the first or second subpopulation were the same to both.

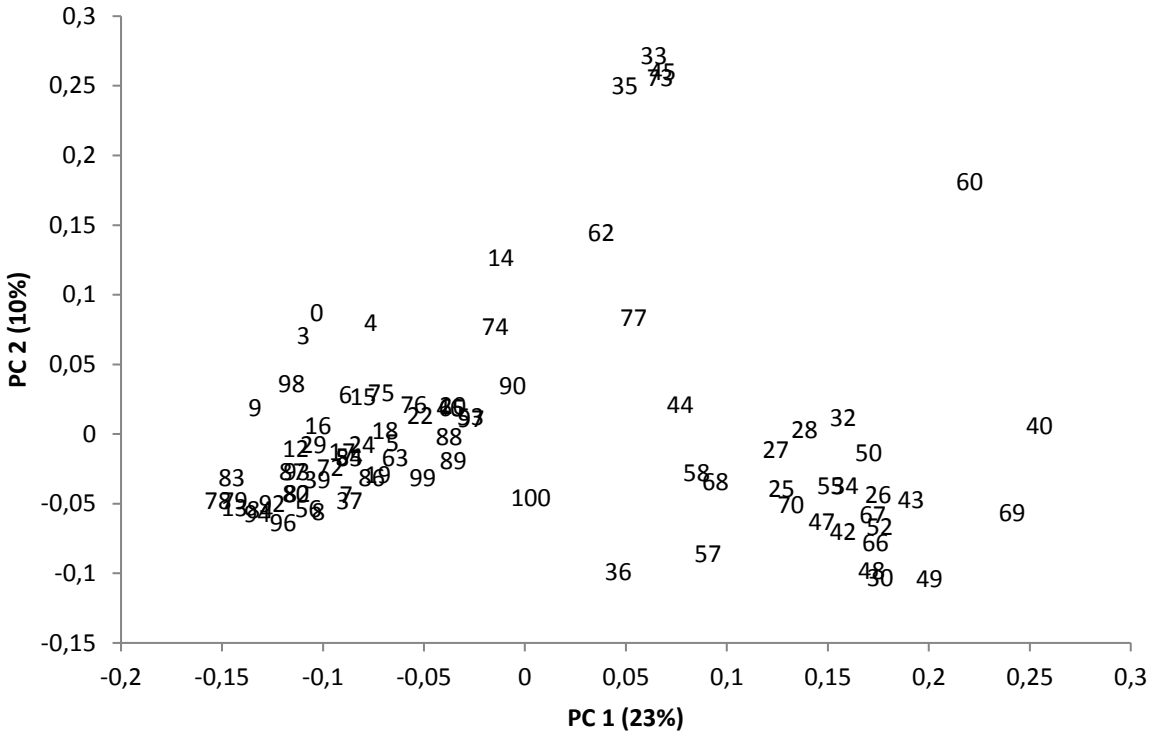

Figure III.5 Principal Coordinate Analysis for the Flint genepool. The first principal coordinate is explaining 22 % of the variation while the second principal coordinate is explaining 10 % of the genetic variation

The first principal coordinate was explaining 22 % and the second principal coordinate was explaining 10 % of the genetic variation and distance within the genepool. In total the first ten principal coordinates explained 65 % of the genetic variation and distance. As Table III.3 shows were the first ten principal coordinates necessary to explain the population structure of the Flint genepool.

Table III.3 Eigenvalues of the first five principal coordinates and its proportion of variance of the Flint genepool

Principal coordinate	eigenvalue	Proportion of variance (%)
1	1.41	22.68
2	0.51	10.15
3	0.32	6.35
4	0.27	5.43
5	0.24	4.77
6	0.20	4.00
7	0.18	3.63
8	0.16	3.24
9	0.16	3.14
10	0.14	2.72

3.2 Genome-wide Association mapping: sugar content in the stover (BRIX)

The BRIX measurement was done to analyzed the sugar content in the stover. With % BRIX the sucrose content in the measured sample was named.

For the genome-wide association mapping, the last measurement of BRIX, directly before harvest was taken. Furthermore was the BRIX given for the whole plant, no differentiation was made between above and below the corn cob.

3.2.1 Dent Lines

General Linear Model

The general linear model was done to analyze significant associations between SNPs and the trait BRIX. As the Q-Q-plot of expected vs. observed p-values (under a Gaussian distribution) is showing, was the model fitting well with the expected p-values (Figure III.6). The observed p-values were showing a normal distribution and were lying nearly exactly on the line of observed p-values. The outliers on the top of the line were showing significant associations, between SNP and BRIX.

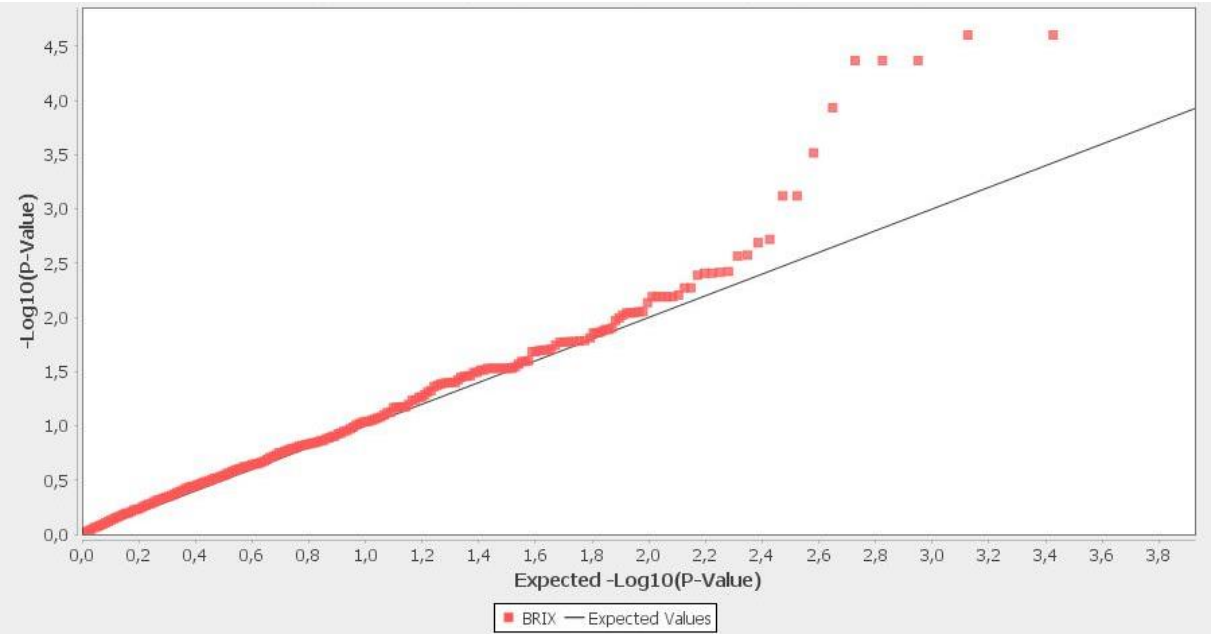

Figure III.6: Quantile-Quantile plot for the BRIX (sugar content of stover), comparing the observed p-values (-Log10(p-value)) with the expected p-values (-Log(10(p-value))

Figure III.7 is showing the associations between SNPs and the trait BRIX for each chromosome. Especially on chromosome 2 a peak was visible, at a low level of p-values. Another peak was found on chromosome 4, which was showing just one dot. Chromosome 3 ws also showing a peak, but at a lower level, compared to chromosome 2 and chromosome 4.

Chromosome 1, chromosome 8, chromosome 9 and chromosome 10 were showing no peaks. Here the associations were mainly in the second part of the chromosome, at a low p- level.

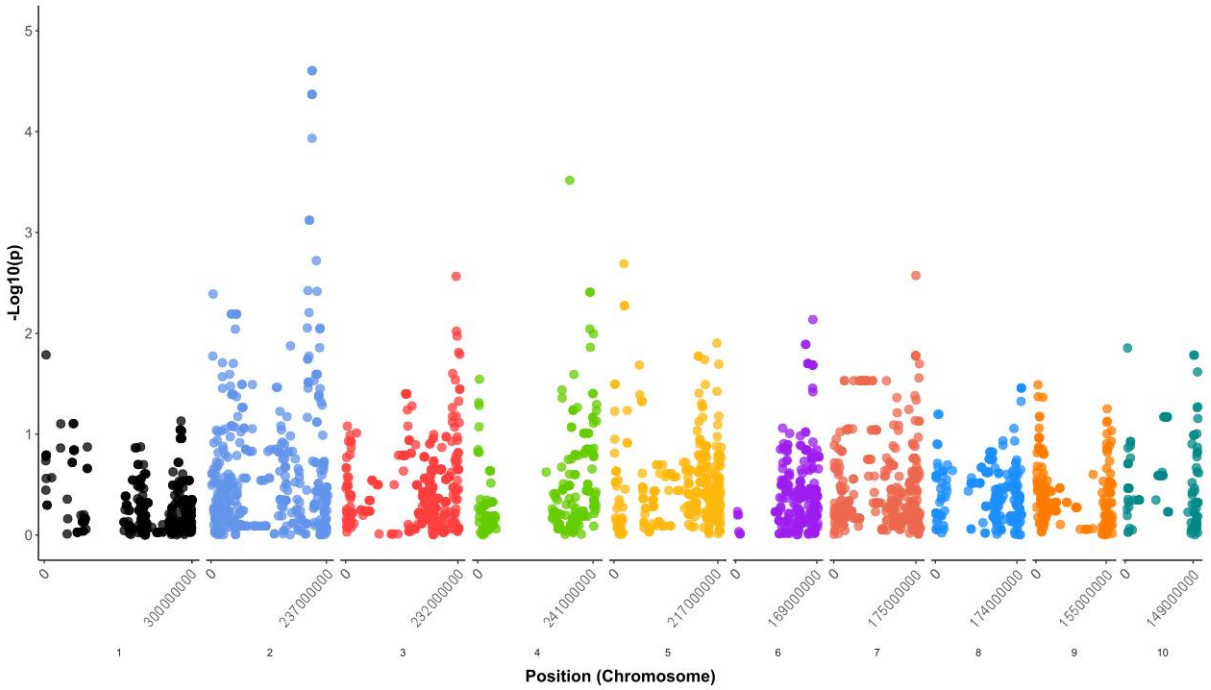

Figure III.7 (Manhattan Plot for BRIX (sugar content in stover), showing the observed p-values of the SNPs for each chromosome.

The general linear model identified seven significant associations between SNP and trait BRIX, with a false discovery rate of 20 %. Table III.4 is showing the marker with significant associations.

Table III.4 Significant Marker for the trait BRIX and their belonging chromosome, alleles, lines and effects

Marker	Chromosome	Position (bp)	Allele	Lines	Marker p-value	Allele Effect
SYN24153	2	205290868	A	42	0.0000248	0.6413
			G	39		0
SYN15092	2	205429390	A	39	0.0000248	-0.6413
			G	42		0
SYN5375	2	205085470	A	44	0.0000427	0.6230
			G	37		0
PZE-102157814	2	205138853	A	37	0.0000427	-0.6230
			C	44		0
SYN24149	2	205357748	A	37	0.0000427	-0.6230
			G	44		0
SYN12074	2	205144830	A	43	0.0001164	0.5781
			G	38		0
PZE-104110312	4	186766394	A	75	0.0003040	-0.9525
			G	5		0

Six significant associations of SNPs were found on chromosome 2. Depending on the marker different genotypes were possible. Five (SYN24153; SYN15092; SYN5375; SYN24149; SYN12074) of the six significant markers for chromosome 2 contained the genotypes AA and GG while the two genotypes were found nearly in the same rate between the lines. Only the marker PZE-102157814 contained the genotypes AA and CC. The allele effect of the markers on chromosome 2 was high, giving a high additive effect. The markers were showing same p-values and allele effects, indicating that the markers were probably linked with each other.

On chromosome 4 one significant association between marker and BRIX was found. The belonging genotypes were AA and GG, while most lines contain genotype AA. The allele effect was high, indicating an high additive effect.

Mixed Linear Model

As the quantile-quantile plot of expected vs. observed p-values (under a Gaussian distribution) for the mixed linear model was showing, are the observed p-values were lower than the expected p-values (Figure III.8). The model was correcting for the population structure and familial relatedness and was therefore showing a different Q-Q-plot. The model was as well-fitting well for the trait, even though the observed values were estimated lower.

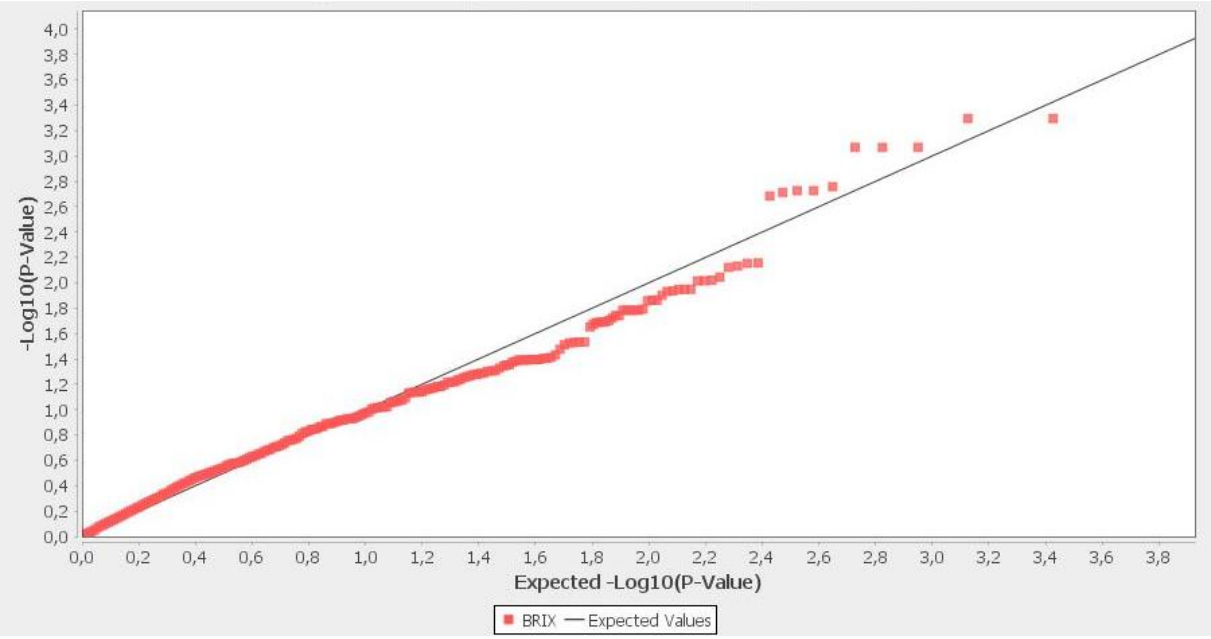

Figure III.8 Quantile-Quantile plot for the BRIX (sugar content of stover), comparing the observed p-values (-Log10(p-value)) with the expected p-values (-Log(10(p-value))

Associations between SNPs and BRIX are showing in Figure III.9 for each chromosome. Chromosome 2 was still showing the highest peak for the trait BRIX. Even though the markers, were not significant. Chromosome 4 was showing also a peak, at the same p-level than chromosome 3.

Chromosome 5 and chromosome 6 were showing small peaks, at a lower level compared to chromosome 3 and chromosome 4. Furthermore was chromosome 7 containing an outlier at the behind part. Chromosome 8, chromosome 9 and chromosome 10 were showing no peaks, but a range of associations on a high p-level, splitting at the front and back part of the chromosome.

The mixed linear model was identifying no significant associations between marker and trait, at a false discovery rate of 20 %.

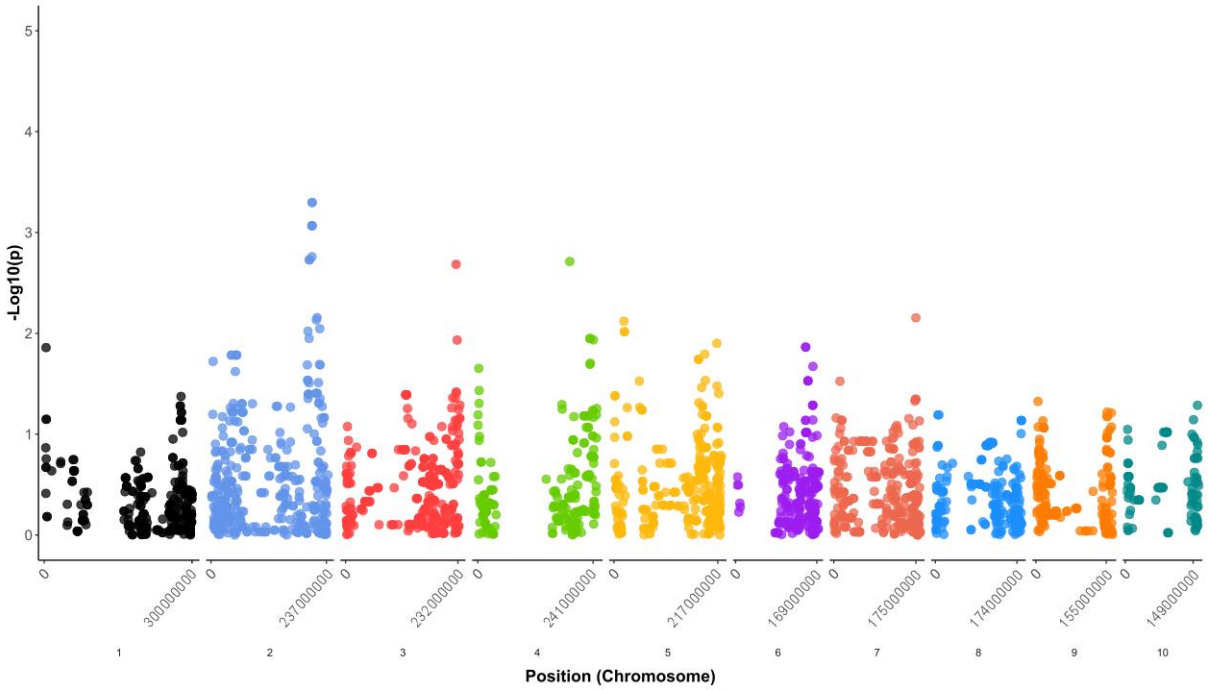

Figure III.9 Manhattan Plot for BRIX (sugar content in stover), showing the observed p-values of the SNPs for each chromosome

Comparing General Linear Model and Mixed Linear Model

Comparing the two models used for the same trait it was shown that the models were fitting both well for the observed values. The Q-Q-plot of expected vs. observed p-values (under a Gaussian distribution) for the general linear model was showing that the observed and expected p-values were a little higher compared to the expected ones. For the mixed linear model the Q-Q-plot of expected vs. observed p-values (under a Gaussian distribution) was showing that the observed p-values were lower than the expected p-values. The difference between the observed p-values and the expected p-values was for both models very small.

Comparing the Manhattan plots of the GLM and MLM with each other, they were looking very similar to each other. But small differences between the plots of the two models were visible.

The y-axis, which was showing the –Log10(p), was much shorter in Manhattan plot for the mixed linear model compared to the Manhattan plot of general linear model. Therefore the p-values were also higher for the mixed linear model compared to the general linear model. With the used FDR of 20 %, no significant markers were found in the mixed linear model, compared to the general linear model, where seven significant associations have been found.

Second, both plots were showing peaks on chromosome 2 and chromosome 4, while for the Manhattan plot of the mixed linear model another peak was found on chromosome 3, at a comparable level than the peak was found on chromosome 4. Comparing the similar peaks on chromosome 2 and chromosome 4 it was shown that the significant markers in the general linear model were also forming the peaks in the mixed linear model. As Table III.5 was showing, were the significant markers for the general linear model also having the lowest p-values in the mixed linear model. Furthermore were all markers on chromosome 2 showing an additive effects in the general linear model and in the mixed linear model.

Comparing the significant marker on chromosome 4, a high allele effect are shown in the general linear model. In the mixed linear model the allele effect are also the highest of all found significant associations.

Table III.5 Comparing significant markers for BRIX and their effects of the general linear model with the mixed linear model

Marker	Chromosome	Allele	General Linear Model		Mixed Linear Model	
			Marker p-value	Allele effect	Marker p-value	Allele effect
SYN24153	2	A G	0.0000248	0.6413 0	0.0005047	0.5909 0
SYN15092	2	A G	0.0000248	-0.6413 0	0.0005047	-0.5909
SYN5375	2	A G	0.0000427	0.6230 0	0.0008570	0.5683 0
PZE-102157814	2	A C	0.0000427	-0.6230 0	0.0008570	-0.5683 0
SYN24149	2	A G	0.0000427	-0.6230 0	0.0008570	-0.5683 0
SYN12074	2	A G	0.0001164	0.5781 0	0.0017400	0.5179 0
PZE-104110312	4	A G	0.0003040	-0.9525 0	0.0019400	-0.8712 0

The peak on chromosome 3 was also found in the general linear model, but much smaller compared to the peaks of chromosome 2 and chromosome 4. In the mixed linear model on chromosome 3 a peak was found, which was compared to chromosome 4 at a comparable level. Even though there were no significant markers found in the mixed linear model, the marker, forming the peak on chromosome 3 (PZE-103179207) had low p-value (0.0020700). In the general linear model the marker was showing a comparable p-value (0.00272).

Third, on chromosome 7 one small peak was shown in the general linear model. This seemed to be an outlier, which was found in the mixed linear model as well.

Forth, in the mixed linear model chromosome 1 was showing an outlier in the front part of the chromosome. This outlier was found in the general linear model as well, but not that obvious compared to the mixed linear model.

Comparing general linear model and mixed linear model with each other, it was shown that the differences between the models are very low. Just the p-values for the mixed linear model were much higher compared to the general linear model, lead to no significances for the model. On the other hand were the markers, showing associations with the trait BRIX were in both models the same.

3.2.2 Flint Lines

General Linear Model

Associations between SNPs and the trait BRIX have been identify with the general linear model. The Q-Q-plot of expected vs. observed p-values (under a Gaussian distribution) was showing, was the model fitting well. Most observed p-values were nearly the same compared with the expected p-values. Some of the p-values were lower compared to the expected p-values, but the differences was very low. One outlier was showing a probably significant association between BRIX and SNPs because it laid highly above the line of expected p-values (Figure III.10).

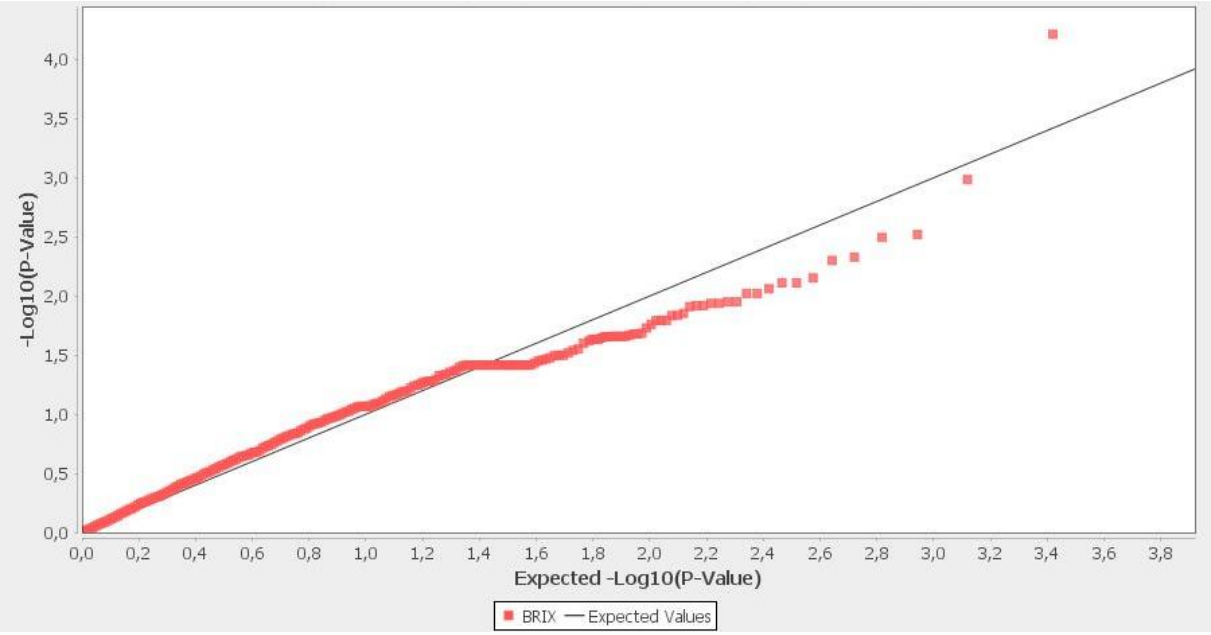

Figure III.10 Quantile-Quantile plot for the BRIX (sugar content of stover), comparing the observed p-values (-Log10(p-value)) with the expected p-values (-Log(10(p-value))

The Manhattan plot of the general linear model (Figure III.11) is showing the associations between SNPs and the trait BRIX for each chromosome. Especially on chromosome 1 an outlier was found in a low p-level. A belonging peak was also visible, but at a lower level, compared to the outlier.

A smaller peak was found on chromosome 10. Here the peak was at a low level, but the associations were forming a peak and not showing a wide range of associations at that level. On chromosome 10 itself were a lot of associations found.

All other chromosomes were showing a wide range of associations on a low level, with a higher impact on the behind part of the chromosome (Figure III.11).

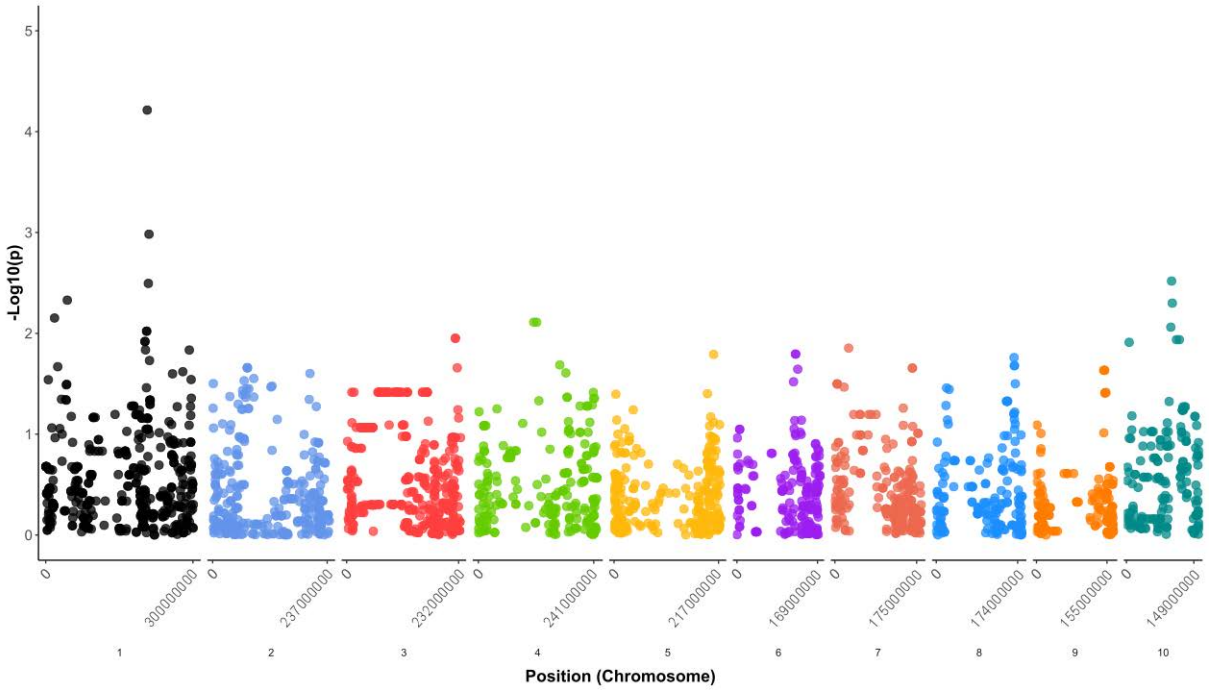

Figure III.11 Manhattan Plot for BRIX (sugar content in stover), showing the observed p-values of the SNPs for each chromosome

With the general linear model one significant associations between SNPs and BRIX have been identified, with a false discovery rate of 20 %. Chromosome 1 was containing the significant marker with a low p-value and a high allele effect (Table III.6).

Table III.6 Significant Marker for the trait BRIX and its belonging chromosome, alleles, lines and effects

Marker	Chromosome	Position (bp)	Allele	Lines	Marker p-value	Allele Effect
PZE-101163539	1	206839486	A	38	0.0000611	0.6142
			G	45		0

The corresponding genotypes were AA and GG, while more lines contained the genotype GG for the marker. The marker was showing a high allele effect effects (Table III.6).

Mixed Linear Model

For the mixed linear model, the quantile-quantile plot of expected vs. observed p-values (under a Gaussian distribution) was showing, the observed p-values are fitting with the expected p-values for a low level. When the p-values were increasing the observed p-values are lying under the line of expected p-values. Even though there was an outlier, laid nearly as high as the expected p-value at a high level (Figure III.12).

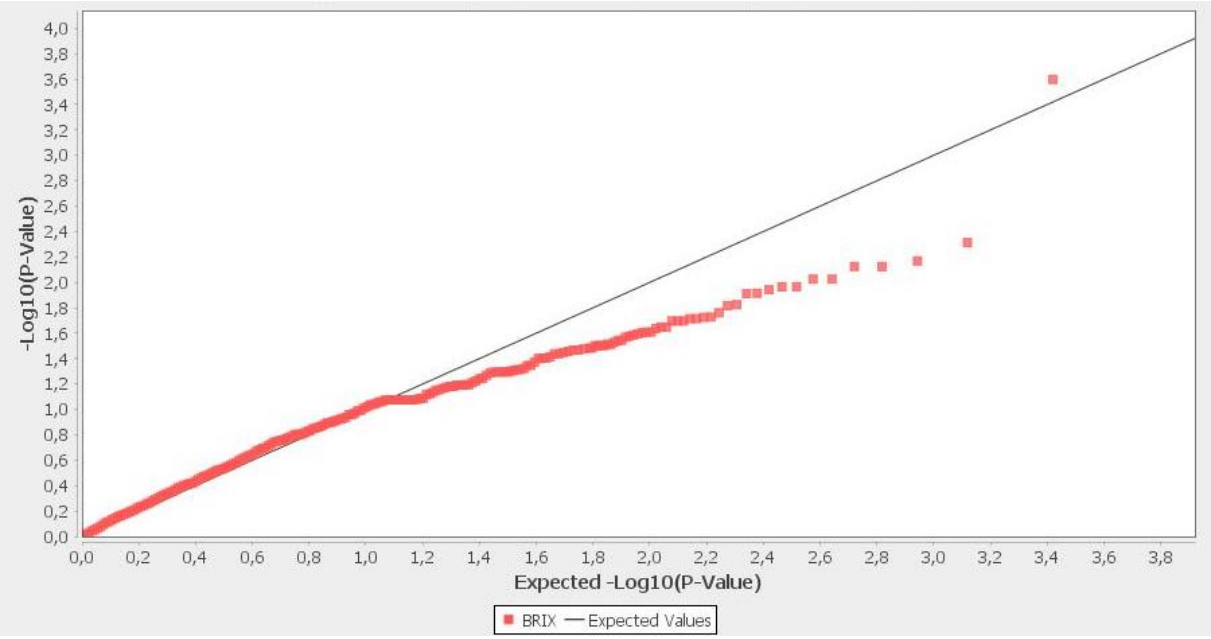

Figure III.12 Quantile-Quantile plot for the BRIX (sugar content of stover), comparing the observed p-values (-Log10(p-value)) with the expected p-values (-Log(10(p-value))

The Manhattan plot for the mixed linear model was showing that chromosome 1 was containing an outlier (Figure III.13) with the lowest p-value. Even though the outlier was not significant.

For chromosome 10 also small peak at a much lower level was found while chromosome 7 was also showing one outlier at a low level (Figure III.13). All other chromosome were showing a wide range of associations at a high p-level. For the mixed linear model no significant associations were found.

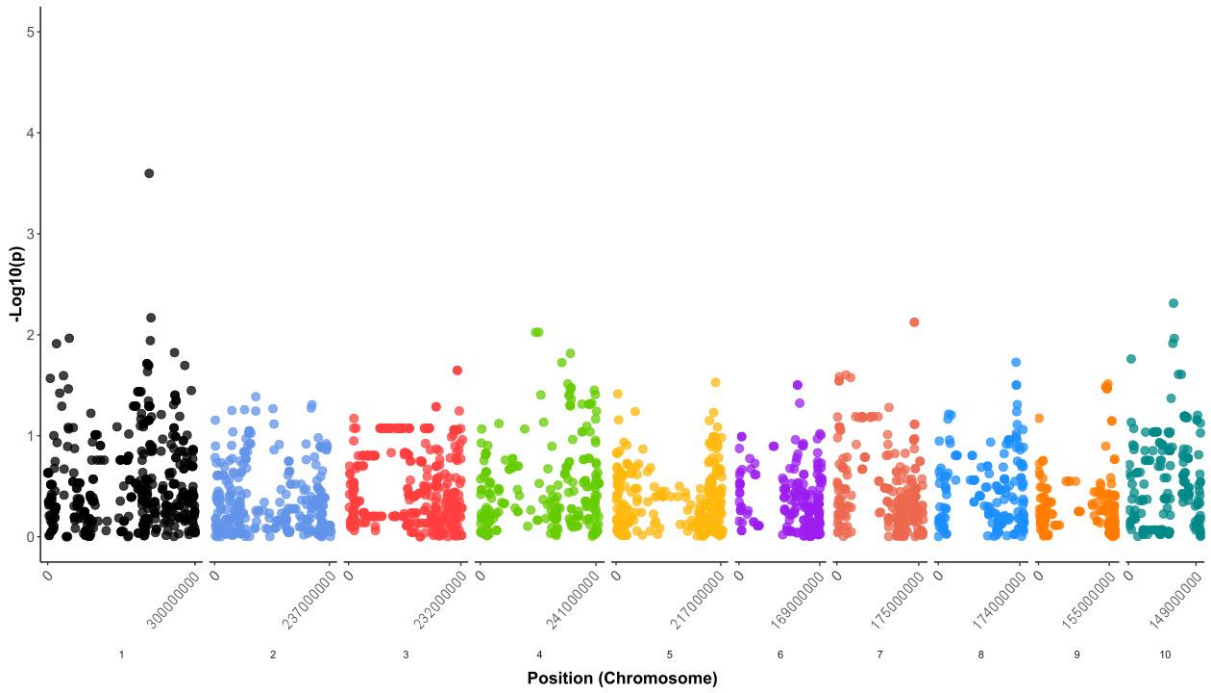

Figure III.13 Manhattan Plot for BRIX (sugar content in stover), showing the observed p-values of the SNPs for each chromosome

Comparing General Linear Model and Mixed Linear Model

While comparing the general linear model and the mixed linear model with each other small differences between the models were visible. At first the Q-Q-plot of expected vs. observed p-values (under a Gaussian distribution) for the general linear model and mixed linear model were compared with each other. Both plots were showing that the models are fitting well. The Q-Q-plot of expected vs. observed p-values (under a Gaussian distribution) for the general linear model were showing that the observed p-values are closer to the expected p-values-line compared to the mixed linear model. Furthermore were all outliers in the general linear model closer to the expected p-values than for the mixed linear model.

Comparing the Manhattan plots for the general linear model and mixed linear model, differences were not obvious. For the mixed linear model, the y-axis of the Manhattan plot was shorter, compared to the general linear model, therefore the –Log10(p)values were higher overall in the mixed linear model, compared to the general linear model. On chromosome 1, there was an outlier found in the general linear model. This marker was also found in the mixed linear model, as an outlier. With the used FDR of 20 % no significant markers were identified in both models. But in the general linear model one marker (PZE-101163539) significant for the trait BRIX. This marker was also found in the mixed linear model, with having the lowest p-value (Table III.7). The analyzed allele effect was nearly the same in both models.

Table III.7 Comparing marker, showing a tendency for significance, for BRIX and its effects analyzed in the general linear model with the mixed linear model

Marker	Chromosome	Allele	General Linear Model		Mixed Linear Model	
			Marker p-value	Allele effect	Marker p-value	Allele effect
PZE-101163539	1	A G	0.0000611	0.6142 0	0.0002523	0.6114 0

Another similarity was found on chromosome 10. Here in both plots a second peak was found, but much smaller in the mixed linear model compared to the general linear model.

Differences were found on chromosome 7. For the general linear model no peak was found on chromosome 7, while a peak was visible in the mixed linear model for the same chromosome.

All other chromosome were showing the same wide range of associations at a low level in the general linear model and in the mixed linear model.

Comparing general linear model and mixed linear model with each other, it was shown that the differences between the models were very small. The p-values in the mixed linear model were lower, compared to the general linear model, lead to a more clinched Manhattan plot for the mixed linear model. Significant markers in the general linear model were also showing the lowest p-value in the mixed linear model, even though it was not significant anymore at a FDR of 20 %.

3.3 Comparing Genome-wide Association Mapping of sugar content in the stover (BRIX) between Dent Lines and Flint Lines

The genome-wide association mapping for the trait sugar content in the stover (BRIX) of the two genepools Dent and Flint were showing different results. Five significant associations between SNPs and trait BRIX have been identified for the Dent lines, while the Flint lines were showing one SNP, which has a tendency for significance.

Comparing the two models for both lines, it was shown that the general linear model for the Dent lines is fitting best, while the mixed linear model for the Dent lines was showing that the observed p-values are lower than the expected p-values. The Flint lines were showing in similar Q-Q-plots for general linear model and mixed linear model, also the general linear model was fitting a better, compared to the mixed linear model. Still, were the observed p-values lower than the expected p-values, for both models. It was shown that for both genepools the general linear model is fitting better, compared to the mixed linear model.

The Manhattan plots of general linear model and mixed linear model of Dent lines and Flint lines were showing similar results, even though were the mixed linear models, correcting for population structure and familial relatedness had higher p-values compared to the general linear models. Significant associations or associations in the general linear models were found in the mixed linear models, as associations showed a tendency for significance.

Significant associations between SNPs and the trait BRIX were found for the Dent lines in the general linear model. The mixed linear model was supporting this findings, even though no significant associations were found. The found associations were on chromosome 2 and chromosome 4.

Table III.8 was showing the significant associations between SNPs and BRIX identified with the general linear model of the Dent lines compared with the result of the analysis of the Flint lines. As shown, just the marker SYN 15092, on chromosome 2, was also used for the general linear model of the Flint lines. Comparing it with the Flint lines, the marker was showing a high, not significant p-value while its allele effect is low, compared to the Dent lines. The six other markers (SYN 24153, SYN 5375, PZE-102157814, SYN 24149, SYN24149, PZE-104110312) were filtered out for the analysis of the Flint lines.

For the Flint lines one significant associations was found on chromosome 1 in the general linear model. The mixed linear model was supporting this finding, by showing a tendency for significance for this marker.

The significant SNP, PZE-101163539, identified in the Flint lines on chromosome 1 is compared to the corresponding analysis of the Dent lines in Table III.9. For the Dent lines, the marker was shown a high p-value, as a low allele effect compared with the Flint liens.

The results of Dent lines and Flint lines for associations between SNPs and the trait BRIX are not comparable. This indicates that the different genepools were containing different genes, responsible for the trait sugar content in the stover (BRIX).

Table III.8 Comparing Markers identified with the general linear model for the Dent lines with corresponding anaylsis of the Flint lines for the trait BRIX

Line	Marker	Chromosome	Position (bp)	Allel	Marker p-value	Allel effect	Line	Marker	Chromosome	Position (bp)	Allel	Marker p-value	Allel effect
Dent	**SYN24153**	2	205290868	A G	0.0000248	0.6413 0	**Flint**	**SYN24153**	NaN	NaN	NaN	NaN	NaN
Dent	**SYN15092**	2	205429390	A G	0.0000248	-0.6413 0	**Flint**	**SYN15092**	2	205429390	A G	0.4898	0.096 1 0
Dent	**SYN5375**	2	205085470	A G	0.0000427	0.6230 0	**Flint**	**SYN5375**	NaN	NaN	NaN	NaN	NaN
Dent	**PZE-102157814**	2	205138853	A C	0.0000427	-0.6230 0	**Flint**	**PZE-102157814**	NaN	NaN	NaN	NaN	NaN
Dent	**SYN24149**	2	205357748	A G	0.0000427	-0.6230 0	**Flint**	**SYN24149**	NaN	NaN	NaN	NaN	NaN
Dent	**SYN12074**	2	205144830	A G	0.0001164	0.5781 0	**Flint**	**SYN12074**	NaN	NaN	NaN	NaN	NaN
Dent	**PZE-104110312**	4	186766394	A G	0.0003040	-0.9525 0	**Flint**	**PZE-104110312**	NaN	NaN	NaN	NaN	NaN

The significance level is given by a false discovery rate of 20 %.

Table III.9 Comparing Markers identified in the general linear model for the Flint Lines with the corresponding analysis of the Dent lines for the trait BRIX

Line	Marker	Chromosome	Position (bp)	Allel	Marker p-value	Allel effect	Line	Marker	Chromosome	Position (bp)	Allel	Marker p-value	Allel effect
Flint	**PZE-101163539**	1	206839486	A G	0.0000611	0.6142 0	**Dent**	**PZE-101163539**	1	206839486	A G	0.74999	-0.0705 0

The significance level is given by a false discovery rate of 20 %.

3.4 Candidate Genes for the sugar content in the stover (BRIX)

The sugar content in the stover of maize was not of interest during the last years. Genetic studies to identify QTLs or genome-wide association mapping for the trait BRIX are few done. Recently Bian et al. studied sugar-related traits in populations of recombinant inbred lines of maize, to identify QTLs for stover sugar content and to map dynamic QTLs of stover sugar content in different growth stages of maize (Bian et al. 2014, Bian et al. 2015).

Based on 202 recombinant inbred lines (RILs, $F_{7:8}$) developed by a single seed descent method from a cross between YXD503, with a high sugar content, and Y6-1, with a low sugar content, and 200 SSR and 12 ALPF marker, Bian et al. (2014) identified QTLs on chromosome 1, chromosome 2, chromosome 6 and chromosome 9. Especially the QTLs found on chromosome 1, chromosome 2 and chromosome 9 had positive additive effects on the sugar content of the stover. The suggestion that the parent YXD053, with a high sugar content, may made a higher contribute to the BRIX alleles, compared to the second parent Y6-1. The QTL for an increased BRIX content, found on chromosome 6, on the other hand, was contributed by the parent Y6-1 (Bian et al. 2014). During the study, Bian et al. (2014/2015) identified one major QTL on chromosome 2, qSSC-2.1 (Bian et al. 2014, Bian et al. 2015). In the second study of Bian et al. (2015), QTLs for BRIX were identified at different growth stages of maize. Bian et al. separated the found QTL in conditional QTLs, which are referring the cumulative effects of QTLs from time t-1 to time t, and unconditional QTLs, indicating cumulative effets of QTLs from the intital time to time t (Bian et al. 2015). During the study in total 21 unconditional QTLs have been mapped, where eight out of 21 are found on chromosome 2 and three out of 21 are found on chromosome 1. Furthermore were chromosome 9 (3/21) and chromosome 6 (2/21) containing unconditional QTLs. Analyzing conditional QTLs at different growth stages, Bian et al. (2015) showed that around half of the found conditional QTLs were already mapped in within the unconditional QTLs (Bian et al. 2015). Moreover the major QTL, qSSC-2.1 on chromosome 2 was supported and found in the unconditional and conditional method to identify QTLs (Bian et al. 2015).

Comparing those results with the results of the genome-wide association mapping in this study, it is shown that significant associations on chromosome 2 in the Dent lines were found. All SNPs were showing high allele effects and low p-values in the general linear model and mixed linear model. Those markers are pointing on the major QLT, Bian et al. (2014/2015) found in his studies. The fact, that the mixed linear model was supporting the findings of the general linear model was also showing that the Dent lines were containing associations between markers and BRIX. An unconditional QTL and a conditional QTL was found on chromosome 4 as well (Bian et al. 2015). During the genome-wide association mapping, a significant associations was found on chromosome 4, probably pointing on those QTLs. For chromosome 1, chromosome 6 and chromosome 9 no associations were found, in the Dent lines.

For the Flint lines, on chromosome 1 a SNP was found, showing a tendency for significance in general linear model. Bian et al. (2015) found conditional QTLs and unconditional QTLs on chromosome 1 as well (Bian et al. 2015). No more associations have been found in the Flint lines. The small peak found on chromosome 10 was indicating an unconditional QTL, Bian et al. (2015) found. This was supporting the idea of that the BRIX varies during the different growth states and that genes controlling BRIX were selectively expressed at different growth states. The accumulation of maize stover sugar content was simultaneously controlled by major genes and polygenes (Bian et al. 2015).

3.5 Genome-wide Association mapping: stay-green behavior (SPAD)

The SPAD measurement was done to identify the stay-green behavior of the genotypes. The SPAD measurement was giving an idea about the chlorophyll content in the leaves because the SPAD-value und the chlorophyll content in the leaves were closely correlated with each other.

For the genome-wide association mapping, the first measurement of SPAD, in the middle of August and the last measurement of SPAD, directly before harvest was taken.

3.5.1 Dent Lines

General Linear Model

The general linear model was done to analyze significant associations between SNPs and the traits SPAD 1 (first measurement) and SPAD 8 (last measurement). As the Q-Q-plot of expected vs. observed p-values (under a Gaussian distribution) was showing, was there a difference between the two traits and fitting of the model (Figure III.14).The observed p-values for the trait SPAD 1 were higher compared to the expected p-values. Therefore some overestimations are possible. For the trait SPAD 8 the observed p-values were fitting well the expected p-values.

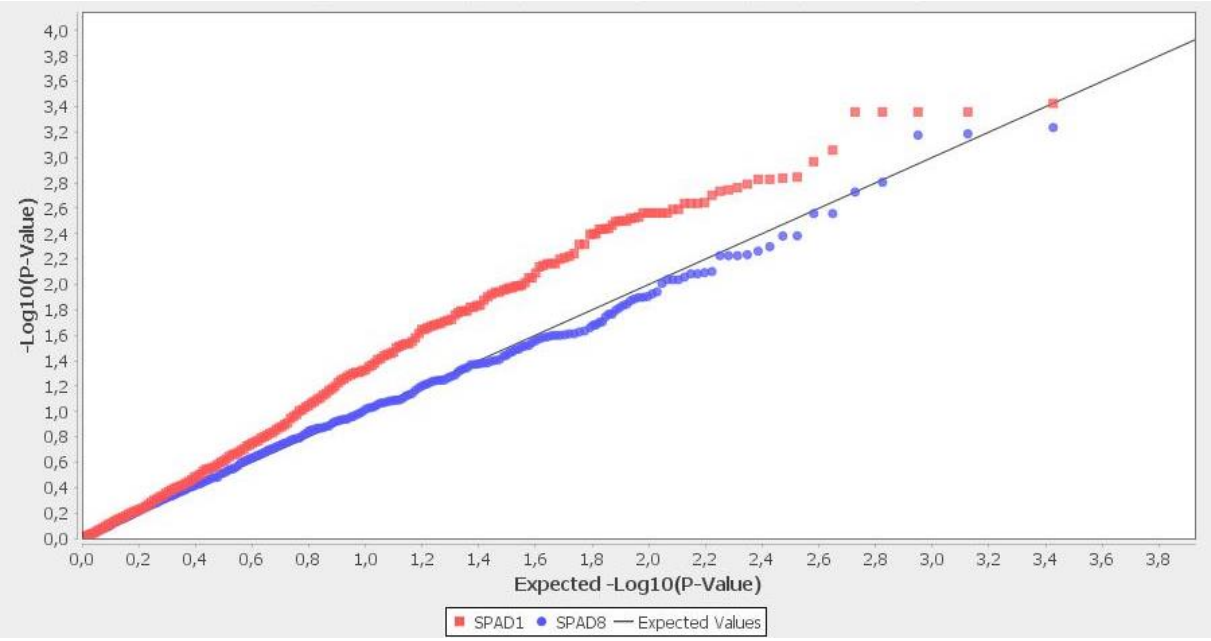

Figure III.14 Quantile-Quantile plot for SPAD1 (first measurement) and SPAD8 (last measurement), comparing the observed p-values (-Log10(p-value)) with the expected p-values (-Log(10(p-value))

The Manhattan plot of the general linear model was showing the associations between SNPs and the trait SPAD1 for each chromosome (Figure III.15). On chromosome 2, chromosome 3 and chromosome 7 a peak was shown. Furthermore were small peaks found on chromosome 5, chromosome 6, chromosome 8, chromosome 9 and chromosome 10.

Only chromosome 1 and chromosome 4 contained nearly no peaks, but showed a bride range of associations at a low level.

Chromosome 2, which was having also a peak in the ahead part of the chromosome was also showing the most associations, in a wide range on the chromosome, while the associations on the

other chromosomes were mostly in the ahead part or the behind part, but with a lower cover in the middle part.

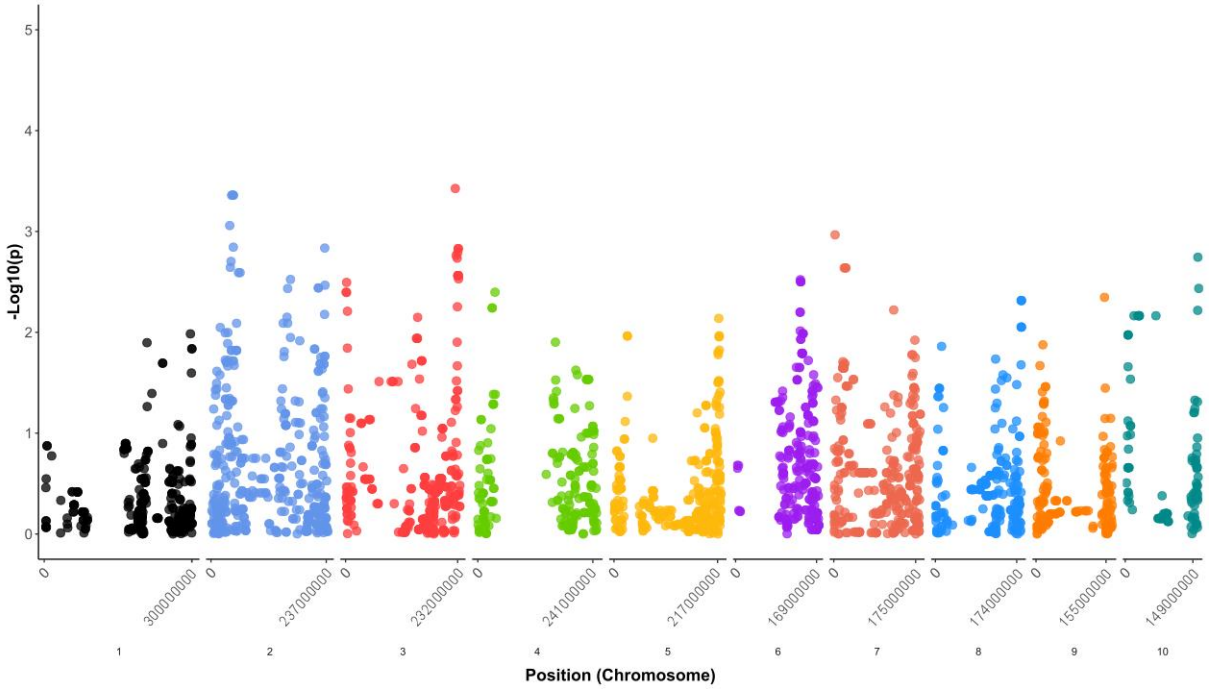

Figure III.15 Manhattan Plot for SPAD 1 (first measurement), showing the observed p-values of the SNPs for each chromosome

The general linear model identified no significant associations between SNP and trait SPAD1, with a false discovery rate of 20 %. The two SNPs, forming the peaks on chromosome 2 and chromosome 3 were showing a tendency for significance. Table III.10 was showing the SNPs and their belonging facts.

Table III.10 Markers, showing a tendency for significance, for the trait SPAD1 and its belonging chromosome, alleles, lines and effects

Marker	Chromosome	Position (bp)	Allele	Lines	Marker p-value	Allele Effect
SYN34350	3	222837682	A C	61 20	0.0003749	-1.1722 0
PZE-102062746	2	41853032	A G	25 56	0.0004368	1.3172 0

One association of SNPs was found on chromosome 2. The belonging genotypes were AA and GG, while most lines (56) were containing genotypes GG. The allele effect was very high, with a low p-value of the marker. The second associations, shown a tendency for significant was found on chromosome 3. Here the belonging genotypes were AA and CC, while most lines were observed with the genotype AA. The marker p-values was the lowest of the whole model and the allele effect was high (Table III.10).

The Manhattan plot of the general linear model for the associations between SNPs and SPAD8 for each chromosome (Figure III.16) was looking different compared to the Manhattan plot of SPAD1. On chromosome 8 a peak was shown. On chromosome 2 a second peak was found, formed by one outlier. Chromosome 5, chromosome 6 and chromosome 7 were also showing small peaks at the behind part of the chromosome. All other chromosome were having no peak. Chromosome 10 was

showing a only a very small amount of associations, at a low level. Chromosome 1 and chromosome 4 were showing a wide range of associations on the ahead and behind part of the chromosome.

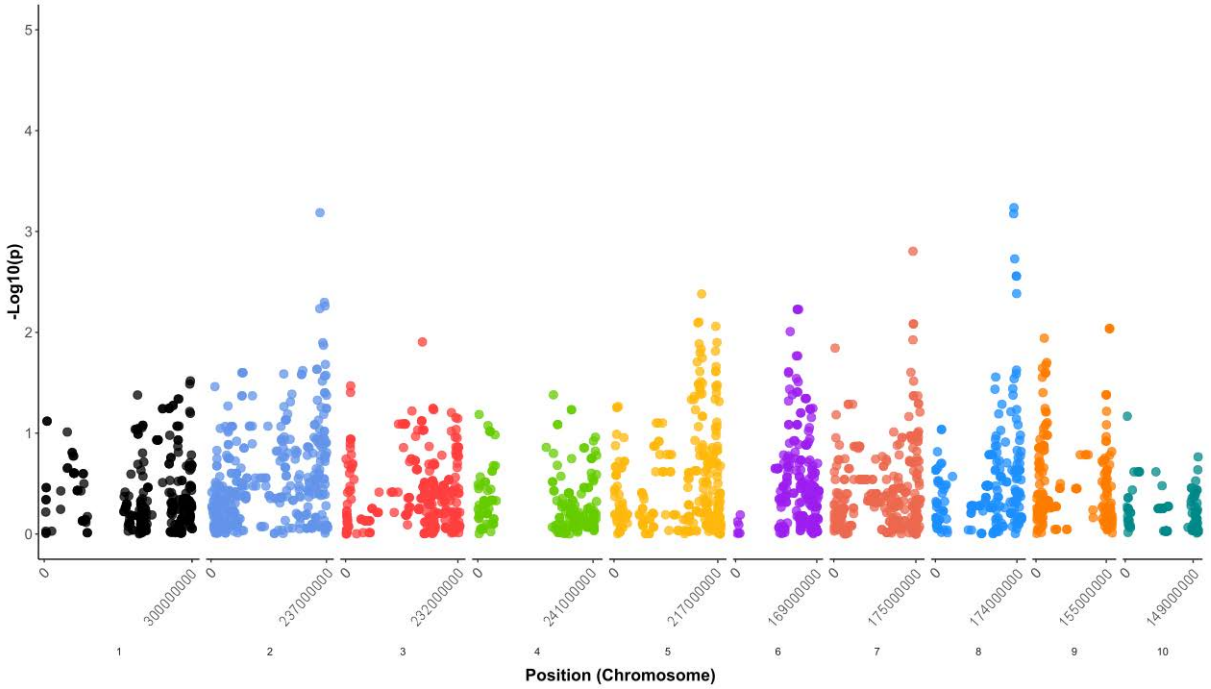

Figure III.16 Manhattan Plot for SPAD 8 (last measurement), showing the observed p-values of the SNPs for each chromosome

For the trait SPAD 8 no significant associations were identified. Even though the markers, forming the peak on chromosome 8 and chromosome 2 were showing a tendency for significance , with having the lowest p-values of all markers (Table III.11).

Table III.11 Markers, showing a tendency for significance for the trait SPAD 8 and their belonging chromosome, alleles, lines and effects

Marker	Chromosome	Position (bp)	Allele	Lines	Marker p-value	Allele Effect
PZE-108105381	8	159526711	A G	7 74	0.0005802	4.44106 0
PZE-102178194	2	221433785	A G	57 24	0.0006504	-3.0215 0
PZE-108104106	8	158942170	A G	9 72	0.0006669	3.8325 0

The corresponding genotypes were AA and GG, for all three markers. For the two markers found on chromosome 8 (PZE-108105381, PZE- 108104106) most lines were observed with the genotype GG while for the identified marker on chromosome 2 most lines contained the genotype AA. For all markers was the allele effect high, with a low p-value.

Mixed Linear Model

For analyzing significant associations between SNPs and traits SPAD 1 (first measurement) and SPAD 8 (last measurement), while taking familial relatedness and population structure into account, a mixed linear model was done. The Q-Q-plot of expected vs. observed p-values (under a Gaussian distribution) was showing, that both traits are have lower observed p-values than expected (Figure III.17).

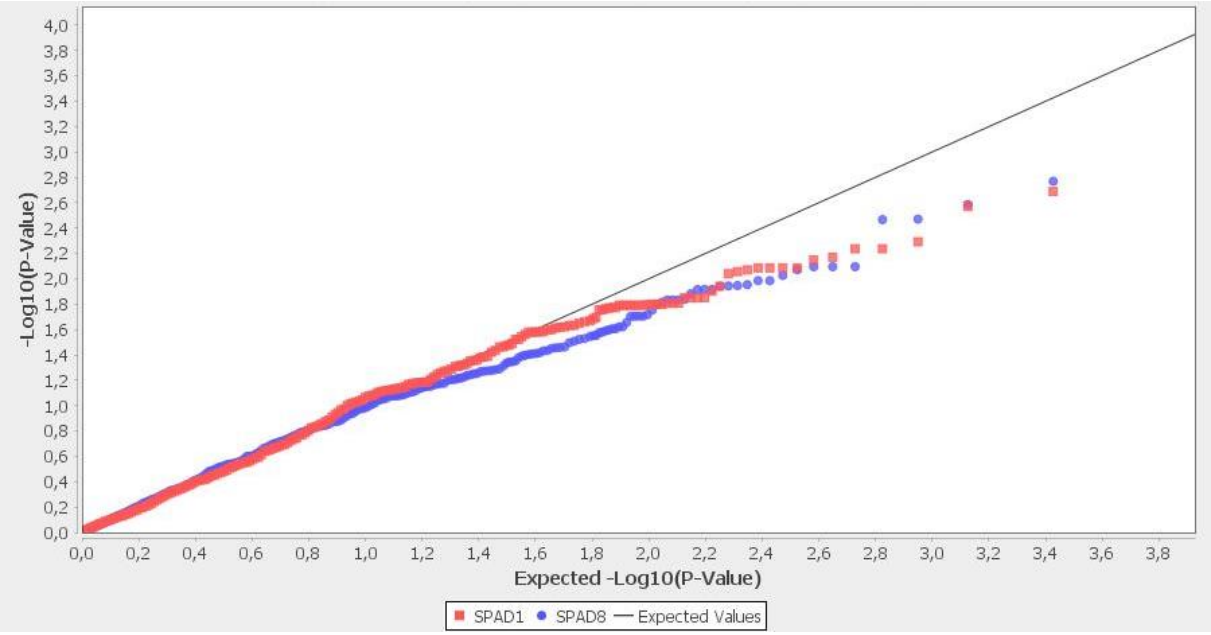

Figure III.17 Quantile-Quantile plot for SPAD 1 (first measurement) and SPAD 8 (last measurement), comparing the observed p-values (-Log10(p-value)) with the expected p-values (-Log(10(p-value))

Associations between SNPs and the trait SPAD 1 were found on all chromosomes. As the Manhattan plot of the mixed linear model was showing, chromosome 3, chromosome 9 and chromosome 10 are having the highest peaks (Figure III.18).

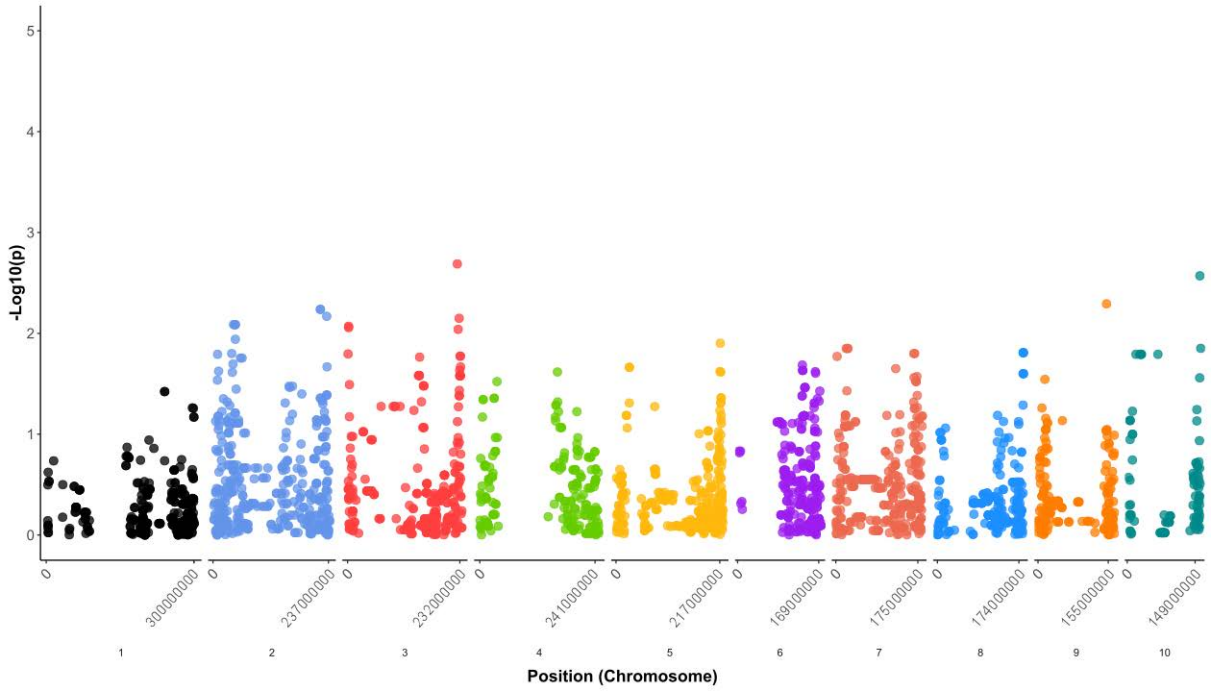

Figure III.18 Manhattan Plot for SPAD 1 (first measurement), showing the observed p-values of the SNPs for each chromosome

Chromosome 2, chromosome 5 and chromosome 6 were showing smaller peaks as well, while chromosome 1 and chromosome 4 were having a wide range of associations on a low level, especially in the behind part of the chromosome. For the trait SPAD 1 no significant associations were found. The p-values analyzed in the model were very high for all markers.

The trait SPAD 8 and its associations with the SNPs are shown in Figure III.19. Comparing the plots of SPAD 1 with the plot of SPAD 8 differences were seen.

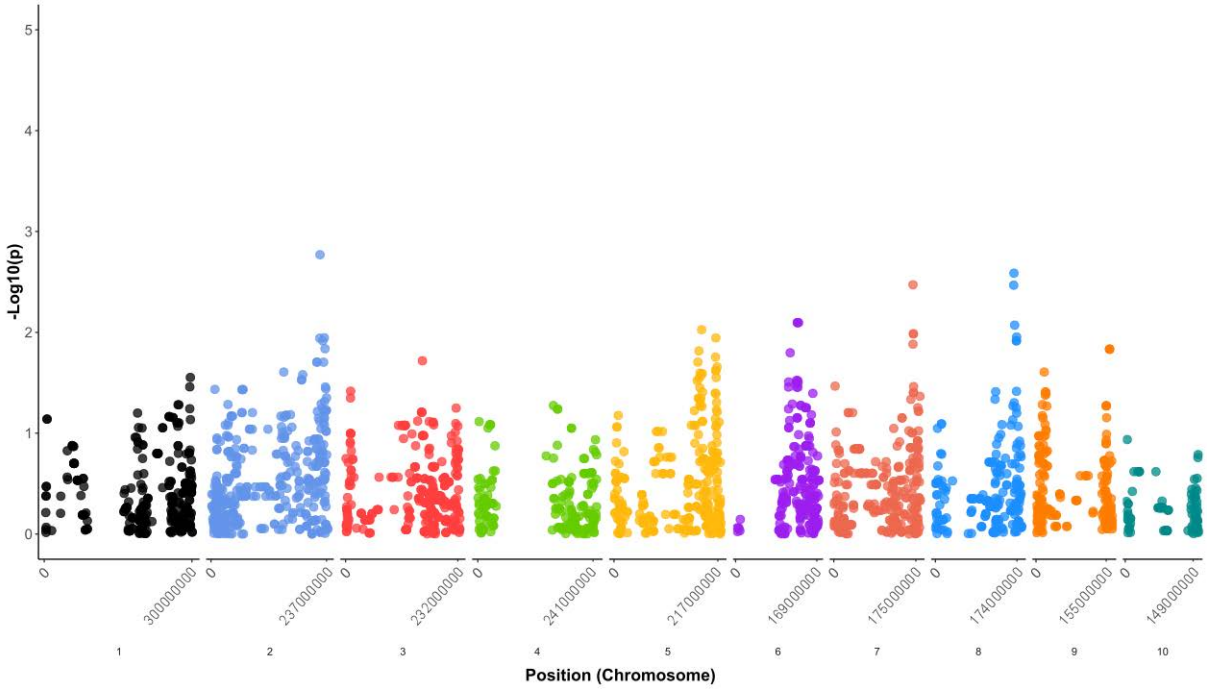

Figure III.19 Manhattan Plot for SPAD 8 (last measurement), showing the observed p-values of the SNPs for each chromosome

There was a peak shown in chromosome 2, formed by an outlier. Chromosome 5, chromosome 6, chromosome 7, chromosome 8 and chromosome 9 were also showing peaks, formed by more SNPs. Chromosome 1, chromosome 4 and chromosome 10 were showing no peaks at all. No significant associations were found between the trait SPAD 8 and SNPs.

Comparing General Linear Model and Mixed Linear Model

Comparing the general linear model and the mixed linear model with each other for the two traits SPAD 1 and SPAD 8, differences were shown, not only between the model, but also between the traits.

For the trait SPAD 1, the Q-Q-plot of expected vs. observed p-values (under a Gaussian distribution) for the general linear model was showing that the observed p-values are higher compared to the expected p-values. The mixed linear model was showing in its Q-Q-plot of expected vs. observed p-values (under a Gaussian distribution) that the observed p-values were lower than the expected p-values for the trait SPAD 1.

The Manhattan plot of the general linear model was also looking a little different, compared with the mixed linear model, for the trait SPAD 1. The general linear model was showing a peak on chromosome 2 and chromosome 7, while those peaks were not clearly visible anymore in the mixed linear model. The peaks on chromosome 3 and 10 were still visible. The peak of chromosome 9 was even higher than before. Chromosome 6 was showing no clear peak anymore in the mixed linear model compared to the general linear model.

Comparing the markers showing a tendency for significance for SPAD 1 found in the general linear model, with the mixed linear model it was shown that the marker on chromosome 3 is still had the

lowest p-value. The SNPs identified on chromosome 2 was also having a low p-value, but it was not possible to identify this marker easily in the mixed linear model. In total were the p-values in the mixed linear model higher, compared to the general linear model. Both markers were still showing a great allele effect independent of the used model (Table III.12).

Table III.12 Comparing significant marker for SPAD 1 and its effects of the general linear model with the mixed linear model

Marker	Chromosome	Allele	General Linear Model		Mixed Linear Model	
			Marker p-value	Allele effect	Marker p-value	Allele effect
SYN34350	3	A C	0.0003749	-1.1722 0	0.00205	-1.1437 0
PZE-102062746	2	A G	0.0004368	1.3172 0	0.0082	1.1863 0

Comparing the general linear model and mixed linear model for the trait SPAD 8, the differences between the models was smaller. The Q-Q-plot of expected vs. observed p-values (under a Gaussian distribution) for the trait SPAD 8 was showing for the general linear model and mixed linear model nearly the same results. The observed p-values were lower compared to the expected p-values for both models.

The Manhattan plot of the general linear model and mixed linear model for the trait SPAD 8 were looking similar. Both plots were showing a peak on chromosome 2 and chromosome 8. Chromosome 5, chromosome 6, chromosome 7 and chromosome 9 were also showing peaks, which a lower compared to chromosome 2 and chromosome 8. In both models, no significant associations were found for the SPAD 8. But the general linear model was identifying three markers which were showing a tendency for significance. Those markers were also forming the peaks on chromosome 2 and chromosome 8. Table III.13 is comparing the markers for general linear model and mixed linear model. All markers were also showing the lowest p-values in the mixed linear model. The allele effect was for both models and all markers high .

Table III.13 Comparing marker, showing a tendency for significance, for SPAD 8 and its effects of the general linear model with the mixed linear model

Marker	Chromosome	Allele	General Linear Model		Mixed Linear Model	
			Marker p-value	Allele effect	Marker p-value	Allele effect
PZE-108105381	8	A G	0.0005802	4.44106 0	0.00259	4.2315 0
PZE-102178194	2	A G	0.0006504	-3.0215 0	0.0017	-3.0715 0
PZE-108104106	8	A G	0.0006669	3.8325 0	0.00341	3.6136 0

Comparing general linear model and mixed linear model with each other, it was shown that the differences between the models were very low. Just the p-values for the general linear model were much lower compared to the mixed linear model.

3.5.2 Flint Lines

General Linear Model

The general linear model was used to identify significant associations between SNPs and the traits SPAD 1 (first measurement) and SPAD 8 (last measurement) for the Flint lines. As the Q-Q-plot of expected vs. observed p-values (under a Gaussian distribution) was showing, was the model fitting for both traits well. The observed p-values of both traits were fitting nearly exactly with the expected p-values calculated for the general linear model (Figure III.20).

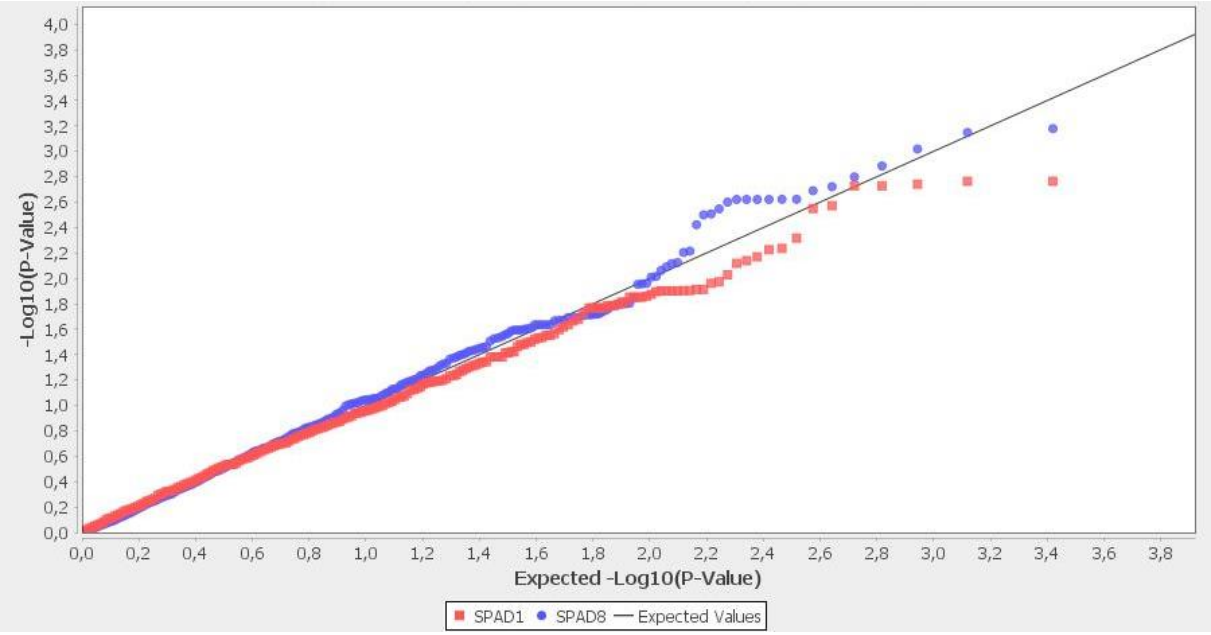

Figure III.20 Quantile-Quantile plot for SPAD1 (first measurement) and SPAD8 (last measurement), comparing the observed p-values (-Log10(p-value)) with the expected p-values (-Log(10(p-value))

Figure III.21 was showing the associations between SNPs and SPAD 1 on each chromosome. The p-values of SPAD 1 were high. On chromosome 1 a small range of peaks was shown, distributed over the whole chromosome. Also were chromosome 3 and chromosome 6 showing peaks for the trait SPAD 1.

Chromosome 5, chromosome 8, chromosome 9 and chromosome 10 were also showing peaks on one end of the chromosome but the peaks were very low and just for a few markers.

Chromosome 2, chromosome 4 and chromosome 7 were showing a wide range of associations, with a tendency for stronger associations on the behind part of the chromosome.

No significant associations were found between SNPs and SPAD 1 in the general linear model also depending on the high p-values.

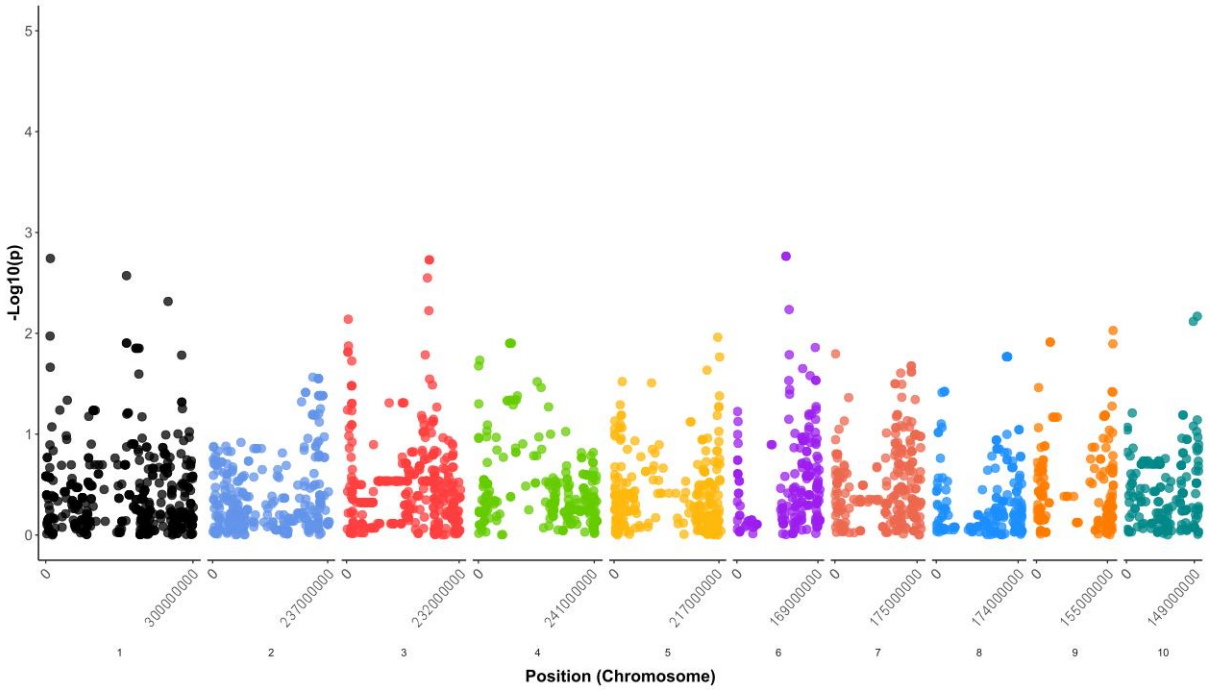

Figure III.21 Manhattan Plot for SPAD 1 (first measurement), showing the observed p-values of the SNPs for each chromosome

The Manhattan plot of the general linear model for the associations between SNPs and SPAD8 for each chromosome (Figure III.22) was looking different compared to the Manhattan plot of SPAD1. Chromosome 2 and chromosome 9 were showing a long peak, while on chromosome 1, chromosome 5 and chromosome 6 outliers were found, which were forming also smaller peaks. The other chromosomes were showing a range of associations on a lower level.

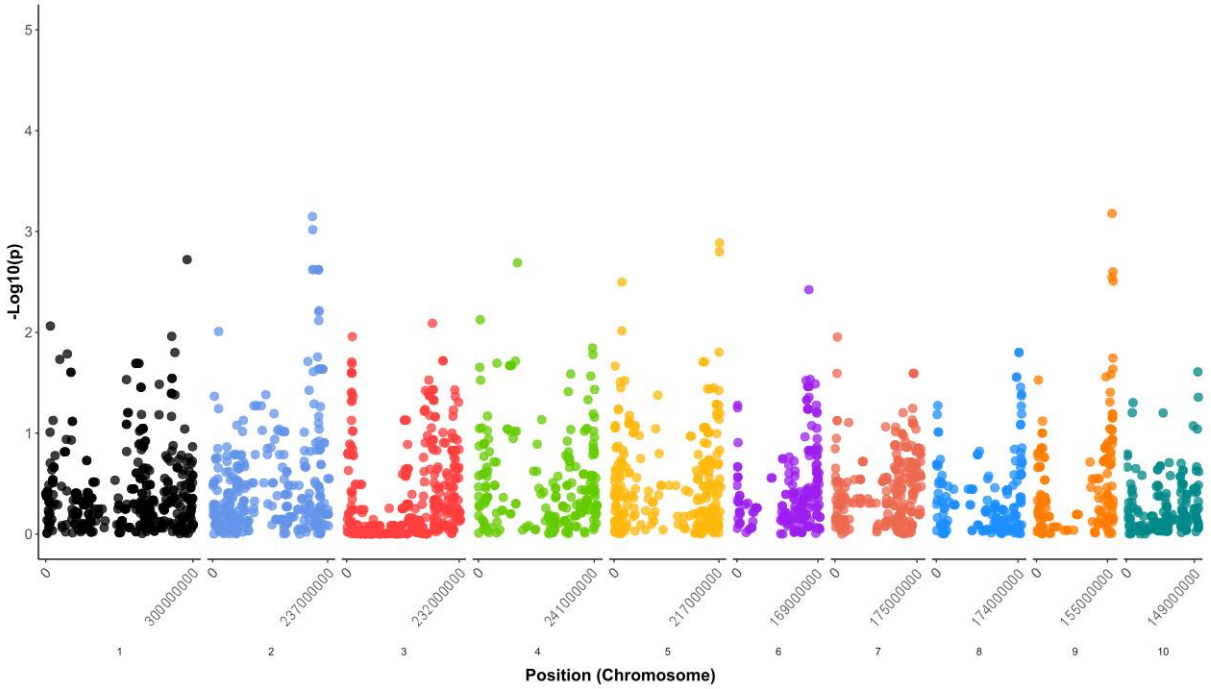

Figure III.22 Manhattan Plot for SPAD 8 (first measurement), showing the observed p-values of the SNPs for each chromosome

No significant associations between SNPs and SPAD 8 were found. The peak on chromosome 2 and chromosome 9 was identifying some associations, which were showing a tendency for significance.

The markers, formed this peaks were having the lowest p-values for the trait SPAD 8 and were also showing a tendency for significance. Table III.14 is showing the markers and their belonging facts.

Table III.14 Markers, showing a tendency for significance for the trait SPAD 8 and their belonging chromosome, alleles, lines and effects

Marker	Chromosome	Position (bp)	Allele	Lines	Marker p-value	Allele Effect
SYN15971	9	153876976	A	52	0.0006635	2.5521
			G	31		0
PZE-102155296	2	203383454	A	5	0.0007109	4.6885
			G	78		0
SYN19366	2	204173443	A	47	0.0009603	2.5511
			C	36		0

The corresponding genotypes for the markers found on chromosome 2 were AA, GG and CC. Most lines were containing the genotype GG, while the genotype CC is observed less. Here the p-value was very low, while the allele effect was high. For the marker PZE-102155296 was the allele effect even nearly as double as high compared to the second marker on chromosome 2 (SYN19366). The SNP formed the association on chromosome 8 was showing the lowest p-value for the whole model. Moreover were the belonging genotypes AA and GG, while most lines were containing genotype AA. The allele effect was also high.

Mixed Linear Model

For the mixed linear model, the Q-Q plot of expected vs. observed p-values (under a Gaussian distribution) was showing, that the observed p-values were lower compared to the expected p-values for a low level. For SPAD 1 and SPAD 8 the observed p-values were lower, even though the differences was just small (Figure III.24).

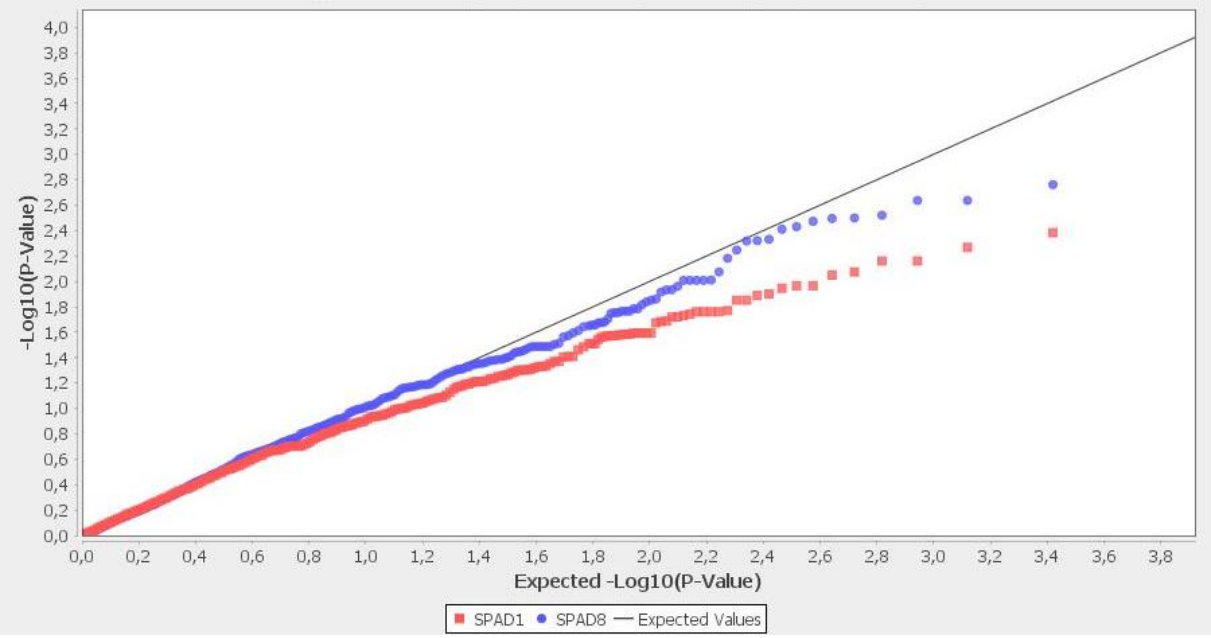

Figure III.23 Quantile-Quantile plot for SPAD1 (first measurement) and SPAD8 (last measurement), comparing the observed p-values (-Log10(p-value)) with the expected p-values (-Log(10(p-value))

Figure III.24 is showing the Manhattan plot of the trait SPAD 1 of the mixed linear model. On chromosome 1 a small range of peaks wasfound, while chromosome 6 and chromosome 9 were

showing a real peak. Chromosome 3, chromosome 5, chromosome 8 and chromosome 10 were also showing small peaks, but on a lower level compared to chromosome 1 and chromosome 9. Chromosome 2 and chromosome 7 were showing a wide range of associations on a high p-level.

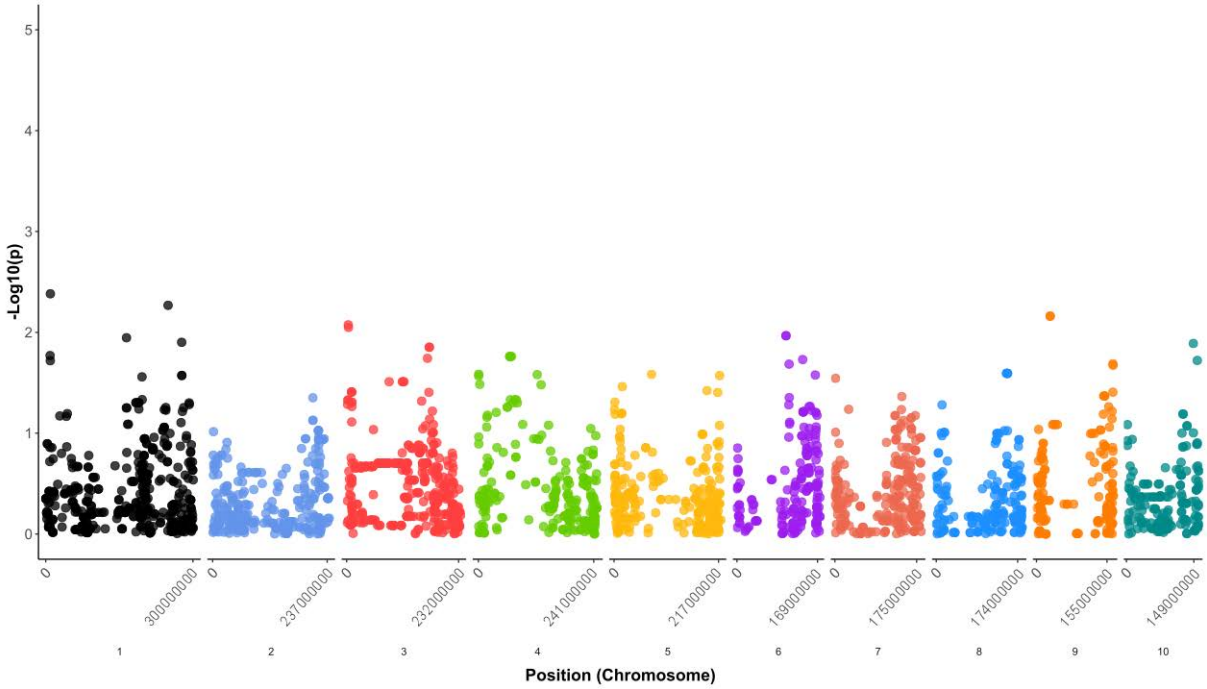

Figure III.24 Manhattan Plot for SPAD 1 (first measurement), showing the observed p-values of the SNPs for each chromosome

The Manhattan plot of the mixed linear model for the associations between SNPs and SPAD 8 for each chromosome (Figure III.25) was looking different compared to the Manhattan plot of SPAD1.

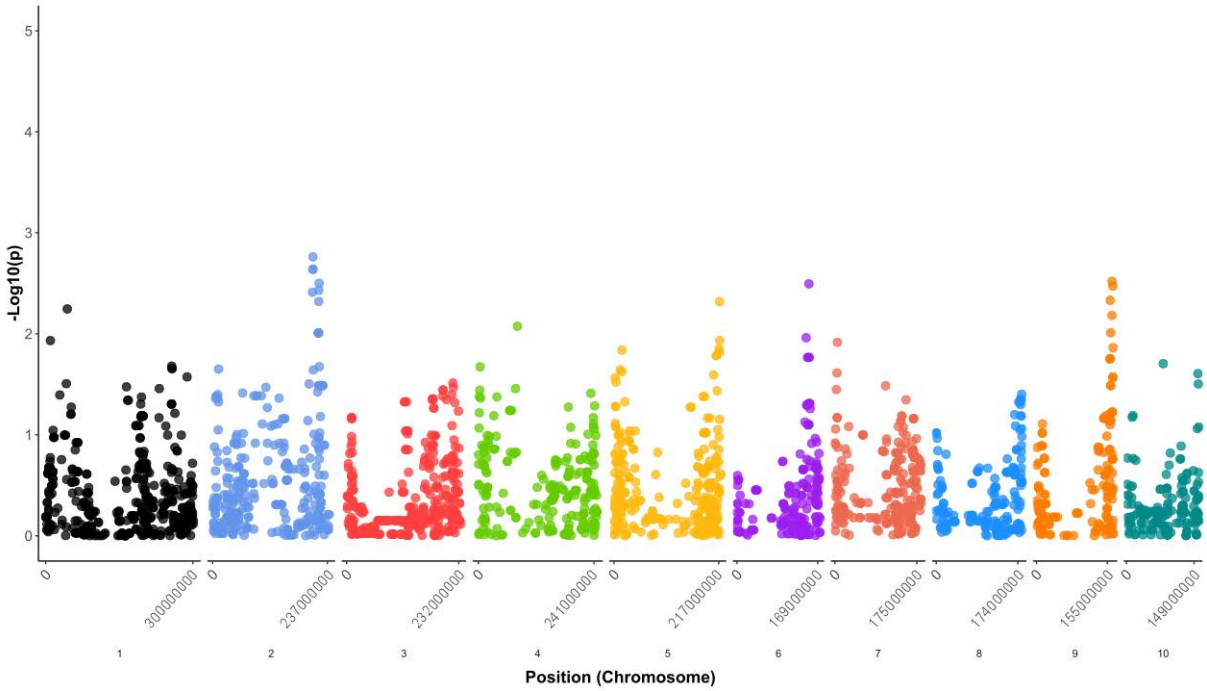

Figure III.25 Manhattan Plot for SPAD 1 (first measurement), showing the observed p-values of the SNPs for each chromosome

A high peak was seen on chromosome 2 and chromosome 9, while chromosome 5 and chromosome 6 were also showing a high peak Chromosome 1 was showing one small outlier while chromosome 3, chromosome 4 and chromosome 8 were showing no peaks at all.

Comparing General Linear Model and Mixed Linear Model

Comparing the general linear model and mixed linear model with each other for the two traits SPAD 1 and SPAD 8, differences were shown, not only between the model, but also between the traits.

Both traits were fitting well for the general linear model. The observed p-values of SPAD 1 were lying a little under the expected p-values while for SPAD 8 observed p-values and expected p-values are at the same level. For the mixed linear model, the observed p-values of SPAD 8 were also lying under the expected p-values, while for SPAD 1 nearly nothing was changed.

Comparing the trait SPAD 1 analyzed with the general linear model and mixed linear model nearly no differences were shown. Both Manhattan plots had the same range of p-values, which was very low, and no markers were identified with significant associations for SPAD 1. Chromosome 1 and chromosome 9 were showing peaks with nearly the same height in both models. On the other hand chromosome 6 had a higher peak in the general linear model than in the mixed linear model.

The trait SPAD 8 was showing also very similar Manhattan plots of general linear model and mixed linear model. Chromosome 2 and chromosome 9 were showing a peak in both models. While chromosome 5 and chromosome 6 were showing clearer peaks in the mixed linear model compared to the general linear model.

For both models, no significant associations were found with a false discovery rate of 20 %.For the general linear model three markers have been identified, showing a tendency for significance. Those markers were also showing the lowest p-values in the mixed linear model. Table III.15 was comparing the markers between the models. The allele effect was much higher in the general linear model compared to the mixed linear model.

Table III.15 Markers, showing a tendency for significance for SPAD 8 and their effects of the general linear model with the mixed linear model

Marker	Chromosome	Allele	General Linear Model		Mixed Linear Model	
			Marker p-value	Allele effect	Marker p-value	Allele effect
SYN15971	9	A G	0.0006635	2.5521 0	0.06064	0.7864 0
PZE-102155296	2	A G	0.0007109	4.6885 0	0.16714	1.0146 0
SYN19366	2	A C	0.0009603	2.5511 0	0.4455	0.8374 0

Comparing general linear model and mixed linear model with each other, it was shown that the differences between the models are very small. For the trait SPAD 1 not even a difference in the p-values was shown. For the trait SPAD 8 the p-values of the general linear model were higher compared to the mixed linear model; leadto differences in the Manhattan plots. For both models no significant associations between SNPs and traits were found.

3.6 Comparing Genome-wide Association Mapping of the stay-green behavior (SPAD) between Dent Lines and Flint Lines

The genome-wide association mapping for the traits SPAD 1 and SPAD 8 of the two genepools Dent and Flint were showing different results. Two associations, showing a tendency for significance between SNPs and trait SPAD 1 have been detected for the Dent lines, while the Flint lines were showing no significant associations between SNPs and SPAD 1. For the trait SPAD 8 three SNPs with a tendency for significance wre identified for the Dent lines and the Flint lines.

Comparing the results for the trait SPAD 1 it was shown that the mixed linear model was fitting better for the Dent lines, compared to the general linear model. For the Flint lines, the general linear model was fitting better, because here the observed p-values were closer to the expected p-values than compared to the mixed linear model.

The Manhattan plots of general linear model and mixed linear model of Dent lines and Flint lines were showing that the p-values of the mixed linear model were overall higher compared to the p-values of the general linear model. In general were the p-values of the Dent lines lower compared to the p-values of the Flint lines. Moreover were the Dent lines showing a wider range of associations between SNPs and SPAD 1 on each chromosome compared to the Flint lines. Comparing the Manhattan plots of both genepools for the trait SPAD 1, it was shown, that on chromosome 3 Dent line and Flint lines were showing a peak.

For the Dent lines, two associations, showed a tendency for significance between SNP and the trait SPAD 1 was found in the general linear model. Comparing the associations detected in the general linear model of the Dent lines for the trait SPAD 1, with the Flint lines, Table III.16 was showing that all SNPs have been filtered out for the Flint lines, because of to many missing calls. For the Flint lines, no associations between SNPs and SPAD 1 were identified.

For the trait SPAD 8, the differences between general linear model and mixed linear model were small therefore both models were fitting well, for the Dent lines. For the Flint lines the Q-Q-plot of the general linear model was showing that the observed p-values were similar to the expected p-values. Comparing the p-values of general linear model and mixed linear model, the p-values of the mixed linear model were always higher compared to the p-values of the general linear model.

For the Dent lines, three SNPs were detected, which were showing a tendency for significance on chromosome 8 and chromosome 2. Comparing the SNPs analyzed in the corresponding model for the Flint lines, it was shown that the SNPs found on chromosome 8 were also used for analysis, but the p-values were higher in the Flint lines compared to the Dent lines. The marker identified on chromosome 2 was not used for analysis of the Flint lines, because of to many missing calls (Table III.17). For the Flint lines, three SNP were identified, that were showing a tendency for significance on chromosome 2 and chromosome 9. Comparing the SNPs with the Dent lines, it iwas shown that all SNPs are filtered out because of to many missing calls (Table III.18)

The results of Dent lines and Flint lines for associations between SNPs and the traits SPAD 1 and SPAD 8 were not comparable. This indicates that the different genepools were containing different genes, responsible for the traits SPAD 1 and SPAD 8.

Table III.16 Comparing Markers identified with the general linear model for the Dent lines with corresponding analysis of the Flint lines for the trait SPAD 1

Line	Marker	Chromosome	Position (bp)	Allele	Marker p-value	Allele effect	Line	Marker	Chromosome	Position (bp)	Allele	Marker p-value	Allele effect
Dent	**SYN34350**	3	222837682	A C	0.0003749	-1.17220	**Flint**	**SYN34350**	NaN	NaN	NaN	NaN	NaN
Dent	**PZE-102062746**	2	41853032	A G	0.0004368	1.31720	**Flint**	**PZE-102062746**	NaN	NaN	NaN	NaN	NaN

The significance level is given by a false discovery rate of 20 %.

Table III.17 Comparing Markers identified with the general linear model for the Dent lines with corresponding analysis of the Flint lines for the trait SPAD 8

Line	Marker	Chromosome	Position (bp)	Allele	Marker p-value	Allele effect	Line	Marker	Chromosome	Position (bp)	Allele	Marker p-value	Allele effect
Dent	**PZE-108105381**	8	159526711	A G	0.005802	4.441060	**Flint**	**PZE-108105381**	8	159526711	A G	0.1444300	1.67950
Dent	**PZE-102178194**	2	221433785	A G	0.0006504	-3.02150	**Flint**	**PZE-102178194**	NaN	NaN	NaN	NaN	NaN
Dent	**PZE-108104106**	8	158942170	A G	0.0006669	3.83250	**Flint**	**PZE-108104106**	8	158942170	A G	0.6398700	-0.42950

The significance level is given by a false discovery rate of 20 %.

Table III.18 Comparing Markers identified with the general linear model for the Flint lines with corresponding analysis of the Dent lines for the trait SPAD 8

Line	Marker	Chromosome	Position (bp)	Allele	Marker p-value	Allele effect	Line	Marker	Chromosome	Position (bp)	Allele	Marker p-value	Allele effect
Flint	**SYN15971**	9	153876976	A G	0.0006635	2.55210	**Dent**	**SYN15971**	NaN	NaN	NaN	NaN	NaN
Flint	**PZE-102155296**	2	203383454	A G	0.0007109	4.68850	**Dent**	**PZE-102155296**	NaN	NaN	NaN	NaN	NaN
Flint	**SYN19366**	2	204173443	A C	0.0009603	2.55110	**Dent**	**SYN19366**	NaN	NaN	NaN	NaN	NaN

The significance level is given by a false discovery rate of 20 %.

3.7 Candidate genes for the stay-green behavior (SPAD)

Stay-green traits and their genetic background have already been studied for years. The idea about interactions between stay-green and other relevant traits, like grain yield and resistance against pest was leading to a high interest in stay-green traits and their genetic background. Recently not only the genetic interaction was studied, but also QTLs for the trait stay-green have been identified and clustered. Those QTLs have been identified mainly with help of simple sequence repeats (SSR) markers in two. Moreover different genetic maps have been built up to cluster the QTLs on the belonging linkage group and compare those maps with other studies (Zheng et al. 2009, Wang et al. 2012a, Belícuas et al. 2014, Kante et al. 2016).

In 2009, Zheng et al. (2009) clustered QTLs mainly on chromosome 1, chromosome 2 and chromosome 5, but also found QTLs on chromosome 3, chromosome 6, chromosome 8 and chromosome 9 (Zheng et al. 2009). Depending on the plant development, the gene expression for stay-green was varying (Zheng et al. 2009). In 2012, Wang et al. (2012a) identified QTLs on nearly the same chromosomes and detected, that the expressed QTLs on the different chromosomes were changing within the season (Wang et al. 2012a). The hypothesis was based on the fact, that some QTLs were found during the whole season, while other were just detected during flowering or after riping (Wang et al. 2012a). By this, Wang et al. (2012a) supported the hypothesis of Zheng et al. (2009) (Zheng et al. 2009, Wang et al. 2012a). Furthermore assumed Wang et al. (2012a) that especially chromosome 1 seemed to be important in controlling and maintaining green leaf area, because most QTLs that were expressed during the whole season were found on chromosome 1 (Wang et al. 2012a). Two year later, Belícuas et al. (2014) was identifying a major QTL on chromosome 1, with the further idea that chromosome 2 and chromosome 5 were also containing most QTLs responsible for stay-green behavior. Even though Belícuas et al. (2014) was not able to detected any QTL on chromosome 5, based on the former studies, they assumed that the chromosome 5 was an important player in terms of stay-green behavior (Belícuas et al. 2014). The used backcrosses between Dent parents, which are showing low stay-green behavior and Flint parents, which are showing an increased stay-green behavior, showed that both parental lines were containing favorable alleles and that the additive effects were more important compared to the dominant effects (Belícuas et al. 2014). Most recently Kante et al. (2016) identified significant markers on chromosome 10, showed a higher frequency of stay-green alleles, compared to the other chromosomes, containing significant markers. The significant markers found in chromosome 1 have been present during the whole study independent of the time measured. This finding again supported the earlier idea of Zheng et al. (2009) and Wang et al. (2012a) promoting that, depending on the stage of the plant during the season different QTLs were involved in the stay-green behavior (Zheng et al. 2009, Wang et al. 2012a, Kante et al. 2016). Furthermore concluded Kante et al. (2016) that QTLs underlying stay-green were not evenly distributed but clustered on chromosome 1, chromosome 2 and chromosome 5 (Kante et al. 2016). With this, Kante et al. (2016) supported the earlier named studies, that found major QTLs on chromosome 1 and clustered QTLs on chromosome 2 and chromosome 5 (Zheng et al. 2009, Wang et al. 2012a, Belícuas et al. 2014, Kante et al. 2016). Moreover, Kante et al. (2016) found overlapping QTLs between grain yield and stay-green, supporting the hypothesis that stay-green was influencing other important breeding traits as well (Kante et al. 2016). The stay-green gene expression was variating with the plant developmental sequences (Zheng et al. 2009, Wang et al. 2012a, Kante et al. 2016).

Table III.19 is summarizing the studies and giving an overview about the chromosomes and the studies finding QTLs on the different chromosomes. As Table III. shows, the only chromosome, where no QTLs are identified, was chromosome 7. Zheng et al. (2009) and Belícuas et al. (2014) were detecting most QTLs on nearly every chromosome. Wang et al. (2012) and Kante et al. (2016) were identifying more specific connections between the stay-green and other traits, but on the other hand supporting the earlier findings, of Zheng et al. (2009) and Belícuas et al. (2014).

Table III.19 Summary of the studies, comparing chromosomes containing QTLs for stay-green

Chromosome	QTL detected in study	Chromosome	QTL detected in study
1	Zheng et al. (2009) Wang et al. (2012) Belicuas et al (2014) Kante et al. (2016)	**6**	Zheng et al. (2009) Belicuas et al (2014)
2	Zheng et al. (2009) Belicuas et al (2014)	**7**	
3	Zheng et al. (2009) Belicuas et al (2014)	**8**	Zheng et al. (2009)
4	Wang et al. (2012) Belicuas et al (2014)	**9**	Zheng et al. (2009) Wang et al. (2012) Belicuas et al (2014)
5	Zheng et al. (2009) Wang et al. (2012), Kante et al. (2016)	**10**	Kante et al. (2016)

Comparing the genome-wide association mapping with the literature, two markers showed a tendency for significance in the general linear model and are found on chromosome 2 and chromosome 3 for the Dent lines and the trait SPAD 1. Even though the Dent lines were showing a low heritability for SPAD 1 (23 %) and SPAD 8 (27 %). Chromosome 2 was containing QTLs for the trait stay-green and the marker found on chromosome 2 was supported by this findings (Kante et al. 2016). While chromosome 3 was also showing QTLs for the trait stay-green (Belícuas et al. 2014). For the trait SPAD 8, three markers have been identified, that were showing a tendency for significance in the Dent lines. Two markers were found on chromosome 8. This was interesting, because only Zheng et al. (2009) detected QTLs on that chromosome before (Zheng et al. 2009). In the study, Zheng et al. (2009) detected the QTLs late in the season (Zheng et al. 2009). SPAD 8 was measured shortly before harvest. Therefore the found peak was supporting the Zheng et al. (2009) (Zheng et al. 2009). While the third one was found on chromosome 2, which was supporting Kante et al. (2016) again, saying that chromosome 2 is one of the major chromosomes where QTLs for the trait stay-green were clustered.

For the Flint lines, two markers were found in chromosome 2 and one marker was found on chromosome 9, for the trait SPAD 8, that were showing a tendency for significance. As already mentioned, several studies were pointing out, that QTLs were clustered, mainly on chromosome 1, chromosome 2 and chromosome 5 (Zheng et al. 2009, Kante et al. 2016). Therefore it was shown, that the genome-wide association mapping was showing potential QTLs for the trait SPAD 8. The used backcrosses in the literature were always based on Flint lines, containing a high stay –green (Belícuas et al. 2014, Kante et al. 2016). Therefore the markers found on chromosome 2, showed a tendency for significance could camouflage potential QTLs. Furthermore were several studies also showing QTLs on chromosome 9, indicating a QTL which is visible after riping (Wang et al. 2012a).

4. Discussion

The usage of genome-wide association mapping has been increasing during the last years (Li and Jiang 2005, Pearson and Manolio 2008, Yan et al. 2011, Wang et al. 2012b). Especially for breeding, knowing the genetic background of pathways and traits is of great interest. For dual use maize, stay-green behavior and sugar content of the stover are important requiremnts. Studies show, that species showing stay-green behavior are also containing a higher basal sugar content (Seale et al. 1986, Subudhi et al. 2000, Murray et al. 2008b, Murray et al. 2008a, Bian et al. 2014, Bian et al. 2015). High sugar content in the energy source is needed to garantee stable biogas production (Beavis et al. 1994, Subudhi et al. 2000, Xu et al. 2000, Bekavac et al. 2007, Zheng et al. 2009, Bian et al. 2014, Bian et al. 2015). Breeding for those traits is possible and pre-breeding based on genetic analysis is reducing time and costs (Kearsey and Farquhar 1998, Peleman and van der Voort 2003, Cockram et al. 2007, Yan et al. 2011).

For the Dent populations, 81 genotypes have been used, while for the Flint populations 84 have been used for genome-wide association mapping. Reducing the total number of genotypes per population was further impossible because the genotyped lines are already very limitied. Comparing the amount of genotypes used, with other genome-wide association studies, it is shown that most studies are using around 300 to 800 genotypes (Yu et al. 2006, Riedelsheimer et al. 2012, Strigens et al. 2013, Hauck et al. 2014). The total number is even though increasing up to 1000 (Belícuas et al. 2014) or 3000 (Amon et al. 2004, Kante et al. 2016), depending on the available material and the traits analyzed. If the NAM population is used for analysis, around 5000 lines, splitted in 25 families are analyzed (McMullen et al. 2009). Based on a few amount of lines, it is shown, that QTLs found in former studies or showing small effects are not detected anymore (Yan et al. 2011, Strigens et al. 2013). The Dent and Flint lines are showing rarely signficiant associations for the two traits BRIX and SPAD. By increasing the number of used lines, the number of associations can be increased, while the results at the same time are more reliable.

Besides, the population structure is influcening the analysis as well (Li and Jiang 2005, Strigens et al. 2013). By correcting the population strucuture, false positive detections can be found (Li and Jiang 2005). Therefore it is important to define carefully the number of used axis during the principal coordinate analysis. As shown in the literature the used number of axis for correcting the populations strucuture is between three and ten principal coordinates. Those principal coordinates should explain most of the variation within the population and seem to be most useful for correction (Strigens et al. 2013). Eventhough the number of principal coordinates used during analysis has be detected for each population itself to avoid over- or undercorrection (Strigens et al. 2013). Limiting genome-wide association mapping by reducing the number of lines and correcting for population structure can lead to association signals, that are most likely below the signficiance level (Strigens et al. 2013). For the Dent and Flint genepool most variation within the genepools is explained by the first ten principal coordinates, which have been used for further analysis. Comparing the population structure between Dent and Flint lines, it seemed to be shown that the Flint genepool is containing two subpopulations, while the Dent lines are forming a small population with a strong familial relatedness between the genotypes. Analyzing the two subpopulations of the Flint lines is impossible, because of the too small number of genotyped lines in the population.

A third aspect that has to be taken into account, while talking about populations and its number of genotypes, is the fact, that the two used models, general linear model and mixed linear model, are

correcting differently for the several aspects. Therefore overestimation for a trait in a population is possible, depending on the factors corrected by the models (Larsson et al. 2013). The general linear model is fixing the effects to test for association (Atlassian Bitbucket 2014b). Optionally the analysis accounts the population structure using it as covariants, indicating the degree of membership in the population (Atlassian Bitbucket 2014b). If there is no correction for population structure via principal coordinates, the general linear model is not taking it into account. On the contrary, the mixed linear model is correcting for population structure and familial relatedness, by using kinship matrix (Yu et al. 2006, Atlassian Bitbucket 2014c). It is including random and fixed effects, while the random effects are giving the mixed linear model the ability to incorporate information about relationships among the genotypes. Therefore it is implementing the method of compression which reduces the dimensionality of kinship matrix and puts every genotype in its own group. While the general linear model is contrary to that and is putting all genotypes in one group (Atlassian Bitbucket 2014c). For the two used genepools, Dent and Flint, the two models are fitting differently. The general linear model is fitting best for the Dent lines. Correcting for population strucuture and familial relatedness, the resulting p-values could be underestimated as the Q-Q plot of expected vs. observed p-values (under a Gaussian distribution) is showing (Voorman et al. 2011). For the Flint lines, it is shown that both models are fitting good, independent whether corrected for familial relatedness. Correction for population structure in both populations is necessary to avoid false positive correlations by controlling for effects (Atlassian Bitbucket 2014b).

The available amount of SNP markers, due to the 12K KWS Illumina Chip, was high. Therefore the number of markers was reduced until the number of genotypes and number of markers was fitting best. Because of the low number of genotypes, all missing calls, full linkage disequilibrium and heterozygous markers could be filtered out. Linkage disequilibrium between the markes can lead to a higher detection of false positive results (Cook et al. 2012). Linkage disequilibrium between a QTL and a marker is necessary to identify genes and their neighbourhoods (Becker 2011) but linkage disequilibrium is found as an association between a pair of markers as well. Therefore linked markers are not usefull for analysis because validity of the linked markers is the same (Morton 2005). Markers in full linkage disequilbrium are showing the same or opposite genotypes and allele effects. Duirng genome-wide association mapping, some markers have been identified to be significant showing opposite allele effects. It seemed that they were in full linkage disequilibrium but not filtered out before. Those SNPs are having some validity because they are not in full linkage disequilibrium with all SNPs they are linked with. Furthermore are the shown results with the limited amount of markers an increased strongness of the model and the best results.

The used false discovery rate (FDR) of 20 % is commonly used to identify significant markers during genome-wide association mapping (Benjamini and Hochberg 1995, Bender et al. 2007). By controlling with FDR, it is stated, that on average the false discovery rate for the experiment, replicated many times, is not bigger than the expected false discovery rate (Genovese et al. 2002). The false discovery rate is more powerful because of less strict controlling for false discoveries and allows controlling for the proportion of effort (Reiner et al. 2003). Even though it is shown that the false discovery rate is highly useful for the discovery of differential genetic expressions (Reiner et al. 2003). The second possibility, the Bonferroni correction, is not used in the study, because of the low number of genotypes and the high p-values. Moreover, correcting with Bonferroni is stronger compared to the false discovery rate (Miller 1981, Benjamini and Yekutieli 2001, Reiner et al. 2003). Caused by the fact, that with the false discovery rate, already few significant associations are found, the Bonferroni

correction would be too strong. Less significant markers have been identified due to high p-values with a false discovery rate of 20 %. Caused by this, the false discovery rate could be set up to 30 % or higher. This was done in earlier studies, depending on the analyzed traits (Biscarini et al. 2016). Also because the false discovery rate is depending on the data and it should be determined what is a tolerable rate of false discoveries. A rate between 0.1-0.2 is reasonable for many reasons (Benjamini and Yekutieli 2001, Genovese et al. 2002). For the two traits and the Dent and Flint lines a false discovery rate of 20 % was used, because it is reasonable, as Benajmini and Yekutieli (2001) stated. Moreover, a discovery rate of 20 % for false positive is already very high and suitable for the low amount of tested genotypes.

The genetic background for the two traits SPAD and BRIX has been analyzed during genome-wide association mapping for the Dent and Flint lines with the general linear model and mixed linear model. During the analysis, significant associations between SNPs and phenotype have been found in the general linear model, supported by a tendency to significane of those SNPs in the mixed linear model. The found association are corresponding to the found QTLs in former studies (Zheng et al. 2009, Wang et al. 2012a, Belícuas et al. 2014, Bian et al. 2014, Bian et al. 2015, Kante et al. 2016).

As important requirements for dual use maize, yield of grain and stover are defiend. Leaf strucuture as well as the senescence of leaves can strongly influence the grain yield and the quality of the grain yield (Xu et al. 2000, Zheng et al. 2009, Wang et al. 2012a, Bekavac et al. 2007). Kante et al. (2016) showed that candidate genes for stay-green behavior are found in the same regions than QTLs for grain yield (Kante et al. 2016). Therefore stay-green behavior is also an important requirement in terms of dual use maize. Most QTLs coding for stay-green behavior are clustered on chromosome 1, chromosome 2 and chromosoem 5 (Zheng et al. 2009, Wang et al. 2012a, Belícuas et al. 2014, Kante et al. 2016). Genome-wide association mapping of Dent and Flint lines are mainly associations on chromosome 2, which are showing a tendency for significane, for Dent and Flint lines. Zheng et al. (2009) stated that the expression of stay-green genes is depending on the plant developmental sequence (Zheng et al. 2009). Therefore SPAD 1 (eight weeks before harvest) and SPAD 8 (one week before harvest) have been measured. As the genome-wide association mapping is showing different assocations between SPAD 1 and SPAD 8 and SNPs are found. Moreover, the manhatten plots are showing different associations on the chromosomes, but chromosome 2 is showing the most associations for boths measurements. As studies are showing chromosome 2 is containing a major QTL for stay-green (Zheng et al. 2009, Belícuas et al. 2014), which is found confirmed by the done genome-wide association mapping. Depending on the plant developmental sequence different QTLs are expressed for stay-green behavior. Therefore it could be interesting to identify the different genes, responsible for the stay-green behavior in maize. Hence analyzing the genetic background SPAD during the season could be helpful, starting eight weeks before harvest until harvest.

For the usage as dual use maize, the sugar content of the stover is an important requirement, to garantuee stable biogas production processes. As Subudhi et al. (2000) showed grass species are potential energy sources (Subudhi et al. 2000). The sugar content of maize stover, analyzed with the BRIX method has not been studied a lot before. During genome-wide association mapping, signficiant associations for the sugar content of the stover have been found in the Dent lines and Flint lines. Especially chromosome 2 is showing a lot of significant associations for the Dent lines. Studies of Bian et al. (2014/2015) have been identified a major QTL for the sugar content in the stover on chromsome 2 (Bian et al. 2014, Bian et al. 2015) as well. The phenotyping of the BRIX showed, that the sugar content is changing during the season. Therefore it is of great interest to know if more

major genes controlling the sugar content during its plant developmental sequences. As seen for the Flint lines, chromosome 1 is showing significant associations, instead of chromosome 2. Bian et al. (2015) found unconditional QTLs on chromosome 1, which are selectively expressed at different growth stages (Bian et al. 2015). By analyzing the genetic background of BRIX, identifying significant associations, developing special markers for sugar content and finding major QTLs for the trait, would be an effort in terms of breeding. The BRIX-method is destructive and time-consuming. Even though the sugar content of the stover is only measurable shortly before harvest. Therefore it could be cheaper and less time-consuming having special markers, detecting major QTLs controlling for the sugar content. Already in early plant developmental stages, those QTLs could be detected and more lines and testcrosses could be tested for the the QTLs.

Significant associations are found mainly on chromosome 2 for BRIX and SPAD. Studies are showing that a major QTL for BRIX is located on chromosome 2 (Bian et al. 2014, Bian et al. 2015). QTLs for stay-green are clustered on chromosome 1, chromosome 2 and chromosome 5 (Zheng et al. 2009, Wang et al. 2012a, Belícuas et al. 2014, Kante et al. 2016). Further it is known that candidate genes for stay-green are found in the same region than QTLs for grain yield (Kante et al. 2016) and that the basal sugar content is increasing if the plants are showing stay-green behavior (Seale et al. 1986, Subudhi et al. 2000, Murray et al. 2008b, Murray et al. 2008a, Bian et al. 2014, Bian et al. 2015). The results leading to the idea, that there are also overlapping candidate genes for the two traits SPAD and BRIX on chromosome 1 or chromosome 2. Therefore further study has to be done, including development of sugar content markers and QTL mapping for both traits. The nested association mapping (NAM) population is forming the basis for clarifying the genetic architecture of several traits of interest in maize (McMullen et al. 2009, Tian et al. 2011, Wallace et al. 2014). The NAM population is offering the opportunity to dissect QTLs and on the other hand to use genome-wide association mapping. Several QTLs for quantitative traits have been found already (Veldboom et al. 1994, Cook et al. 2012, Riedelsheimer et al. 2013, Wallace et al. 2014).

Identifiying the genetic background of stay-green behavior and sugar content of the stover is of great interest for dual use maize. As shown associations are mainly found on chromosome 2 for both traits. Comparing the found associations with literature, it is confirmed that the studied populations are containing candidate genes for the SPAD and BRIX. By putting effort into the development of special markers to detect the sugar content of the stover, probably in combination with stay-green behavior, genome-wide association mapping and QTL mapping can be strong breeding tools for dual use maize.

IV. General Discussion

In Germany maize is most important for energy production and also for producing feed. While using maize as energy source, the available arable land is no longer used for production of food (BMEL. 2015). Furthermore, has an increasing population a need for infrastrucuture and settlements, while the arable land is already limited (Destatis 2014b). This is leading to an ambivalent opinion in public (Zschache et al. 2009, Linhardt and Dhungel 2013). Biomass is the only resource that is regrowable and usable in different energy parts and in usage chains (Baur 2010), instead of fossil resources. The resulting food/feed energy conflict for arable land has a great impact on the cultivation of crops.

Maize is an important energy crop because of its easy handling and the total methane yield which is high while the total costs are low (Oechsner 2005, Moeser 2013, Falter et al. 2015). Comparing it to other crops, maize is showing a lot of advantages, like high yielding hybrid cultivars using Dent and Flint genepools and low need of pesticides. Moreover it has been used already for years as feed, before using it as biogas substrate. Those cultivation needs are not changing depending on the different kind of usage as energy (Oechsner 2005, Stolzenburg 2012).

With dual use maize as a combination of grain maize and energy maize, the food/feed conflict can be mitigated, while environmental resources are better used and the economic value is increasing as well, by selling grains and stover seperatly from each other. By studying the objectives

1. Testing different maize genotypes for the usage as dual use maize (preformance tests)
2. Developing dual use maize cultivars (selection)
3. Identify significant associations between SNPs and stay-green behavior and sugar content (Genome-wide association mapping)

it is investigated in the requirements for if dual use maize, to switched from grain maize **or** energy maize production to grain maize **and** energy maize production.

IV.1 Performance and Selection

Maize is one of the main cultivated crops in Germany. Depending on the usage of maize as silage maize, energy maize or grain maize, it is supposed to have different breeding programs (Oechsner et al. 2003). Dual use maize is combining the different requirements, for grain maize and energy maize, in one cultivar. The most important requirements are:

1. High grain yield
2. High yield of stem and leaves (stover), including
    a. High methane yield
    b. High water content
    c. High sugar content
3. Long photosynthetic activity (stay-green behavior)

Breeding for high yield performance is common in the breeding process of maize. A combination of high grain yield and high stover yield is difficult to reach because both traits are partly contrary to each other. Problematic could be the negative correlation between stover and grain yield and between dry matter content and high methane yield (Weiland 2003, Li et al. 2011). As shown, the grain dry matter yield and the stover dry matter yield are negatively correlated with each other, but at a low and non-significant level. Therefore breeding for dual use maize, combining a high grain dry matter yield and a high stover dry matter yield, is possible.

For a stable wet fermentation in the biogas plants, a dry matter content of 10 % to 13 % is propsed (Weissbach 2000, Weiland 2003, Fernández et al. 2008), therefore the water content of the stover has to be high enough, while the dry matter of the plants has to be high enough (Hugger 2005). Wet fermentation is used for feedstocks that can percolated well because of their low solids content (GICON 2017) and is most commonly used in Germany (mifratis.de 2017). Furthermore a dry matter content of 28 %-35 % is aimed for effective use of biogas production with maize stover (Weiland 2003, Kaiser 2007, Fernández et al. 2008, Li et al. 2011). The tested material (Dent, Flint, factorial crosses) is showing a high water content of the stover. Comparing the dry matter content to the wanted range between 28 % - 35 %, the results are showing dry matter contents within the range, indicating that the biogas production can be effective and stable.

The sugar content of the stover has been measured with the BRIX method. With the BRIX method only the sucrose content is measured. This is giving the highest part of sugar in maize, while fructose and glucose are also present in maize, but with much lower amount (Loomis 1945, nährwertrechner.de 2017). Nowadays the sugar content in sweet corn is measured with BRIX, which is, compared to silage maize, much higher (van Waes et al. 1998, Mok et al. 2014). The BRIX value is decreasing during the season, in a non linear way. Therefore the last measurement shortly before harvest is of greatest interest, because the stover should be used for biogas production and therefore the sugar content has to be high. There is a strong interaction between location and year with the % BRIX found, while the heritabilities are moderate.

For measuring the stay-green behavior of the plant, the SPAD measurement was chosen. It has to be taken into account, that the SPAD measurement is not directly measuring the photosynthetic activity, but the greenness of the leave. The color of the leaves is correlated with the chlorophyll content in the leaves, and therefore a correlation to the photosynthetic activity is given (Konica Minolta Optics, Inc. 2009). Using the SPAD-value as an indication for stay-green behavior is common and was done in several studies before (Bekavac et al. 1998, Thomas and Howarth 2000, Bekavac et al. 2007, Zheng et al. 2009, Wang et al. 2012b, Wang et al. 2012a, Belícuas et al. 2014, Thomas and Ougham 2014, Kante et al. 2016). It is assumed that genotypes, showing a stay-green behavior after grain filling will assimilate more sugar in the stem, which is used as a sink then (Rajendran et al. 2000, White et al. 2011). The correlation between SPAD and BRIX in the study is shown to be very low. A high SPAD value does not necessarily correlate with a high BRIX value.

Dual use maize cultivars have different requirements, compared to grain, silage or energy maize. Therefore a specific breeding program for them is necessary, as suitable genotypes are just found during dual use maize harvest. The results show that genotypes can be selected with a great potential to become dual use maize cultivars.

IV.2 Genome-wide association mapping

Identifying the genetic background of stay-green behavior and sugar content in the stover is a useful tool for breeding programs for of dual use maize. Due to marker-assisted breeding, costs can be reduced and an increase in efficacy can be made (Peleman and van der Voort 2003) especially to identifiying potential genotypes in early developmental stages.

Two different populations, Dent and Flint, have been studied. The belonging genotypes are coming from the actual breeding material of KWS SAAT SE. The population structure, as well as the familial

relatedness is influencing the study of genome-wide association mapping (Li and Jiang 2005, Strigens et al. 2013). Caused by the fact, that the used material is containing lines coming from different crosses it is difficult to comprehend the structure and its familial relatedness easily. With the help of a principal coordinate analysis and a kinship the population structure and familial relatedness is taken into account (Romay et al. 2013, Strigens et al. 2013). The first ten principal coordinates are showing most of the variation within the Dent and Flint populations. The population strucuture is different for the two genepools. The Flint lines are showing two subpopulations, which are not futher studied, because of a two low total number of lines in the population, while the Dent lines seemed to be closely related with each other.

The genome-wide association mapping was conducted with 81 genotypes of the Dent population and 84 genotypes of the Flint population. This is a low number compared to earlier studies, with 300 to 800 genotypes (Yu et al. 2006, Riedelsheimer et al. 2012, Strigens et al. 2013, Bian et al. 2014, Hauck et al. 2014, Bian et al. 2015), or even up to 1000 (Belícuas et al. 2014) to 3000 (Amon et al. 2004, Kante et al. 2016). Therefore the analysis is probably less powerful because the associations found are based on the small population. The phenotypic data was taken form testcrosses between the different lines of Dent and Flint with one tester from the other genepool. Caused by the fact that the pollen donor has been coming from the other population, it is expected that the genetic variation of the tester is representative for the whole tester population (Goodman et al. 2014). The found genetic variation in the testcrosses is then coming from the mother. But probably recessive alleles are hiding behind dominate alleles of the pollen donor and are not detected.

Especially the genetic background of the sugar content of the stover is rarely. Therefore comparisons between literature and results are difficult, even though two studies have been found, identifying QTLs for the sugar content in the stover (Bian et al. 2014, Bian et al. 2015). Bian et al. (2014) found a major QTL on chromosome 2 (Bian et al. 2014). Comparing the found QTL with the genome-wide association mapping, it is shown, that chromosome 2 is also containing most signficiant associations between SNPs and BRIX in the Dent lines. Due to the fact that the sugar content is decreasing non-linear during the season, it is suggested that major genes are controlling the sugar content during the plant developmental sequences (Bian et al. 2015). In the 20th century already two candidate genes have been identified, controlling the change of sucrose during the season from stem to leaf (Loomis 1945). Unconditional QTLs, which are expressed depending on the plant developmental sequence for the sugar content in the stem, are found on several chromosomes (Bian et al. 2015). The Flint lines are showing significant associations on chromosome 1, which is probably indicating an unconditional QTL.

Candidate genes for stay-green behavior have been found in the regions than QTLs for yield (Kante et al. 2016), making stay-green behavior to another important requirement for dual use maize. Earlier studies already showed that the grain yield and stover yield is influenced by the senescence of leaves (Xu et al. 2000, Bekavac et al. 2007, Zheng et al. 2009, Wang et al. 2012a). Depending on the plant developmental sequence different stay-green genes are expressed (Zheng et al. 2009). Therefore the two measurements of SPAD, eight weeks before harvest and one week before harvest, are analyzed, showing two different points in time during the developmental sequence of the plant. Associations are shown for both traits on chromosome 2 and both populations. Zheng et al. (2009) and Belícuas et al. (2014) are showing that chromosome 2 is containing major QTL for stay-green behavior (Zheng et al. 2009, Belícuas et al. 2014).

The genome-wide association mapping is showing associations, between SNPs and traits which are supporting QTLs in other studies (Loomis 1945, Zheng et al. 2009, Wang et al. 2012a, Belícuas et al. 2014, Bian et al. 2014, Bian et al. 2015, Kante et al. 2016) and showing that even with a small amount of lines, important QTLs can be identified. Sugar content of the stover, needed for a stable biogas production, and stay-green behavior, needed because of the interaction with yield, are two requirements for dual use maize, which are highly important. Significant associations between SNPs and BRIX and SPAD due to genome-wide association mapping are, which can be used as helpful breeding tool.

IV.3 Remarks and Outlook

For technical reasons the performance test of dual use maize and silage maize harvest, have been done at different lcoations than the measurement of SPAD and BRIX. As shown in the analysis of variance the environmental conditions are causing some variation in the traits. This is also shown in the literature (Amon et al. 2003, Oechsner et al. 2003). By taking an average over years and locations a comparison is possible. Furthermore, the performance tests for the Dent and Flint testcrosses were done in 2014, while in 2015 the factorial crosses have been tested for their performance. The traits SPAD and BRIX have been evaluated over two (factorial crosses) to three (Dent and Flint testcrosses) years. As known, the environmental conditions are different between years and locations (Amon et al. 2003, Oechsner et al. 2003). For maize, the yield in 2015 was much lower all over Germany compared to 2014 (Destatis 2016b, DMK e.V. 2016b). For better comparable results the performance test of the Dent and Flint testcrosses could have been done over two years instead of one year, to identify stress resistant genotypes as well as weaknesses of the tested genotypes.

The usage of dual use maize is of high interest. Even though the harvest is difficult compared to the common harvest of silage maize or grain maize. Harvesting dual use maize requires two steps. At first the grain has to be taken, while in a second step stem and leaves have to be taken from the ground. Therefore it is important that as much as possible of the plant material is taken while the pollution with soil has to be as low as possible (Holzhammer 2016). Right around 50 % of the maize stover is rescued from the field (Fleschhut et al. 2016). By testing different methods an increase of the rescue value (Fleschhut 2015, Neumann 2015, Fleschhut et al. 2016) and a method of efficient dual use maize harvest is required and further research on harvest technology is necessary.

Another topic of further research is the usability of maize stover as bioenergy source. As the study is showing, all requirements are fulfilled, even though other studies are showing that a combination of maize stover with co-substrates like chicken manure or kitchen waste is giving a more stable biogas production process (Li et al. 2013, Neumann 2015). For indirect selection, the part of corn cob can be measured during silage maize harvest. The part of corn cob in the whole silage maize will give an idea about the amount of stover and grain of the genotype.

Beyond it is stated that trace elements are showing a high impact on stable biogas production. Deficiency of trace elements and a small organic load can lead to instable biogas production from maize silage for long-term (Lebuhn et al. 2008). Further research should focus on the need of trace elements in the biogas substrate and its effect on biogas production.

To identify more and probably significant associations on the different chromosomes for the traits sugar content of the stover and stay-green behavior, a higher number of genotypes lines used for the genome-wide association mapping should be used. Further effort should be put into developing specific markers, identifying QTLs for BRIX and SPAD for the tested material and to identify potential candidate genes. This could be a great step in breeding for higher sugar content in the stem and stay-green behavior, making phenotyping of the traits already in earlier developmental stage possible.

IV.4 Conclusion

The main goal of this study was to investigate the requirements of efficiently development of dual use maize, for switchting from grain maize **or** energy maize production to grain maize **and** energy maize production. As shown, breeding for dual use maize is possible, by fulfilling the different requirements given to a dual use maize cultivar. An effienct use of environmental resources and a higher economic value for the farmers are favorable effects of dual use maize. It can become a meaningful alternative to mitigate the conflict between food and energy production.

By testing different maize genotypes of current breeding material for their usage as dual use maize in two performance tests (silage maize and dual use maize), it is shown that it is possible to select genotypes showing a high grain dry matter yield while having a high stover dry matter yield as well. Furthermore, the water content and sugar content of the stover is high enough for a stable biogas production. Stay-green genotypes are not always indicating a high sugar content in the stover as expected, but still is stay-green behavior an important trait, because of its correlation with yield and its indication of being more resistant to stresses. Indirect selection by silage maize harvest is not possible. The performance of genotypes during silage maize harvest is giving no idea about the grain dry matter yield of the genotype during dual use maize harvest. Indirect selection during silage maize harvest is risking false positive selection.

Identifying the regions of potential QTLs with help of genome-wide association mapping for the two traits traits sugar content of the stover and stay-green behavior the genetic background is analyzed. Both traits are showing that mainly chromosome 2 is associated with marker alleles. By developing specific markers, explaining most variation, for sugar content of the stover, probably in combination with stay-green behavior, marker-based selection can become an efficient breeding tool.

V. Summary

Arable land resources for the production of food, feed and energy are limited. The increasing use of maize for bioenergy production is critically discussed in public. By developing dual use maize varieties combining the use of grains for feeding animals with the use of leaves and stem as substrate for biogas production, the conflict between food/feed and energy production will be mitigated. With the development of dual use maize cultivars an interesting alternative has been found to increase the economic and environmental value of maize in Germany.

Present cultivars cannot be used as dual use maize because of their low water content and sugar content in the stover. Dual use maize varieties have to combine characteristics different from grain maize, silage maize or energy maize, like:

1. High grain yield (nearly as high as grain maize)
2. High yield of stover (stem and leaves) with a:
    a. High water content of stover
    b. High sugar content of stover
3. Stay-green behavior indicating long photosynthetic activity even when the grains are mature

The combination of different characteristics for dual use maize cultivars is asking to investigate breeding methods for dual use maize cultivars. The switch from grain maize **or** energy maize production to grain maize **and** energy maize production is only possible if the requirments for an efficient development are given. Eventhough the genetic background of sugar content in the stover and stay-green behavior is of great interest while investigating in breeding methods.

To breed for dual use maize cultivars 178 testcrosses (89 Dent line x Flint tester and 89 Flint lines x Dent tester), coming from the actual breeding material of KWS SAAT SE have been cultivated in 2014 at three different locations in two different performance tests (silage maize and dual use maize) in Southern Germany. In 2015, 88 factorial crosses, received by the best lines have been tested at three different locations in Southern Germany for their performance as silage maize and dual use maize.

The ‘stay-green’ behavior and the sugar content in the stover of the Dent and Flint testcrosses was measured in observations tests at two locations (Einbeck and Göttingen) over three years. Both traits have been measured in the factorial crosses as well for two years at the same two locations (Einbeck and Göttingen).

The selection of the best lines was based on the their ‘stay-green behavior’, grain dry matter yield, total dry matter yield, water content of the stover and sugar content of the stover, compared to the grain dry matter content and total dry matter content. Seven Dent and thirteen Flint lines have been selected

High grain dry matter yield and high yield of stover are not easy to combine, but it is found that the correlation is low between the two traits. The sugar content of the stover was measured with the BRIX method, showing the sucrose content of the sample in % BRIX. The stay-green behavior was measured with the SPAD-method, which is highly correlated with the chlorophyll content. Both traits have been showing a high heritability but a low correlation to each other. Genotypes are found that are showing stay-green behavior during dual use maize harvest, in combination with a high grain dry matter yield and stover dry matter yield. The water content and the sugar content of the stover are high, to garantuee a stable biogas production.

On the other hand, genotypes showing a high total dry matter yield during silage maize harvest, are not necessarily the best preforming genotypes in dual use maize harvest. Therefore indirect selection for dual use maize by selection during silage maize harvest is not possible. The response to this indirect selection is very low, and therefore the complicated and time consuming dual use maize harvest is necessary to conduct the best results.
After finishing the second experimental year one promising genotype has been submitted by KWS SAAT SE to official variety tests. These cultivar can be used as grain maize with additional use of stover for biogas production.

In a second part of the study, genome-wide association mapping was conducted for 81 Dent lines and 84 Flint lines. The lines were genotyped with 8917 single nucleotide polymorphisms (SNPs) using the 12K KWS Illumina Chip.

The population structure was analyzed with a principal coordinate analysis. The first ten principal coordinates have been used to correct for population structure. The Dent population is showing a high familial relatedness, while the population structure of the Flint population indicating two subpopulations.

Genome-wide association mapping was done with the general linear model, corrected for population structure and the mixed linear model, correcting for population structure and familial relatedness in the program TASSEL Version 5.0.

The general linear model is showing several statistically significant associations between marker allels and variation in sugar content and chlorophyll content, which are supported by the mixed linear model. For the sugar content of the stover (BRIX) the Dent population is showing significant associations on chromosome 2 and chromosome 4. The Flint population is showing associations for the sugar content on chromosome 1. ‘Stay-green’ behavior was measured two times, eight weeks before harvest and one week before harvest. For the first measurement (eight weeks before harvest) associations are found on chromosome 2 and chromosome 3 for the Dent lines, while the Flint lines are showing no significant associations. For the second measurement (one week before harvest) both populations are showing associations on chromosome 2. The Dent lines are showing as well on chromosome 8 associations, while the Flint lines are having associations on chromosome 9 as well. Furthermore it is shown that the findings are supported by already done studies, finding QTLs for stay-green behavior and sugar content in the stover.

Identifying methods of breeding for dual use maize have been the main objective. Switching from grain maize or energy maize production to grain maize and energy maize production is a great option, because of the high environmental and economic value for farmers. To become a meaningful alternative to mitigate the conflict between food and energy production more research should be investigated in better harvest systems and silaging of maize stover in biogas plants, by analyzing the methane yield of the stover. Usage of additional trace elements for stable biogas production and editing other co-substrates has to be analyzed as well. The genetic background of the traits sugar content of the stover and stay-green behavior should be studied further, with developing specific markers and QTL mapping to get a powerful breeding tool.

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VII. Appendix

Table VII.1: Entry Name and further information about the mother lines of experiment 1, Dent genepool

Entry Name	Mother Line	Father Line	Information Mother Line	Entry Name	Mother Line	Father Line	Information Mother Line
RICARDINIO				**KXB3352**			
2	DENT	FLINT	156+158/2	**52**	DENT	FLINT	156+158/52
KXB3331				**53**	DENT	FLINT	156+158/53
4	DENT	FLINT	156+158/4	**54**	DENT	FLINT	156+158/54
5	DENT	FLINT	156+158/5	**55**	DENT	FLINT	156+158/55
6	DENT	FLINT	156+158/6	**56**	DENT	FLINT	156+158/56
7	DENT	FLINT	156+158/7	**57**	DENT	FLINT	156+158/57
8	DENT	FLINT	156+158/8	**58**	DENT	FLINT	156+158/58
9	DENT	FLINT	156+158/9	**59**	DENT	FLINT	156+158/59
10	DENT	FLINT	156+158/10	**60**	DENT	FLINT	156+158/60
COLISEE				**MILLESIM**			
12	DENT	FLINT	156+158/12	**62**	DENT	FLINT	156+158/62
13	DENT	FLINT	156+158/13	**63**	DENT	FLINT	156+158/63
14	DENT	FLINT	156+158/14	**64**	DENT	FLINT	156+158/64
15	DENT	FLINT	156+158/15	**65**	DENT	FLINT	156+158/65
16	DENT	FLINT	156+158/16	**66**	DENT	FLINT	156+158/66
17	DENT	FLINT	156+158/17	**67**	DENT	FLINT	156+158/67
18	DENT	FLINT	156+158/18	**68**	DENT	FLINT	156+158/68
19	DENT	FLINT	156+158/19	**69**	DENT	FLINT	156+158/69
20	DENT	FLINT	156+158/20	**70**	DENT	FLINT	156+158/70
KXB2007	.			**GROSSO**			
22	DENT	FLINT	156+158/22	**72**	DENT	FLINT	156+158/72
23	DENT	FLINT	156+158/23	**73**	DENT	FLINT	156+158/73
24	DENT	FLINT	156+158/24	**74**	DENT	FLINT	156+158/74
25	DENT	FLINT	156+158/25	**75**	DENT	FLINT	156+158/75
26	DENT	FLINT	156+158/26	**76**	DENT	FLINT	156+158/76
27	DENT	FLINT	156+158/27	**77**	DENT	FLINT	156+158/77
28	DENT	FLINT	156+158/28	**78**	DENT	FLINT	156+158/78
29	DENT	FLINT	156+158/29	**79**	DENT	FLINT	156+158/79
30	DENT	FLINT	156+158/30	**80**	DENT	FLINT	156+158/80
KXB3151				**KWS2322**			
32	DENT	FLINT	156+158/32	**82**	DENT	FLINT	156+158/82
33	DENT	FLINT	156+158/33	**83**	DENT	FLINT	156+158/83
34	DENT	FLINT	156+158/34	**84**	DENT	FLINT	156+158/84
35	DENT	FLINT	156+158/35	**85**	DENT	FLINT	156+158/85
36	DENT	FLINT	156+158/36	**86**	DENT	FLINT	156+158/86
37	DENT	FLINT	156+158/37	**87**	DENT	FLINT	156+158/87
38	DENT	FLINT	156+158/38	**88**	DENT	FLINT	156+158/88
39	DENT	FLINT	156+158/39	**89**	DENT	FLINT	156+158/89
40	DENT	FLINT	156+158/40	**90**	DENT	FLINT	156+158/90
SIMPATICO KWS				**KXB3229**			
42	DENT	FLINT	156+158/42	**92**	DENT	FLINT	156+158/92
43	DENT	FLINT	156+158/43	**93**	DENT	FLINT	156+158/93
44	DENT	FLINT	156+158/44	**94**	DENT	FLINT	156+158/94+95
45	DENT	FLINT	156+158/45	**95**	DENT	FLINT	156+158/94+95
46	DENT	FLINT	156+158/46	**96**	DENT	FLINT	156+158/
47	DENT	FLINT	156+158/47	**97**	DENT	FLINT	156+158/
48	DENT	FLINT	156+158/48	**98**	DENT	FLINT	156+158/98
49	DENT	FLINT	156+158/49	**99**	DENT	FLINT	156+158/99
50	DENT	FLINT	156+158/50	**100**	DENT	FLINT	156+158/100

Table VII.2: Entry Name and further information about the mother lines of experiment 2, Flint genepool

Entry Name	Mother Line	Father Line	Information Mother Line	Entry Name	Mother Line	Father Line	Information Mother Line
RICARDINIO				**KXB 3352**			
KXB 3331				**52**	FLINT	DENT	155+157/52
3	FLINT	DENT	155+157/3	**53**	FLINT	DENT	155+157/53
4	FLINT	DENT	155+157/4	**54**	FLINT	DENT	155+157/54
5	FLINT	DENT	155+157/5	**55**	FLINT	DENT	155+157/55
6	FLINT	DENT	155+157/6	**56**	FLINT	DENT	155+157/56
7	FLINT	DENT	155+157/7	**57**	FLINT	DENT	155+157/57
8	FLINT	DENT	155+157/8	**58**	FLINT	DENT	155+157/58
9	FLINT	DENT	155+157/9	**59**	FLINT	DENT	155+157/59
10	FLINT	DENT	155+157/10	**60**	FLINT	DENT	155+157/60
COLISEE				**MILLESIM**			
12	FLINT	DENT	155+157/12	**62**	FLINT	DENT	155+157/62
13	FLINT	DENT	155+157/13	**63**	FLINT	DENT	155+157/63
14	FLINT	DENT	155+157/14	**64**	FLINT	DENT	155+157/64
15	FLINT	DENT	155+157/15	**65**	FLINT	DENT	155+157/65
16	FLINT	DENT	155+157/16	**66**	FLINT	DENT	155+157/66
17	FLINT	DENT	155+157/17	**67**	FLINT	DENT	155+157/67
18	FLINT	DENT	155+157/18	**68**	FLINT	DENT	155+157/68
19	FLINT	DENT	155+157/19	**69**	FLINT	DENT	155+157/69
20	FLINT	DENT	155+157/20	**70**	FLINT	DENT	155+157/70
KXB 2007				**GROSSO**			
22	FLINT	DENT	155+157/22	**72**	FLINT	DENT	155+157/72
23	FLINT	DENT	155+157/23	**73**	FLINT	DENT	155+157/73
24	FLINT	DENT	155+157/24	**74**	FLINT	DENT	155+157/74
25	FLINT	DENT	155+157/25	**75**	FLINT	DENT	155+157/75
26	FLINT	DENT	155+157/26	**76**	FLINT	DENT	155+157/76
27	FLINT	DENT	155+157/27	**77**	FLINT	DENT	155+157/77
28	FLINT	DENT	155+157/28	**78**	FLINT	DENT	155+157/78
29	FLINT	DENT	155+157/29	**79**	FLINT	DENT	155+157/79
30	FLINT	DENT	155+157/30	**80**	FLINT	DENT	155+157/80
KXB 3151				**KWS 2322**			
32	FLINT	DENT	155+157/32	**82**	FLINT	DENT	155+157/82
33	FLINT	DENT	155+157/33	**83**	FLINT	DENT	155+157/83
34	FLINT	DENT	155+157/34	**84**	FLINT	DENT	155+157/84
35	FLINT	DENT	155+157/35	**85**	FLINT	DENT	155+157/85
36	FLINT	DENT	155+157/36	**86**	FLINT	DENT	155+157/86
37	FLINT	DENT	155+157/37	**87**	FLINT	DENT	155+157/87
38	FLINT	DENT	155+157/38	**88**	FLINT	DENT	155+157/88
39	FLINT	DENT	155+157/39	**89**	FLINT	DENT	155+157/89
40	FLINT	DENT	155+157/40	**90**	FLINT	DENT	155+157/90
SIMPATICO KWS				**KXB 3229**			
42	FLINT	DENT	155+157/42	**92**	FLINT	DENT	155+157/92
43	FLINT	DENT	155+157/43	**93**	FLINT	DENT	155+157/93
44	FLINT	DENT	155+157/44	**94**	FLINT	DENT	155+157/94
45	FLINT	DENT	155+157/45	**95**	FLINT	DENT	155+157/95
46	FLINT	DENT	155+157/46	**96**	FLINT	DENT	155+157/96
47	FLINT	DENT	155+157/47	**97**	FLINT	DENT	155+157/97
48	FLINT	DENT	155+157/48	**98**	FLINT	DENT	155+157/98
49	FLINT	DENT	155+157/49	**99**	FLINT	DENT	155+157/99
50	FLINT	DENT	155+157/50	**100**	FLINT	DENT	155+157/100

Table VII.3 List of Genotyps and belonging Genepool for the genome-wide association mapping

Genotype	Genepool	Genotype	Genepool	Genotype	Genepool	Genotype	Genepool
G14-156/2	Dent	G14-156/50	Dent	G14-155/3	Flint	G14-155/50	Flint
G14-156/4	Dent	G14-156/52	Dent	G14-155/4	Flint	G14-155/52	Flint
G14-156/5	Dent	G14-156/53	Dent	G14-155/5	Flint	G14-155/53	Flint
G14-156/6	Dent	G14-156/54	Dent	G14-155/6	Flint	G14-155/54	Flint
G14-156/7	Dent	G14-156/55	Dent	G14-155/7	Flint	G14-155/55	Flint
G14-156/9	Dent	G14-156/56	Dent	G14-155/8	Flint	G14-155/56	Flint
G14-156/10	Dent	G14-156/57	Dent	G14-155/9	Flint	G14-155/57	Flint
G14-156/12	Dent	G14-156/58	Dent	G14-155/100	Flint	G14-155/58	Flint
G14-156/13	Dent	G14-156/59	Dent	G14-155/12	Flint	G14-155/60	Flint
G14-156/14	Dent	G14-156/60	Dent	G14-155/13	Flint	G14-155/62	Flint
G14-156/15	Dent	G14-156/62	Dent	G14-155/14	Flint	G14-155/63	Flint
G14-156/16	Dent	G14-156/63	Dent	G14-155/15	Flint	G14-155/65	Flint
G14-156/17	Dent	G14-156/64	Dent	G14-155/16	Flint	G14-155/66	Flint
G14-156/18	Dent	G14-156/65	Dent	G14-155/17	Flint	G14-155/67	Flint
G14-156/19	Dent	G14-156/66	Dent	G14-155/18	Flint	G14-155/68	Flint
G14-156/20	Dent	G14-156/67	Dent	G14-155/19	Flint	G14-155/69	Flint
G14-156/22	Dent	G14-156/68	Dent	G14-155/20	Flint	G14-155/70	Flint
G14-156/23	Dent	G14-156/69	Dent	G14-155/22	Flint	G14-155/72	Flint
G14-156/24	Dent	G14-156/70	Dent	G14-155/24	Flint	G14-155/73	Flint
G14-156/25	Dent	G14-156/72	Dent	G14-155/25	Flint	G14-155/74	Flint
G14-156/26	Dent	G14-156/73	Dent	G14-155/26	Flint	G14-155/75	Flint
G14-156/27	Dent	G14-156/74	Dent	G14-155/27	Flint	G14-155/76	Flint
G14-156/28	Dent	G14-156/75	Dent	G14-155/28	Flint	G14-155/77	Flint
G14-156/29	Dent	G14-156/76	Dent	G14-155/29	Flint	G14-155/78	Flint
G14-156/30	Dent	G14-156/77	Dent	G14-155/30	Flint	G14-155/79	Flint
G14-156/32	Dent	G14-156/78	Dent	G14-155/32	Flint	G14-155/80	Flint
G14-156/33	Dent	G14-156/79	Dent	G14-155/33	Flint	G14-155/82	Flint
G14-156/34	Dent	G14-156/80	Dent	G14-155/34	Flint	G14-155/83	Flint
G14-156/35	Dent	G14-156/82	Dent	G14-155/35	Flint	G14-155/84	Flint

Genotype	Genepool	Genotype	Genepool	Genotype	Genepool	Genotype	Genepool
G14-156/36	**Dent**	**G14-156/83**	**Dent**	**G14-155/36**	**Flint**	**G14-155/85**	**Flint**
G14-156/38	**Dent**	**G14-156/84**	**Dent**	**G14-155/37**	**Flint**	**G14-155/86**	**Flint**
G14-156/39	**Dent**	**G14-156/85**	**Dent**	**G14-155/39**	**Flint**	**G14-155/87**	**Flint**
G14-156/40	**Dent**	**G14-156/86**	**Dent**	**G14-155/40**	**Flint**	**G14-155/88**	**Flint**
G14-156/42	**Dent**	**G14-156/87**	**Dent**	**G14-155/42**	**Flint**	**G14-155/89**	**Flint**
G14-156/43	**Dent**	**G14-156/88**	**Dent**	**G14-155/43**	**Flint**	**G14-155/90**	**Flint**
G14-156/44	**Dent**	**G14-156/89**	**Dent**	**G14-155/44**	**Flint**	**G14-155/92**	**Flint**
G14-156/45	**Dent**	**G14-156/90**	**Dent**	**G14-155/45**	**Flint**	**G14-155/93**	**Flint**
G14-156/46	**Dent**	**G14-156/92**	**Dent**	**G14-155/46**	**Flint**	**G14-155/94**	**Flint**
G14-156/47	**Dent**	**G14-156/93**	**Dent**	**G14-155/47**	**Flint**	**G14-155/96**	**Flint**
G14-156/48	**Dent**	**G14-156/94**	**Dent**	**G14-155/48**	**Flint**	**G14-155/97**	**Flint**
G14-156/49	**Dent**	G14-156/37	Dent	**G14-155/49**	**Flint**	**G14-155/98**	**Flint**
G14-156/8	Dent	G14-156/98	Dent	**G14-155/99**	**Flint**	G14-155/10	Flint

Table VII.4 Analysis of Variance for the trait number of plants per plot (STACO) during silage maize harvest of the Dent Genepool

Source	DF	MS	Var.cp	F-value	LSD5
Location	1	1689.9212	13.9375	396.48**	0.58
Genotype	98	22.0870	8.9123	5.18**	4.10
Location-Genotype	98	4.2623	-0.5890	0.88	6.14
Error	192	4.8513	4.8153		
Heritability 80%					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

Table VII.5 Analysis of Variance for the trait Total fresh matter (TFMs) in dt/ha during silage maize harvest of the Dent Genepool

Source	DF	MS	Var.cp	F-value	LSD5
Location	1	1084.5901	10.9070	226.15**	0.62
Genotype	98	21.4898	8.3469	4.48**	4.35
Location-Genotype	98	4.7959	1.1429	1.31+	5.34
Error	156	3.6530	3.6530		
Heritability 78%					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

Table VII.6 Analysis of Variance for the trait Total fresh matter (TFMs) in dt/ha during dual use maize harvest of the Dent Genepool

Source	DF	MS	Var.cp	F-value	LSD5
Location	2	276.7558	2.6831	32.75**	0.81
Genotype	99	33.2866	8.2789	3.94**	4.68
Location-Genotype	196	8.4500	3.1227	1.59**	6.44
Error	205	5.3273	5.3273		
Heritability 75%					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

Table VII.7 Analysis of Variance for the trait Total dry matter content (TDC) in % during dual use maize harvest of the Dent Genepool

Source	DF	MS	Var.cp	F-value	LSD5
Location	2	198.2722	1.9426	49.44**	0.56
Genotype	99	24.9520	6.9805	6.22**	3.22
Location-Genotype	196	4.0106	1.2193	1.44**	4.66
Error	205	2.7912	2.7912		
Heritability 84%					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

Table VII.8 Analysis of Variance for the trait Grain fresh matter (GFM) in dt/ha during dual use maize harvest of the Dent Genepool

Source	DF	MS	Var.cp	F-value	LSD5
Location	2	92.3904	0.9169	131.07**	0.23
Genotype	99	2.1969	0.4973	3.12**	1.35
Location-Genotype	196	0.7049	0.3255	1.86**	1.72
Error	205	0.3794	0.3794		
Heritability 68%					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

Table VII.9 Analysis of Variance for the trait Stover fresh matter (SFM) in dt/ha during dual use maize harvest of the Dent Genepool

Source	DF	MS	Var.cp	F-value	LSD5
Location	2	6672.7031	58.2806	7.90**	8.11
Genotype	99	3198.0372	784.4656	3.79**	46.80
Location-Genotype	196	844.6403	257.8539	1.44**	67.54
Error	205	586.7864	586.7864		
Heritability 74%					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

Table VII.10 Analysis of Variance for the trait Stover dry matter content (SDC) in % during dual use maize harvest of the Dent Genepool

Source	DF	MS	Var.cp	F-value	LSD5
Location	2	529.8326	5.1835	46.15**	0.94
Genotype	99	25.0527	4.5241	2.18**	5.46
Location-Genotype	196	11.4803	4.2187	1.58**	7.51
Error	240	7.2617	7.2617		
Heritability 54%					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

Table VII.11 Analysis of Variance for the trait Total fresh matter (TFM) in dt/ha during silage maize harvest of the Flint Genepool

Source	DF	MS	Var.cp	F-value	LSD5
Location	2	1849.0170	18.4190	259.82**	0.74
Genotype	99	38.9165	10.5999	5.47**	4.30
Location-Genotype	198	7.1166	2.4059	1.51**	6.05
Error	241	4.7108	4.7108		
Heritability 82%					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

Table VII.12 Analysis of Variance for the trait Total fresh matter (TFM) in dt/ha during dual use maize harvest of the Flint Genepool

Source	DF	MS	Var.cp	F-value	LSD5
Location	1	429.5967	4.2119	51.07**	0.81
Genotype	99	24.0641	7.8264	2.86**	5.75
Location-Genotype	99	8.4113	3.5293	1.72**	6.17
Error	148	4.8820	4.8820		
Heritability 65%					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

Table VII.13 Analysis of Variance for the trait Total dry matter content (TDC) in % during dual use maize harvest of the Flint Genepool

Source	DF	MS	Var.cp	F-value	LSD5
Location	1	11.6403	0.0803	3.22+	0.53
Genotype	99	12.9490	4.6684	3.58**	3.77
Location-Genotype	99	3.6122	2.2060	2.57**	3.31
Error	166	1.4063	1.4063		
Heritability 72%					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

Table VII.14 Analysis of Variance for the trait Grain fresh matter (GFM) in dt/ha during dual use maize harvest of the Flint Genepool

Source	DF	MS	Var.cp	F-value	LSD5
Location	1	103.8817	1.0317	146.00**	0.24
Genotype	99	1.6763	0.4824	2.36**	1.67
Location-Genotype	99	0.7115	0.3903	2.21**	1.58
Error	148	0.3213	0.3213		
Heritability 58%					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

Table VII.15 Analysis of Variance for the trait Total dry matter yield (TDY) in dt/ha during dual use maize harvest of the Flint Genepool

Source	DF	MS	Var.cp	F-value	LSD5
Location	1	7917.3495	77.0760	37.75**	4.06
Genotype	99	271.8691	31.0574	1.30+	28.74
Location-Genotype	99	209.7543	84.0891	1.67**	31.33
Error	148	125.6652	125.6652		
Heritability 23%					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

Table VII.16 Analysis of Variance for the trait Stover fresh matter (SFM) in dt/ha during dual use maize harvest of the Flint Genepool

Source	DF	MS	Var.cp	F-value	LSD5
Location	1	13700.5861	128.9416	16.99**	7.97
Genotype	99	2180.4049	686.9898	2.70**	56.35
Location-Genotype	99	806.4252	245.6464	1.44**	66.18
Error	148	560.7788	560.7788		
Heritability 63%					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

Table VII.17 Analysis of Variance for the trait Stover dry matter content (SDC) in % during dual use maize harvest of the Flint Genepool

Source	DF	MS	Var.cp	F-value	LSD5
Location	1	85.9506	0.7535	8.11**	0.91
Genotype	99	15.5584	2.4779	1.47*	6.46
Location-Genotype	99	10.6026	4.2261	1.66**	7.05
Error	162	6.3765	6.3765		
Heritability 32%					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

Table VII.18 Analysis of Variance for the trait Total fresh matter (TFM) in dt/ha during silage maize harvest of the factorial crosses

Source	DF	MS	Var.cp	F-value	LSD5
Location	2	8159.2491	81.4311	505.48**	1.12
Genotype	99	38.2701	7.3762	2.37**	6.47
Location-Genotype	198	16.1417	7.9120	1.96**	7.99
Error	257	8.2297	8.2297		
Heritability 58%					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

Table VII.19 Analysis of Variance for the trait Total fresh matter (TFM) in dt/ha during dual use maize harvest of the factorial crosses

Source	DF	MS	Var.cp	F-value	LSD5
Location	1	6360.4535	63.4360	377.45**	1.15
Genotype	99	31.4090	7.2790	1.86**	8.15
Location-Genotype	99	16.8511	8.2822	1.97**	8.18
Error	160	8.5688	8.5688		
Heritability 46%					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

Table VII.20 Analysis of Variance for the trait Total dry matter content (TDC) in % during dual use maize harvest of the factorial crosses

Source	DF	MS	Var.cp	F-value	LSD5
Location	1	4557.1042	45.4021	269.72**	1.15
Genotype	99	30.3769	6.7407	1.80**	8.16
Location-Genotype	97	16.8955	9.8321	2.39**	7.43
Error	136	7.0635	7.0635		
Heritability 44%					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

Table VII.21 Analysis of Variance for the trait Total dry matter yield (TDY) in % during dual use maize harvest of the factorial crosses

Source	DF	MS	Var.cp	F-value	LSD5
Location	1	33241.7301	328.9674	96.36**	5.21
Genotype	99	449.0184	52.0138	1.30+	36.86
Location-Genotype	97	344.9908	173.0403	2.01**	36.67
Error	136	171.9505	171.9505		
Heritability 23%					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

Table VII.22 Analysis of Variance for the trait Grain fresh matter (GFM) in dt/ha during dual use maize harvest of the factorial crosses

Source	DF	MS	Var.cp	F-value	LSD5
Location	1	849.8024	8.4815	515.30**	0.36
Genotype	99	2.8088	0.5765	1.70**	2.55
Location-Genotype	98	1.6492	0.7845	1.91**	2.60
Error	159	0.8647	0.8647		
Heritability 41%					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

Table VII.23 Analysis of Variance for the trait Stover fresh matter (SFM) in dt/ha during dual use maize harvest of the factorial crosses

Source	DF	MS	Var.cp	F-value	LSD5
Location	1	319012.4701	3177.2499	247.78**	10.07
Genotype	99	3015.7732	864.1475	2.34**	71.21
Location-Genotype	98	1287.4782	589.8396	1.85**	73.77
Error	159	697.6386	697.6386		
Heritability 57%					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

Table VII.24 Analysis of Variance for the trait Stover dry matter content (SDC) in % during dual use maize harvest of the factorial crosses

Source	DF	MS	Var.cp	F-value	LSD5
Location	1	8418.5344	83.8571	256.45**	1.61
Genotype	99	41.0762	4.1244	1.25	11.37
Location-Genotype	96	32.8273	17.2355	2.11**	11.04
Error	136	15.5918	15.5918		
Heritability 20%					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

Table VII.25 Analysis of Variance for the trait BRIX above corn cob measuring time 1 (BRIXa1) in %BRIX of the factorial crosses

Source	DF	MS	Var.cp	F-value	LSD5
Year	1	206.7413	1.0295	244.55**	0.18
Location	1	36.3308	-0.3212	0.36	12.74
Location-Year	1	100.5708	0.9973	118.96**	0.26
Genotype	99	2.4496	0.3710	2.54**	1.38
Genotype-Location	99	0.8026	-0.0214	0.95	1.82
Genotype-Year	99	0.9656	0.0601	1.14	1.82
Genotype-Location-Year	99	0.8454	0.2014	1.31*	2.23
Error	342	0.6440	0.6440		
Heritability 61%					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

Table VII.26 Analysis of Variance for the trait BRIX below corn cob measuring time 1 (BRIXb1) in %BRIX of the factorial crosses

Source	DF	MS	Var.cp	F-value	LSD5
Year	1	179.1716	0.8925	270.81**	0.16
Location	1	6.6952	-0.8475	0.04	16.87
Location-Year	1	176.1858	1.7552	266.29**	0.23
Genotype	99	1.9459	0.2942	2.53**	1.23
Genotype-Location	99	0.6500	-0.058	0.98	1.61
Genotype-Year	99	0.7691	0.0537	1.16	1.61
Genotype-Location-Year	99	0.6616	0.1602	1.32*	1.97
Error	342	0.5015	0.5015		
Heritability 60%					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

Table VII.27 Analysis of Variance for the trait BRIX total measuring time 1 (BRIX1) in %BRIX of the factorial crosses

Source	DF	MS	Var.cp	F-value	LSD5
Year	1	192.688	192.6822	349.95**	0.15
Location	1	18.5675	-0.5858	0.14	14.80
Location-Year	1	135.7225	1.3517	246.50**	0.21
Genotype	99	1.9124	0.3335	3.31**	1.07
Genotype-Location	99	0.5288	-0.0109	0.96	1.47
Genotype-Year	99	0.5785	0.0139	1.05	1.47
Genotype-Location-Year	99	0.5506	0.1585	1.40*	1.74
Error	342	0.3921	0.3921		
Heritability 70%					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

Table VII.28 Analysis of Variance for the trait BRIX above corn cob measuring time 2 (BRIXa2) in %BRIX of the factorial crosses

Source	DF	MS	Var.cp	F-value	LSD5
Year	1	402.6243	2.0083	417.86**	0.19
Location	1	129.9942	-0.1465	0.82	16.04
Location-Year	1	159.3023	1.5834	165.33**	0.28
Genotype	99	2.9203	0.3200	1.78**	1.80
Genotype-Location	99	1.1838	0.1101	1.23	1.95
Genotype-Year	99	1.6403	0.3384	1.70**	1.95
Genotype-Location-Year	99	0.9635	0.0337	1.04	2.68
Error	341	0.9298	0.9298		
Heritability 44%					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

Table VII.29 Analysis of Variance for the trait BRIX below corn cob measuring time 2 (BRIXb2) in %BRIX of the factorial crosses

Source	DF	MS	Var.cp	F-value	LSD5
Year	1	534.8119	2.6697	617.96**	0.18
Location	1	118.3091	-1.1455	0.34	23.68
Location-Year	1	347.4123	3.4655	401.42**	0.26
Genotype	99	4.3178	0.6759	2.67**	1.78
Genotype-Location	99	1.1354	0.1350	1.31+	1.85
Genotype-Year	99	1.6144	0.3745	1.87**	1.85
Genotype-Location-Year	99	0.8654	-0.1941	0.82	2.86
Error	341	1.0596	1.0596		
Heritability 63%					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

Table VII.30 Analysis of Variance for the trait BRIX above corn cob measuring time 2 (BRIXb2) in %BRIX of the factorial crosses

Source	DF	MS	Var.cp	F-value	LSD5
Year	2	865.7960	4.3242	912.30**	0.19
Location	1	253.0062	0.6229	3.83	2.86
Location-Year	2	66.1410	0.6519	69.69**	0.27
Genotype	99	1.8254	0.0970	1.47**	1.27
Genotype-Location	99	0.9261	-0.0076	0.98	1.57
Genotype-Year	198	1.2434	0.1472	1.31*	1.92
Genotype-Location-Year	198	0.9490	0.2147	1.29*	2.38
Error	485	0.7343	0.7343		
Heritability 32%					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

Table VII.31 Analysis of Variance for the trait BRIX below corn cob measuring time 2 (BRIXb2) in %BRIX of the factorial crosses

Source	DF	MS	Var.cp	F-value	LSD5
Year	2	1111.2081	5.5505	1010.71**	0.21
Location	1	240.3121	-0.0678	0.92	5.67
Location-Year	2	260.6455	2.5955	237.07**	0.29
Genotype	99	2.6624	0.1934	1.77**	1.40
Genotype-Location	99	0.8815	-0.0726	0.80	1.69
Genotype-Year	198	1.5017	0.2011	1.37*	2.07
Genotype-Location-Year	198	1.0994	0.2902	1.36**	2.50
Error	486	0.8092	0.8092		
Heritability 44%					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

Table VII.32 Analysis of Variance for the trait BRIX above corn cob measuring time 2 (BRIXa2) in %BRIX of the factorial crosses

Source	DF	MS	Var.cp	F-value	LSD5
Year	2	350.1997	1.7458	334.55**	0.20
Location	1	335.2238	0.3965	1.55	5.17
Location-Year	2	216.2887	2.1524	206.62**	0.29
Genotype	99	3.2678	0.3299	2.54**	1.29
Genotype-Location	99	1.2423	.0679	1.19	1.65
Genotype-Year	198	1.2883	0.1207	1.23+	2.02
Genotype-Location-Year	198	1.0468	0.3800	1.57**	2.27
Error	485	0.6668	0.6668		
Heritability 61%					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

Table VII.33 Analysis of Variance for the trait BRIX below corn cob measuring time 2 (BRIXb2) in %BRIX of the factorial crosses

Source	DF	MS	Var.cp	F-value	LSD5
Year	2	362.0483	1.8029	246.59**	0.24
Location	1	324.3997	0.0499	1.05	6.18
Location-Year	2	309.4310	3.0796	210.75**	0.34
Genotype	99	4.1201	0.3648	2.13**	1.58
Genotype-Location	99	1.6042	0.0453	1.09	1.95
Genotype-Year	198	1.9312	0.2315	1.32*	2.39
Genotype-Location-Year	198	1.4682	0.5273	1.56**	2.70
Error	485	0.9409	0.9409		
Heritability 53%					

DF: Degree of Freedom; MS: Mean square; Var.cp: Variance component; F: F-value significance level *p=0.05, **p=0.01; +p=0.1; LSD5: least significant difference

Table VII.34 Table of Correlation for all traits of the Dent testcrosses

Total dry matter content[a]	-0.82**																			
Total dry matter yield[a]	0.65**	-0.11																		
Grain fresh matter[a]	0.57**	-0.31**	0.62**																	
Grain dry matter content[a]	-0.58**	0.66	-0.16	-0.45**																
Grain dry matter yield[a]	0.43**	-0.11	0.63**	0.95**	-0.15															
Stover dry matter yield	0.49**	-0.07	0.75**	-0.01	-0.08	-0.04														
Stover fresh matter	0.97**	-0.85**	0.56**	0.36**	-0.53**	0.21*	0.56**													
Stover dry matter content	-0.57**	0.86**	0.13	-0.44**	0.53**	-0.30**	0.40**	-0.52**												
Water content of stover	0.57**	-0.86**	-0.13	0.44**	-0.53**	0.30	-0.40**	0.52**	-1.00**											
BRIX 1 above corn cob[b]	0.22*	-0.22*	0.08	0.05	-0.12	0.00	0.12	0.23*	-0.13	0.17										
BRIX 1 below corn cob[b]	0.14	-0.10	0.13	0.16	-0.18	0.12	0.08	0.11	-0.06	0.06	0.76**									
BRIX 1	0.19	-0.16	0.12	0.11	-0.17	0.07	0.10	0.18	-0.09	0.09	0.93**	0.94**								
BRIX 2 above corn cob[b]	0.45*	-0.40**	0.24*	0.21*	-0.23*	0.12	0.20*	0.45**	-0.28**	0.28**	0.40**	0.40**	0.43**							
BRIX 2 below corn cob[b]	0.52**	-0.49**	0.25*	0.25*	-0.31**	0.16	0.20*	0.52**	-0.36**	0.36**	0.35**	0.31**	0.36**	0.87**						
BRIX 2	0.50**	-0.46**	0.26*	0.24*	-0.28**	0.15	0.21*	0.50**	-0.33**	0.33**	0.39**	0.37**	0.41**	0.96**	0.97**					
SPAD 1[b]	0.01	-0.05	-0.02	0.05	-0.09	0.02	-0.05	0.00	-0.06	0.06	0.00	0.00	0.00	0.18	0.17	0.18				
SPAD 2[b]	0.23**	-0.26*	0.12	0.23*	-0.32**	0.14	0.08	0.26**	-0.19	0.19	0.22*	0.20*	0.22*	0.16	0.30**	0.24*	0.32**			
Total dry matter content[c]	-0.80**	-0.80**	-0.31**	-0.33**	0.65**	-0.12	-0.30**	-0.80**	0.58**	-0.58*	-0.18	-0.20	-0.20*	-0.33**	-0.43**	-0.40**	0.01	-0.27**		
Total fresh matter[c]	0.74**	-0.57**	0.55**	0.55**	-0.53**	0.41*	0.35**	0.68**	-0.40**	0.40**	0.14	0.17	0.17	0.36**	0.41**	0.40**	0.01	0.24*	-0.72**	
Total dry matter yield[c]	0.23*	0.03	0.47**	0.45	-0.08	0.47**	0.20	0.13	0.04	-0.04	0.02	0.05	0.04	0.17	0.14	0.16	0.06	0.06	0.02	0.67**
	Total fresh matter[a]	**Total dry matter content[a]**	**Total dry matter yield[a]**	**Grain fresh matter[a]**	**Grain dry matter content[a]**	**Grain dry matter yield[a]**	**Stover dry matter yield**	**Stover fresh matter**	**Stover dry matter content**	**Water content of stover**	**BRIX 1 above corn cob[b]**	**BRIX 1 below corn cob[b]**	**BRIX 1**	**BRIX 2 above corn cob[b]**	**BRIX 2 below corn cob[b]**	**BRIX 2**	**SPAD 1[b]**	**SPAD 2[b]**	**Total dry matter content[c]**	**Total fresh matter[c]**

[a] traits are taken during dual use maize harvest/ [b] traits are taken during observation tests/ [c] traits are taken during silage maize harvest/ Significance level: **p=0.01, *p=0.05, +p=0.1

Table VII.35 Table of Correlation for all traits of the Flint testcrosses

	Total fresh matter[a]	Total dry matter content[a]	Total dry matter yield[a]	Grain fresh matter[a]	Grain dry matter content[a]	Grain dry matter yield[a]	Stover dry matter yield	Stover fresh matter	Stover dry matter content	Water content of stover	BRIX 1 above corn cob[b]	BRIX 1 below corn cob[b]	BRIX 1	BRIX 2 above corn cob[b]	BRIX 2 below corn cob[b]	BRIX 2	SPAD 1[b]	SPAD 2[b]	Total dry matter content[c]	Total fresh matter[c]
Total dry matter content[a]	-0.76**																			
Total dry matter yield[a]	0.82**	-0.26**																		
Grain fresh matter[a]	0.64**	-0.39**	0.61**																	
Grain dry matter content[a]	-0.52**	0.40**	-0.44**	-0.73**																
Grain dry matter yield[a]	0.60**	-0.33**	0.60**	0.94**	-0.47**															
Stover dry matter yield	0.63**	-0.12	0.84**	0.14	-0.21*	0.14														
Stover fresh matter	0.97**	-0.77**	0.77**	0.43**	-0.38**	0.41**	0.69**													
Stover dry matter content	-0.29**	0.76**	0.25*	-0.32**	0.16	-0.32**	0.52**	-0.24*												
Water content of stover	0.29**	-0.76**	-0.25*	0.32**	-0.16	0.-32**	-0.52**	0.24*	-1.00**											
BRIX 1 above corn cob[b]	0.9	-0.06	0.08	-0.04	0.00	0.06	0.11	0.12	0.02	-0.02										
BRIX 1 below corn cob[b]	0.06	-0.06	0.05	-0.04	-0.04	-0.07	0.08	0.09	0.00	0.00	0.78**									
BRIX 1	0.08	-0.06	0.07	-0.04	-0.01	-0.07	0.10	0.11	0.01	-0.01	0.94**	0.94**								
BRIX 2 above corn cob[b]	0.14	-0.15	0.08	-0.06	0.13	-0.02	0.10	0.19	-0.08	0.08	0.47**	0.32**	0.42**							
BRIX 2 below corn cob[b]	0.16	-0.17	0.09	-0.07	0.05	-0.07	0.16	0.21*	-0.05	0.05	0.49**	0.41**	0.48**	0.79**						
BRIX 2	0.17	-0.15	0.09	-0.06	0.09	-0.04	0.15	0.21*	-0.07	0.07	0.50**	0.39**	0.47**	0.94**	0.95**					
SPAD 1[b]	0.30**	-0.30**	0.19	0.02	0.07	0.06	0.19	0.34**	0.00	0.00	0.00	0.06	0.05	0.18	0.21*	0.20*				
SPAD 2[b]	0.50**	-0.45**	0.34*	0.22**	-0.17	0.27*	0.29**	0.51**	0.19*	0.20*	0.08	0.19	0.15	0.19	0.29**	0.25*	0.60**			
Total dry matter content[c]	-0.66**	0.76**	-0.31**	-0.41**	0.46**	-0.34**	-0.20*	-0.64**	0.50**	-0.50*	-0.14	-0.21*	-0.18	-0.08	-0.19	-0.15	-0.26**	-0.50**		
Total fresh matter[c]	0.72**	-0.59**	0.55**	0.51**	-0.51**	0.43**	0.40**	0.68**	-0.25*	0.25*	0.09	0.10	0.10	0.12	0.18	0.16	0.24*	0.42**	-0.72***	
Total dry matter yield[c]	0.44**	-0.17	0.51**	0.36**	-0.31**	0.30**	0.41**	0.41**	0.08	-0.08	0.02	-0.02	0.01	0.10	0.10	0.11	0.13	0.18	-0.15	0.80**

[a] traits are taken during dual use maize harvest/ [b] traits are taken during observation tests/ [c] traits are taken during silage maize harvest/ Significance level: **p=0.01, *p=0.05, +p=0.1

Table VII.36 Table of Correlation for all traits of the factorial test crosses

	Total fresh matter[a]	Total dry matter content[a]	Total dry matter yield[a]	Grain fresh matter[a]	Grain dry matter content[a]	Grain dry matter yield[a]	Stover dry matter yield	Stover fresh matter	Stover dry matter content	Water content of stover	BRIX 1 above corn cob[b]	BRIX 1 below corn cob[b]	BRIX 1	BRIX 2 above corn cob[b]	BRIX 2 below corn cob[b]	BRIX 2	SPAD 1[b]	SPAD 2[b]	Total dry matter content[c]	Total fresh matter[c]
Total dry matter content[a]	-0.68**																			
Total dry matter yield[a]	0.76**	-0.14																		
Grain fresh matter[a]	0.52**	-0.28**	0.51**																	
Grain dry matter content[a]	-0.60**	0.62**	-0.29**	-0.59**																
Grain dry matter yield[a]	0.42**	-0.14	0.49**	0.96**	-0.36**															
Stover dry matter yield	0.62**	-0.11	0.77**	-0.09	-0.12	-0.16														
Stover fresh matter	0.96**	0.68**	0.69**	0.26**	-0.49**	0.14	0.74**													
Stover dry matter content	-0.29**	0.79**	0.18	-0.44**	0.43**	-0.40**	0.46**	-0.18												
Water content of stover	0.29**	-0.79**	-0.18	0.44**	-0.43**	0.40**	-0.46**	0.18	-1.00**											
BRIX 1 above corn cob[b]	0.27**	0.19	0.23*	0.06	-0.33**	-0.03	0.29**	0.29**	0.01	-0.01										
BRIX 1 below corn cob[b]	0.27**	-0.14	0.27**	0.03	-0.35**	-0.10	0.38**	0.30**	0.15	-0.15	0.74**									
BRIX 1	0.29**	-0.18	0.26**	0.04	-0.37**	-0.07	0.36**	0.32**	0.08	-0.08	0.94**	0.93**								
BRIX 2 above corn cob[b]	0.14	0.14	0.09	-0.16	-0.14	-0.23*	0.28**	0.22**	0.08	-0.08	0.54**	0.49**	0.56**							
BRIX 2 below corn cob[b]	0.17	0.07	0.20	-0.11	-0.11	-0.17	0.35**	0.23*	0.16	-0.16	0.56**	0.57**	0.61**	0.89**						
BRIX 2	0.16	-0.01	0.15	-0.13	-0.13	-0.20*	0.33**	0.23*	0.13	-0.13	0.57**	0.55**	0.60**	0.97**	0.98**					
SPAD 1[b]	-0.34**	0.26**	-0.27**	-0.18	0.20*	-0.14	-0.22*	-0.33*	0.15	-0.15	0.06	-0.12	-0.03	0.00	0.00	0.00				
SPAD 2[b]	0.03	0.08	0.10	-0.08	0.01	-0.10	0.21*	0.06	0.21*	-0.21*	0.22*	0.19	0.22*	0.31**	0.36**	0.34**	0.45**			
Total dry matter content[c]	-0.59**	0.67**	0.25*	-0.17	0.60**	-0.01	-0.34**	-0.61**	0.35**	-0.35**	-0.31**	-0.35**	-0.36**	-0.21*	-0.22*	-0.22*	0.25*	-0.09		
Total fresh matter[c]	0.64**	-0.57**	0.38**	0.26*	-0.55**	0.12	0.41**	0.64**	-0.24*	0.24*	0.27**	0.36**	0.33**	0.22*	0.22*	0.22*	-0.36**	-0.05	-0.75**	
Total dry matter yield[c]	0.36**	-0.12	0.38**	0.25**	-0.23**	0.21**	0.30**	0.33**	0.06	-0.06	0.07	0.19	0.14	0.00	0.00	0.00	-0.27**	-0.12	-0.11	0.70**

[a] traits are taken during dual use maize harvest/ [b] traits are taken during observation tests/ [c] traits are taken during silage maize harvest/ Significance level: **p=0.01, *p=0.05, +p=0.1

Table VII.37 General linear model output for the Dent lines of the trait BRIX for the significant markers

Marker	Chromosome	Position (bp)	Marker F-value	Marker p-value	Signficance level	Marker Rsq	Additive F-value	Additive p-value	Marker DF	Marker MS	Error DF	Error MS	Model DF	Model MS	Minor Observations
SYN24153	2	205290868	20.44	0.0000248	0.0000749	0.18080	20.44014	2.48E-05	1	4.56810	69	0.22349	11	0.89504	39
SYN1509	2	205429390	20.44	0.0000248	0.0001497	0.18080	20.44014	2.48E-05	1	4.56810	69	0.22349	11	0.89504	39
SYN5375	2	205085470	19.11	0.0000427	0.0002246	0.17157	19.10741	4.27E-05	1	4.33484	69	0.22687	11	0.87384	37
PZE-102157814	2	205138853	19.11	0.0000427	0.0002994	0.17157	19.10741	4.27E-05	1	4.33484	69	0.22687	11	0.87384	37
SYN24149	2	205357748	19.11	0.0000427	0.0003743	0.17157	19.10741	4.27E-05	1	4.33484	69	0.22687	11	0.87384	37
SYN12074	2	205144830	16.70	0.0001164	0.0004491	0.15418	16.70187	1.16E-04	1	3.89546	69	0.23323	11	0.83389	38
PZE-104110312	4	186766394	14.47	0.0003040	0.0005240	0.13717	14.47304	3.04E-04	1	3.46575	69	0.23946	11	0.79483	5

Table VII.38 Mixed linear model output for the Dent lines of the trait BRIX for the significant markers

Marker	Chromosome	Position (bp)	Degree of freedom	Marker F-value	Marker p-value	Signficance level	Error DF	Marker R^2	Genetic Variance	-2Ln Likelihood
SYN24153	2	205290868	1	13.32707	0.0005047	0.0000749	81	0.16097	0.27671	0.26151
SYN1509	2	205429390	1	13.32707	0.0005047	0.0001497	81	0.16097	0.27671	0.26151
SYN5375	2	205085470	1	12.15239	0.0008570	0.0002246	81	0.14678	0.27671	0.26151
PZE-102157814	2	205138853	1	12.15239	0.0008570	0.0002994	81	0.14678	0.27671	0.26151
SYN24149	2	205357748	1	12.15239	0.0008570	0.0003743	81	0.14678	0.27671	0.26151
SYN12074	2	205144830	1	10.61459	0.0017400	0.0004491	81	0.12820	0.27671	0.26151
PZE-104110312	4	186766394	1	10.39098	0.0019400	0.0006737	81	0.12550	0.27671	0.26151

Table VII.39 General linear model output for the Dent lines of the trait SPAD1 for the markers showing a tendency to significance

Marker	Chromosome	Position (bp)	Marker F-value	Marker p-value	Signficance level	Marker Rsq	Additive F-value	Additive p-value	Marker DF	Marker MS	Error DF	Error MS	Model DF	Model MS	Minor Observations
SYN34350	3	222837682	13.99696	0.0003749	0.0000749	0.13367	13.99696	0.0003749	1	13,75395	69	0,98264	11	3,19048	20
PZE-102062746	2	41853032	13.65173	0.0004368	0.0001497	0.13091	13.65173	0.0004368	1	13,47074	69	0,98674	11	3,16473	25

Table VII.40 Mixed linear model output for the Dent lines of the trait SPAD1 for the markers showing a tendency to significance

Marker	Chromosome	Position (bp)	Degree of freedom	Marker F-value	Marker p-value	Signficance level	Error DF	Marker R^2	Genetic Variance	-2Ln Likelihood
SYN34350	3	222837682	1	10.2666	0.00205	0.0000749	81	0.13257	0.35995	0.69624
PZE-102062746	2	41853032	1	7.41014	0.0082	0.000598802	81	0.09568	0.35995	0.69624

Table VII.41 General linear model output for the Dent lines of the trait SPAD8 for the markers showing a tendency to significance

Marker	Chromosome	Position (bp)	Marker F-value	Marker p-value	Signficance level	Marker Rsq	Additive F-value	Additive p-value	Marker DF	Marker MS	Error DF	Error MS	Model DF	Model MS	Minor Observations
PZE-108105381	8	159526711	13.01541	0.0005802	0.0000749	0.12781	13.01541	5.80E-04	1	90.77752	69	6.97462	11	20.8182	7
PZE-102178194	2	221433785	12.76137	0.0006504	0.0001497	0.12571	12.76137	6.50E-04	1	89.28221	69	6.99629	11	20.6823	24
PZE-108104106	8	158942170	12.70587	0.0006669	0.0002246	0.12524	12.70587	6.67E-04	1	88.95435	69	7.00104	11	20.6525	9

Table VII.422 General linear model output for the Flint lines of the trait BRIX for the significant marker

Marker	Chromosome	Position (bp)	Marker F-value	Marker p-value	Signficance level	Marker Rsq	Additive F-value	Additive p-value	Marker DF	Marker MS	Error DF	Error MS	Model DF	Model MS	Minor Observations
PZE-101163539	1	206839486	18.17011	0.0000611	0.0000761	0.13766	18.17011	0.0000611	1	3.50319	71	0.1928	11	1.06896	38

Table VII.433 Mixed linear model output for the Dent lines of the trait SPAD8 for the markers showing a tendency to significance

Marker	Chromosome	Position (bp)	Degree of freedom	Marker F-value	Marker p-value	Signficance level	Error DF	Marker R^2	Genetic Variance	-2Ln Likelihood
PZE-108105381	8	159526711	1	10.66191	0.0017	0.0000749	81	0.13198	1.2925	7.23804
PZE-102178194	2	221433785	1	9.77044	0.00259	0.0001497	81	0.12094	1.2925	7.23804
PZE-108104106	8	158942170	1	9.19829	0.00341	0.0002994	81	0.11386	1.2925	7.23804

Table VII.44 Mixed linear model output for the Flint lines of the trait BRIX for the significant marker

Marker	Chromosome	Position (bp)	Degree of freedom	Marker F-value	Marker p-value	Signficance level	Error DF	Marker R^2	Genetic Variance	-2Ln Likelihood
PZE-101163539	1	206839486	1	14.85426	0.0002523	0.0000761	83	0.16442	0.13534	0.20659

Table VII.45 General linear model output for the Flint lines of the trait SPAD8 for the markers showing a tendency to signficance

Marker	Chromosome	Position (bp)	Marker F-value	Marker p-value	Signficance level	Marker Rsq	Additive F-value	Additive p-value	Marker DF	Marker MS	Error DF	Error MS	Model DF	Model MS	Minor Observa-tions
SYN15971	9	153876976	12.68324	0.0006635	0.0000761	0.12666	12.68324	0.0006635	1	87.00936	71	6.8602	11	18,17045	31
PZE-102155296	2	203383454	12.53115	0.0007109	0.0001521	0.12537	12.53115	0.0007109	1	86.12253	71	6.8727	11	18,08983	5
SYN19366	2	204173443	11.87299	0.0009603	0.0002282	0.11973	11.87299	0.0009603	1	82.24726	71	6.9273	11	17,73753	36

Table VII.46 Mixed linear model output for the Flint lines of the trait SPAD8 for the markers showing a tendency to signficance

Marker	Chromosome	Position (bp)	Degree of freedom	Marker F-value	Marker p-value	Signficance level	Error DF	Marker R^2	Genetic Variance	-2Ln Likelihood
SYN15971	9	153876976	1	9.43362	0.00302	0.000304298	83	0.11941	4.68488	4.72872
PZE-102155296	2	203383454	1	9.99955	0.00230	0.000228224	83	0.12658	4.68488	4.72872
SYN19366	2	204173443	1	10.60563	0.00173	0.0000761	83	0.13425	4.68488	4.72872

E r k l ä r u n g e n

1. Hiermit erkläre ich, dass diese Arbeit weder in gleicher noch in ähnlicher Form bereits anderen Prüfungsbehörden vorgelegen hat.

   Weiter erkläre ich, dass ich mich an keiner anderen Hochschule um einen Doktorgrad beworben habe.

   Göttingen, den .....

.....

   (Unterschrift)

2. Hiermit erkläre ich eidesstattlich, dass diese Dissertation selbständig und ohne unerlaubte Hilfe angefertigt wurde.

   Göttingen, den .....

.....

   (Unterschrift)

Curriculum Vitae

Personal Information

Luisa Pfalsdorf
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Career

- October 2014- July 2017: Georg-August Universität Göttingen
    - Scientific Stuff/ PhD-Student
        - Supervision of own Project
            - Breeding for Dual Use Maize
        - Work with Technicians and Stuff
        - Cooperation with KWS SAAT SE
- March 2014 – August 2014: Wasservogelreservat Wallnau NABU
    - Scientific Stuff/ Student
        - Supervision of own Project
            - Data Collection and Analysis with Live Monitoring
        - Guided Tours for Children and Adult
        - Monitoring of Birds inside the Reservation
        - Biotope Maintenance
        - Monitoring Assistance in neighbored Reservations

Education

- October 2014 – now (July 2017): Georg-August Universität Göttingen
    - PhD Student
    - Degree: PhD (Dr. agr. sc.)
    - Title: Dual use maize – Corn as food and cob as bioenergy
- September 2012- August 2014: Radboud University Nijmegen (The Netherlands)
    - Degree: Master of Science
    - Subject: Biology
    - Title of Master thesis:
        - Spatial dynamics of plant species in dune grassland patches in one year
        - Who is the enemy? – Predation of two great cormorant (*Phalacrocorax carbo*) colonies by white-tailed eagle (*Haliaeetus albicilla*), fox *(Vulpus vulpus*),

great black-backed gull (*Larus marinus*) and herring gull (*Larus argentatus*) and crow (*Crovus*)

- September 2009 – May 2013: Radboud University Nijmegen (The Netherlands)
    - Degree: Bachelor of Science
    - Subject: (Medical) Biology
    - Title of Bachelor thesis:
        - Influence on Biodiversity through changes in an ecosystem, based on past gravel dedging
- August 2006 - July 2009: Collegium Augustianum Gaesdonck
    - Degree: Abitur

Languages

- German: Mother Tongue
- English: Fluent
- Dutch: Fluent (RU-NT2 Exam)
- Swedish: Basic Knowledge

Stay abroad

- September 2009 - August 2014: The Netherlands
    - Study of Biology: Radboud University, Nijmegen
- August 2013 – January 2014: Sweden
    - Erasmus exchange: University of Gothenburg, Gothenburg

Projects/further Education

- Wasservogelreservat Wallnau NABU (March 2014-August 2014)
    - Guided Tour: Bird Calls
    - Guided Tour: Herbs
    - Guided Tour: Bats
    - Guided Tour: Amphibians
- Master thesis March 2013 – October 2013:
    - Title: Spatial dynamics of plant species in dune grassland patches in one year
    - Work independent
    - Communication with nature reservations
    - Data Collection of Flora
    - Scientific Report
- European Classes Alden Biesen (Januar 2008)
    - International Project week for students of different EU-countries