Relationship between Leaf Greenness, Sugar Contents at R2 growth stage and Stalk Rot tolerance in maize (*Zea mays L*)

MSc Thesis

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Abstract

Among the maize diseases, stalk and ear rots are most harmful diseases with the largest economic losses and occur in all maize growing regions. This study concentrated on the stalk rot of maize, causing stalk breakage, stalk lodging, pre-mature death and root lodging, resulting in yield loses, often estimated at 10 to 30%. In Europe the most common stalk rot diseases isolated from roots of maize are Fusarium Stalk rot (FSR) with the causal agents *Fusarium verticillioides (Fusarium moniliforme)* and *Fusarium culmorum,* Gibberella Stalk rot (GSR) with causal agent *Gibberella zeae (Fusarium graminearum)* and Anthracnose stalk rot (ASR) with the causal agent *Colletotrichum graminicola*. The aim of the study was to analyze, if it is possible to use chlorophyll content and cell wall components in the residual plant at the beginning of grain filling to select maize genotypes that are tolerant to stalk rot pathogens. A field trial was conducted, where the chlorophyll content, sugar content and cell wall components of the residual plant at Blister (R2) development stage of the plant was measured. Between Dent (R5), Physiological Maturity (R6) and Harvest Maturity (HM), the progress of stalk rot disease was assessed regularly. At HM a rating on stalk rot disease was done on the cut stems of the plants. Overall no correlation between the chlorophyll content, sugar content or other cell wall components measured in this study and the stalk rot tolerance could be found. Therefore, we concluded that there is little interest in the use of some of the traits as an indirect selection criterion for stalk rot tolerance in maize breeding programs.

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Acronyms and Abbreviations

ADF	Acid Detergent Fiber, Percent
ASR	Anthracnose Stalk Rot
CF	Crude Fiber, Percent
DCS	In Vitro Digestibility (Aufrere), Percent
DCW	Cell Wall Digestibility (Van Soest), Percent
DIGND	Neutral Detergent Fiber Digestibility, Percent
DINAG	Non-Starch/Non-Sugar Digestibility, Percent
DMO	Digest Overall (Dardenne), Percent
FNSC	Final Stand Count
FNSP	Final Stand Percentage
FSR	Fusarium Stalk Rot
GSR	Gibberella Stalk Rot
HCEL	Hemicellulose (NDF-ADF), Percent
HM	Harvest Maturity
NDF	Neutral Detergent Fiber, Percent
NFC	Non-Fiber Carbohydrates, Percent
NIRS	Near-Infrared Spectroscopy
NS	Not Significant
OADF	Organic Acid Detergent Fiber, Percent
ONDF	Organic Neutral Detergent Fiber, Percent
PROS	Silage Protein At 90 Percent Dry Matter, Percent
R2	Blister developments stage of Maize
R5	Dent development stage of Maize
R6	Physiological Maturity development stage of Maize
S50D	Days after planting, when 50% of the silks are visible
Sev	Severity rating from pathology laboratory
SDM	Silage Dry Matter in Percent
SG	Stag Green
SPAD	Soil Plant Analysis Development
SROTP	Stalk Rot Percentage, counted on the cut stems
SRSR	Stalk Rot Severity Rating
SRSR16_17	Stalk Rot Severity Rating, average rating across 2016 and 2017

SSG	Soluble Sugar, Percent
STLC	Stalk Rot Count, “Push test”
STLP	Stalk Rot Percentage, “Push Test”
_MID	Mid parent value of a Trait

1 Introduction

1.1 Overview of Maize

Maize (*Zea mays L.)* together with rice and wheat are globally the three major cereal crops. Compared to rice and wheat, maize has the highest genetic yield potential. (Fischer & Edmeades, 2010) (Duvick, 2005). In the European Union (EU) the production of maize is mainly for grain and to a smaller extend for silage. In 2018 (EU-28) grain maize was grown on 8.4 million hectares and silage maize on 6.4 million hectares (DMK, 2019). The grain maize production is concentrated on the countries France (South), Italy, Hungary and Romania in the South of Europe classified by Mediterranean and Continental climate. The silage maize production is concentrated with 60% of the acreage in France (North) and Germany, classified by Oceanic climate.

Diseases and pest are major concerns in maize production. In 2001-3 the estimate of loses in maize yield due to diseases (not counting insects or viruses) was about 9% worldwide (Oerke, 2005). The estimated loses differ between regions, with 4% in Northern Europe and 14% in West Africa (CABI, 2019). When considering climate change and the relationship to resistance to pathogens only few modelling approaches and assessment studies are found in literature today. Climatic changes may also improve the crop health situation in maize depending on the environmental requirements of the disease, the present-day and future climatic conditions of the location (Juroszek & von Tiedemann, 2013). Important to mention is the fact that ear rots and associated mycotoxin contamination of maize grain are expected to increase in many countries worldwide. (Juroszek & von Tiedemann, 2013).

1.2 Stalk Rot Diseases in Maize

Among the maize diseases, stalk and ear rots are most harmful diseases with the largest economic losses and occur in all maize growing regions. Especially ear rot infected kernels and pre-harvest infested plants contain numerous mycotoxins that can potentially affect human and animal health (Steyn & Stander, 1999) (Pitt, 2000). Ear rot and stalk rot causal agents are characterized by a wide host range the pathogen suggesting that these are relatively unspecialized (Balint-Kurti & Johal, 2008). Examples of the wide host range are *Fusarium verticillioides* and *Gibberella zeae* infecting the seeds of rice, maize and wheat as well as other grasses (Richard & Payne , 2002). Furthermore, *Fusarium verticillioides*, *Gibberella zeae* and

Stenocarpella maydis are causal agents for stalk rot and ear rot diseases (Balint-Kurti & Johal, 2008).

In this study we concentrated on the stalk rot of maize, causing stalk breakage, stalk lodging, premature death and root lodging, resulting in yield loses, often estimated at 10 to 30%. In Europe the most common stalk rot diseases isolated from roots of maize are Fusarium Stalk rot (FSR) with the causal agents *Fusarium verticillioides (Fusarium moniliforme)* and *Fusarium culmorum,* Gibberella Stalk rot (GSR) with causal agent *Gibberella zeae (Fusarium graminearum)* (Bottalico, 1998) and Anthracnose stalk rot (ASR) with the causal agent *Colletotrichum graminicola* (EPPO, 2005).

1.3 Stalk Rot Diseases and Cell Wall Components

Early studies in maize illustrate a relationship between the sugar level of the plant at grain harvest and the resistance to rot and stalk rot of maize (Mortimore & Ward, 1964) (Craig & Hooker, 1961). It is known that factors causing reduction of the sugar content in the plant could have some impact on the late season stalk strength (Dodd, 1979). This is due to the fact, that after pollination the maize plants start to translocate sugars to the developing ear reducing the sugars in the stalk and tissue resulting in senescence of root cells (Dodd, 1979).

Apart from the sugar content there are reports that describe the role of cell wall components in maize resistance to pest and diseases (Santiago, Barros-Rios, & Malvar, 2013). One study reported higher Acid Detergent Fiber (ADF) and Neutral Detergent Fiber (NDF) contents in the leaves of a resistant maize inbred line compared to a susceptible one and it was shown that this higher content is responsible for the tolerance to the Southern rust of corn (*Puccinia Polyrosa*) (Ji & Yamakawa, 2011). NDF and ADF are together with Acid Detergent Lignin (ADL) structural components of the plant, specially the cell wall, determined with the detergent method. (Van Soest, Nutritional Ecology of the Ruminant, 1994). **Figure 1** shows that the NDF fraction is the total crude fiber with hemicelluloses (HCEL), cellulose, lignin, the ADF fraction is cellulose and lignin and the ADL is the acid insoluble lignin fraction. Hemicelluloses (HCEL) can be determined by NDF minus ADF. A second study suggested the influence of HCEL on the resistance to *Gibberella zeae* (Cao, Reid, Butron, Malvar, & Souto, 2011).

In Grain Sorghum it has been reported that the stalk rot fungi affect Leaf Greenness (SPAD) in a genotype specific way (Bandara, Weerasooriya, Tesso, & Little, 2016). In forage maize the SPAD value was different between “Normal” and so called ”Stay Green” (SG) varieties (Swanckaert, et al., 2017). In the same study the SG was characterized as cosmetic, because the concentration

of sucrose and fructose did not differ between SG and normal varieties. On the other hand, the SG trait influenced the N translocation from the leaves to the ear and this, could be an indication, that the SG trait has an influence on the resistance to stalk rot (Dodd, 1979).

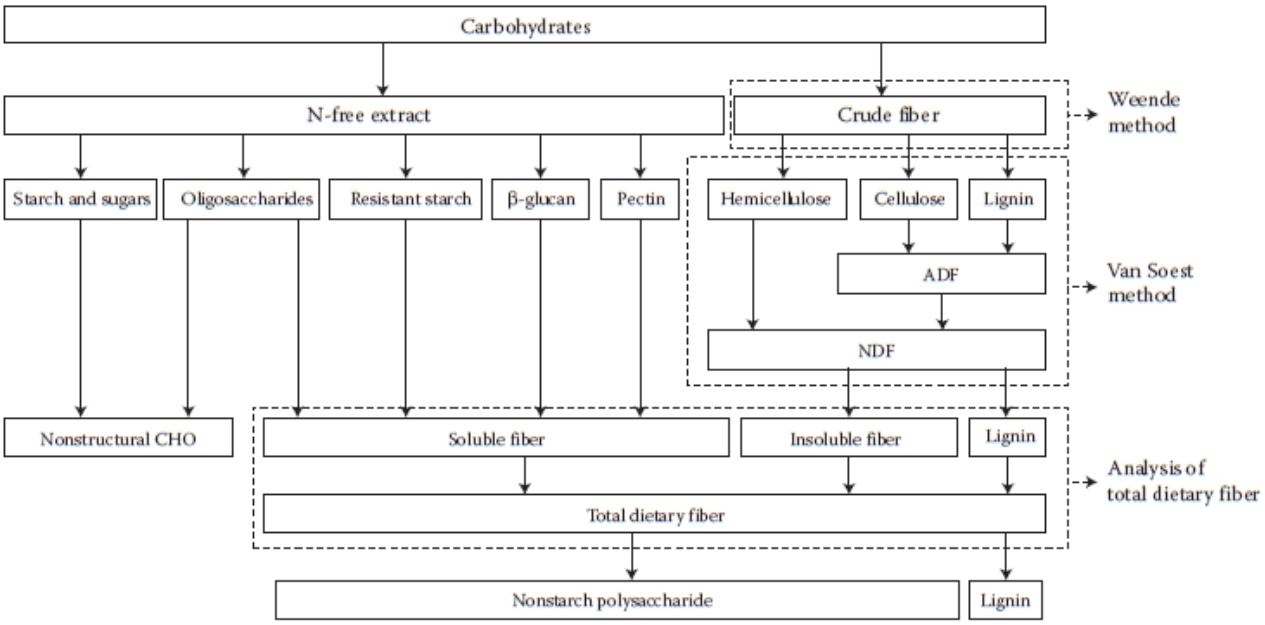


Figure 1 Grouping of the polysaccharides of feed and the composition of dietary fiber as measured by different methods (Schutte, 1991).

1.4 Objective of the Study

The aim of the study was to analyze if it is possible to use chlorophyll content and cell wall components in the residual plant at the beginning of grain filling to select maize genotypes that are tolerant to stalk rot pathogens. A field trial was conducted, where the chlorophyll content, sugar content and cell wall components of the residual plant at Blister (R2) (Bell, 2017) development stage of the plant was measured. Between Dent (R5), Physiological Maturity (R6) and Harvest Maturity (HM, 25 to 30% moisture in the kernels), the progress of stalk rot disease was assessed regularly. At HM a rating on stalk rot disease was done on the cut stems of the plants. A description of the maize development stages is shown in **Figure 2** and **Table 1**.

The experiment was conducted following the specific objectives:

1.) Is there a relationship between chlorophyll content at R2 stage (**Figure 2 and Table 1**) and the tolerance to stalk rot diseases at R6 stage (**Figure 2 and Table 1**)?
2.) Is there a relationship between sugar content at R2 stage and the tolerance to stalk rot diseases at R6 stage?
3.) Is there a relationship between other structural plant component at R2 stage and the tolerance to stalk rot diseases at R6 stage?

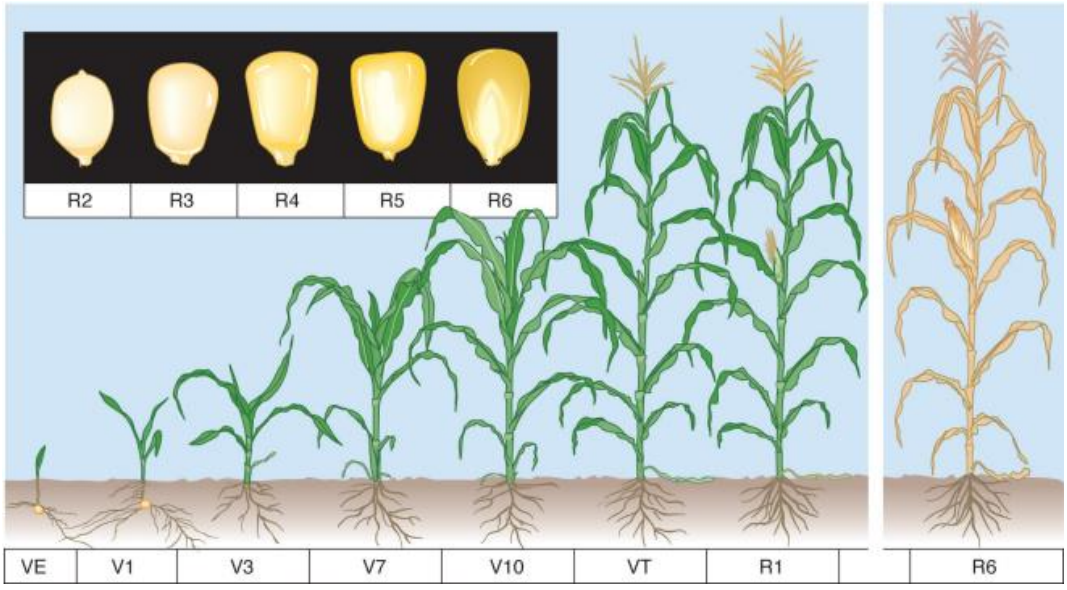


Figure 2 Maize Growth Stages (Nafziger, 2009).

Table 1 Description of Maize Growth Stages in Figure 2 (Nafziger, 2009).

Stage	Description
VE	Emergence
V1	First Leaf collar
V3	Third Leaf collar
V7	Seven Leaf collar
V10	Ten Leaf collar
VT	Tasseling, bottom-most branch of tassel completely visible and silk has not emerged
R1	Silking, silks visible outside the husks
R2	Blister, kernels white on outside, clear liquid inside
R3	Milk, 80% moisture in the kernels, kernel yellow outside, milky white fluid inside
R4	Dought, 70% moisture in the kernels, kernel fluid thick/pasty
R5	Dent, 40% moisture in the kernels, most kernels at least partially dented
R6	Black Layer (Physiological maturity), milk line no longer evident

2 Materials and Methods

2.1 Plant Material

In this study, we used forty-eight experimental maize hybrids, twenty-eight of them were flint x dent hybrids and twenty dent x dent hybrids. Furthermore, we used the parent inbred lines of these forty maize hybrids. The selection of the hybrids used in the experiment was done based on the stalk rot severity rating (SRSR) in previous experiment of the years 2016 and 2017. SRSR is a visual rating from 1, no plants affected by stalk rot, to 9, all plants are affected. For this study we selected hybrids with a relative wide range of SRSR notes from 1 to 5.5. (**Table 2)**. Because continuous selection against stalk rot takes place in every breeding cycle at Bayer Crop Science - Monsanto, a rating above 6 is very rare.

2.2 Experimental Design

The experiment was grown in season 2018 at two Bayer Crop Science - Monsanto breeding sites in Germany. Both sites are in regions with intensive maize cultivation. The Borken site (51.8797296, 6.7921) is in the North-West of Germany and usually characterized by oceanic climate with mild winters and humid summers. The experiment was grown on a sandy soil with 81% sand, 12% silt, 2,6% organic matter and a pH value of 5,6%. The Künzing site (48.6455202, 13.0318344) is in the South-East of Germany described by eastern-continental climate, with cold winters and hot dry summers. The experiment was grown on a loamy soil with 70% silt, 18% clay, 1,9% organic matter and a pH value of 6,1%.

To avoid neighboring effects between strong growing maize hybrids and the smaller maize inbred lines during the growing season, the experiment was spitted in two different sets of experiments, one for the maize hybrids (NCB_KSBW01) and a second one for the maize inbred lines (NCB_KSBW02). The sets were placed in two blocks in the field separated by buffer plots (**Figure 3**). Moreover, we have two additional reps of each set for the two different harvest points. The two sets of experiment were planted like shown in **Figure 3** to allow a mechanical harvest. Each plot consists of one row of 1.4 meters with approximate six to eight plants per plot, the rows were separated by 0.75 meter. The field trials were sown on the 3rd May 2018 at the Borken site and on the the 8rd May 2018 at the Künzing site.

Table 2 Coding of the forty maize hybrids, the crossing pattern, the codes of the corresponding parent inbred lines and the Stalk Rot Severity Rating (SRSR) of the maize hybrids

Code Maize Hybrid	Crossing Pattern	Code Parent 1	Code Parent 2	SRSR[a]
HYB 01	Dent x Flint	INB 04	INB 01	5.0
HYB 02	Dent x Flint	INB 04	INB 06	4.8
HYB 03	Dent x Flint	INB 09	INB 06	4.6
HYB 04	Dent x Flint	INB 10	INB 06	4.8
HYB 05	Dent x Flint	INB 04	INB 07	2.6
HYB 06	Dent x Flint	INB 10	INB 07	1.7
HYB 07	Dent x Flint	INB 03	INB 11	3.6
HYB 08	Dent x Flint	INB 04	INB 11	5.1
HYB 09	Dent x Flint	INB 16	INB 11	3.9
HYB 10	Dent x Flint	INB 03	INB 12	2.0
HYB 11	Dent x Flint	INB 04	INB 12	1.6
HYB 12	Dent x Flint	INB 05	INB 12	3.0
HYB 13	Dent x Flint	INB 09	INB 12	1.3
HYB 14	Dent x Flint	INB 10	INB 12	1.3
HYB 15	Dent x Flint	INB 04	INB 13	3.9
HYB 16	Dent x Flint	INB 03	INB 14	5.3
HYB 17	Dent x Flint	INB 04	INB 14	5.0
HYB 18	Dent x Flint	INB 10	INB 14	4.8
HYB 19	Dent x Flint	INB 16	INB 14	1.6
HYB 20	Dent x Flint	INB 15	INB 02	1.1
HYB 21	Dent x Flint	INB 05	INB 02	1.2
HYB 22	Dent x Flint	INB 05	INB 14	3.2
HYB 23	Dent x Flint	INB 15	INB 14	2.4
HYB 24	Dent x Flint	INB 05	INB 07	1.3
HYB 25	Dent x Flint	INB 15	INB 07	1.0
HYB 26	Dent x Flint	INB 16	INB 07	1.0
HYB 27	Dent x Flint	INB 09	INB 11	4.6
HYB 28	Dent x Flint	INB 16	INB 06	3.2
HYB 29	Dent x Dent	INB 23	INB 21	1.2
HYB 30	Dent x Dent	INB 22	INB 27	2.2
HYB 31	Dent x Dent	INB 19	INB 28	1.2
HYB 32	Dent x Dent	INB 19	INB 29	1.2
HYB 33	Dent x Dent	INB 22	INB 30	4.2
HYB 34	Dent x Dent	INB 17	INB 18	3.0
HYB 35	Dent x Dent	INB 24	INB 18	2.5
HYB 36	Dent x Dent	INB 24	INB 27	3.2
HYB 37	Dent x Dent	INB 24	INB 28	3.5
HYB 38	Dent x Dent	INB 23	INB 29	3.5
HYB 39	Dent x Dent	INB 24	INB 29	3.5
HYB 40	Dent x Dent	INB 17	INB 30	5.5
HYB 41	Dent x Dent	INB 24	INB 30	4.0
HYB 42	Dent x Dent	INB 25	INB 20	1.2
HYB 43	Dent x Dent	INB 26	INB 20	1.2
HYB 44	Dent x Dent	INB 25	INB 21	3.2
HYB 45	Dent x Dent	INB 26	INB 21	5.2
HYB 46	Dent x Dent	INB 25	INB 31	2.2
HYB 47	Dent x Dent	INB 26	INB 31	1.2
HYB 48	Dent x Dent	INB 24	INB 20	2.5

[a] Means of the SRSR in the years 2016 and 2017.

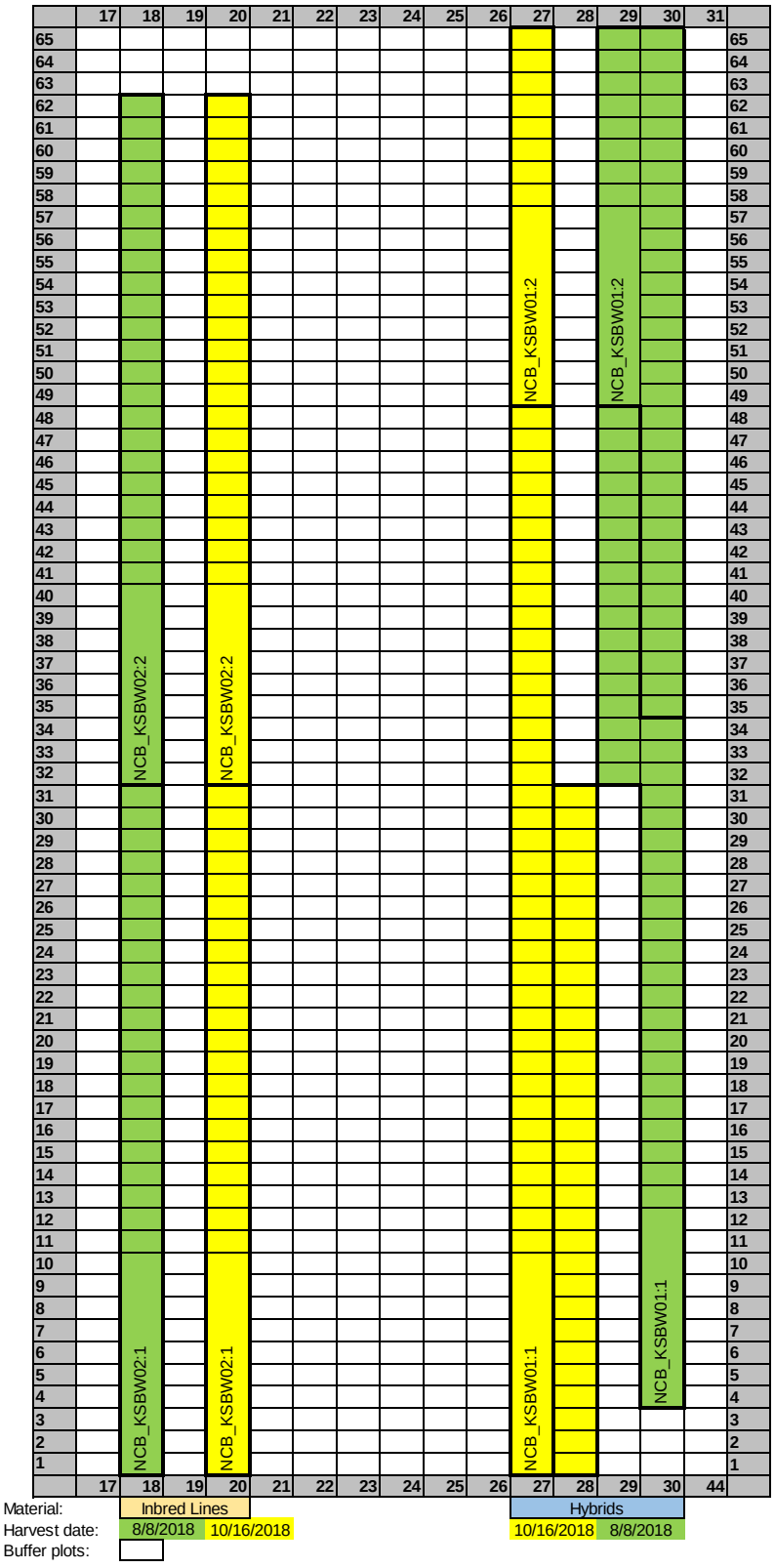


Figure 3 The field map of the trial at the location Borken with the different material types, inbred lines and hybrids and harvest dates.

2.3 Field and Harvest Measurements

2.3.1 Final Stand Count

About two to three weeks after sowing a first evaluation of the seedling emergence was done at both sites. We observed a poor emergence at the Künzing site. Beginning of June, the number of plant per plot was counted and saved in the trait final stand count (FNSC). The experiment at the Künzing site was discarded after the second visit due to a severe attack with wireworms. The number of plants per plot was too heterogenous.

2.3.2 Female Flowering

In July female flowering was captured every two days and correspond to the days after planting when 50% of the female flowers (silks) were visible (S50D).

2.3.3 Evaluate the First Harvest Timing

To evaluate the optimal first harvest time the dry matter content was measured in special border plots with known inbred lines at the beginning of August. The optimal point to harvest is between 20 to 30% of dry mater in the residual plant, approximately 30 to 40 days after flowering.

2.3.4 Chlorophyll Content

Before the mechanical harvest, the chlorophyll content was measured by using the Chlorophyll Meter SPAD-502 Plus (Konica Minolta, Inc.) on three plants per plot (Chapman & Barreto, 1997). The SPAD-502 Plus (Konica Minolta, Inc.) determines the chlorophyll concentration by measuring the leaf absorbance in red and near-infrared regions. The absorbance values of the SPAD-502 Plus was used to calculate a company defined SPAD (Soil Plant Analysis Development) value by division of light transmission intensities at 650 nm by 942 nm. This numerical SPAD value is proportional to the relative content of chlorophyll within the sample leaf. The average of the three measurements was captured in the trait SPAD.

2.3.5 Dry Matter Content of Residual Plant

After the ears were removed from the plants, the residual plants were chopped. Six to eight plants result in a 2,5 to 3 kg sample of chopped plant material. The samples were dried for 48 hours at 60 ˚C. The dry mater content was calculated by dry sample weight divided by fresh sample weight multiplied by 100 and saved in the trait silage dry matter (SDM).

2.3.6 Rating of Stalk Rot Infection

On the 25th of September we started to evaluate the stalk rot pressure in the experiment with a visual rating captured in the trait SRSR and a “push test”. The “push test” was done based on the guidelines for the official trials in Germany (Bundessortenamt, 2008). For the “push test” each plant in a plot was pushed with the same pressure by hand till the tassel touches the neighbor row. Non-flexible plants are suggested to have stalk rot and captured in stalk lodging count (STLC). We repeated the “push test” on the 8th and 16th of October to explore the development of the disease. The percentage of infected plants was captured by notation date in the traits STLP_1, STLP_2 and STLP_3 (Stalk Rot Percentage).
The second harvest took place on the 16th of October. The harvest procedure was the same like for the first harvest. After the second harvest we made a visual rating of stalk rot on the cut stems in the field. Healthy plants will have a clean cut and the cut of plants with stalk rot is looking tattered. The percentage of plants with stalk rot determined by this method was captured in SROTP (Stalk Rot Percentage, rated on the cut stems).

2.4 Laboratory Measurements

2.4.1 Cell Wall Components of the Residual Plant

All dried samples were grinded in two steps to 1 mm maximum particle size. First with a cutting mill (Retsch SM 100) to 3 mm and second with a cross beater mill (Peppink AN 200) to 1mm. The grinded samples were analyzed with a NIRS™ DS2500 F from FOSS. The NIRS™ DS2500 F using Near-infrared spectroscopy (NIRS’) technology across the full wavelength range of 850 to 2500 nm. **Table 3** is showing the abbreviation and the description of the components that were measured.

Table 3 Abbreviation, description and unit of measure of the traits captured with the NIRS instrument

Trait	Description	Unit of Measure
ADF	Acid Detergent Fiber	Percent
ADL	Acid Detergent Lignin	Percent
DCW	Cell Wall Digestibility (Van Soest)	Percent
NDF	Neutral Detergent Fiber	Percent
CF	Crude Fiber	Percent
DCS	In Vitro Digestibility (Aufrere)	Percent
DIGND	Neutral Detergent Fiber Digestibility	Percent
DINAG	Non-Starch/Non-Sugar Digestibility	Percent
DMO	Digest Overall (Dardenne)	Percent
HCEL	Hemicellulose (NDF-ADF) (Van Soest & Wine, Use of detergents in the analysis of fibrous feeds. IV. Determination of plant cell wall constituents J. AOAC Int. 1967, 50, 50– 55, 1967)	Percent
NFC	Non-Fiber Carbohydrates	Percent
OADF	Organic Acid Detergent Fiber	Percent
ONDF	Organic Neutral Detergent Fiber	Percent
PROS	Silage Protein At 90 Percent Dry Matter	Percent
SSG	Percent Soluble Sugar	Percent

2.4.2 Determine the Disease Species

To identify the species, that are present in the experiment, root samples of four hybrids and the corresponding inbred lines were subjected to pathology analysis. The decision on which maize genotypes to send to the lab, was based on the results of SRSR, STLP (all rating dates) and SROTP and their analysis. The goal was to have the complete range of ratings in the root samples. **Table 4** shows the genotypes that were sent to the pathology lab and their disease ratings.

For the analysis the infected stalks were cut into small pieces and the small pieces were soaked in a beaker of 10% bleach solution. Under the sterile hood 3 small pieces were placed on a plate of artificial media. After several days of growing a visual rating was performed based on morphological criteria, like color of the colony, mycelium structure, spores size and shape. The causal agent of the stalk rot disease and the three discrete classes “Mild”, “Moderate” and “Severe” were determined. The three discrete classes are captured in the traits Severity (Sev).

Table 4 Genotypes used for field disease evaluation and their respective ratings

Set	Code	Entry#	Rep#	STLP1	STLP2	STLP3	SROTP
				25-Sep	8-Oct	16-Oct	16-Oct
NCB_KSBW01	HYB 07	7	1	0.0	0.0	0.0	100.0
			2	33.3	66.7	50.0	50.0
	HYB 09	9	1	0.0	0.0	16.7	33.3
			2	16.7	33.3	33.3	66.7
	HYB 19	19	1	0.0	0.0	0.0	20.0
			2	0.0	0.0	0.0	16.7
	HYB 46	46	1	0.0	50.0	50.0	40.0
			2	0.0	0.0	0.0	0.0
NCB_KSBW02	INB 03	3	1	0.0	0.0	0.0	83.3
			2	0.0	0.0	0.0	100.0
	INB 11	11	1	16.7	16.7	33.3	33.3
			2	33.3	50.0	66.7	66.7
	INB 14	14	1	50.0	50.0	66.7	83.3
			2	33.3	33.3	33.3	100.0
	INB 16	16	1	0.0	16.7	0.0	50.0
			2	0.0	0.0	0.0	100.0
	INB 25	25	1	66.7	83.3	100.0	100.0
			2	83.3	100.0	100.0	100.0
	INB 31	31	1	33.3	33.3	100.0	83.3
			2	71.4	71.4	85.7	71.4

2.5 Statistical Analysis

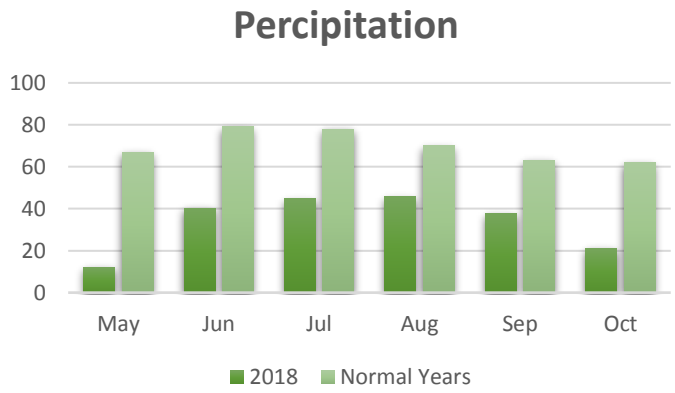
For statistical analysis the package R, version R-3.5.1was used. Combined analysis of Variance (ANOVA) was performed for the different disease ratings and stover contents. In addition, Pearson correlation analyses between different stover components, disease ratings and genotypic backgrounds (inbred or hybrids) was calculated.

3 Results and Discussion

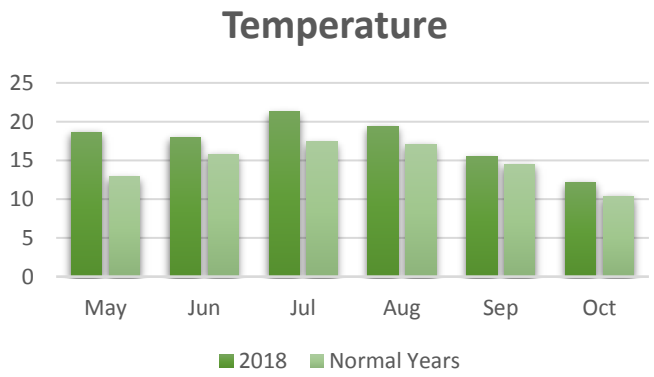
3.1 Growing Conditions Maize Season 2018

The field trial at Künzing site had a severe attack by wireworms and the seed emergence was heterogeneous. After the FNSC evaluation with plants per plot from 0 to 9 the field trial was discarded and no further notations where made.

The year 2018 was concerning the growing conditions for maize an extreme year in Germany. We had severe drought, with up to 90% less rainfall in spring and summer than in average of the years 1961-1990 (**Figure 4a, Figure 5 c-e**). In addition, the temperature in calendar year 2018 was 1,5 to 2˚ C higher than in average of the years 1961 -1990 **(Figure 4b, Figure 5 a, b)**. Because of the missing precipitation in May and June at the Borken site, the field trial was irrigated with 30mm on June 29th, July 11th and 25th.



a.



b.

Figure 4 Comparison of a. Precipitation and b. Temperature at Borken site 2018 (weather station at the site) and in normal years *(Projects, AM Online, 2019)*

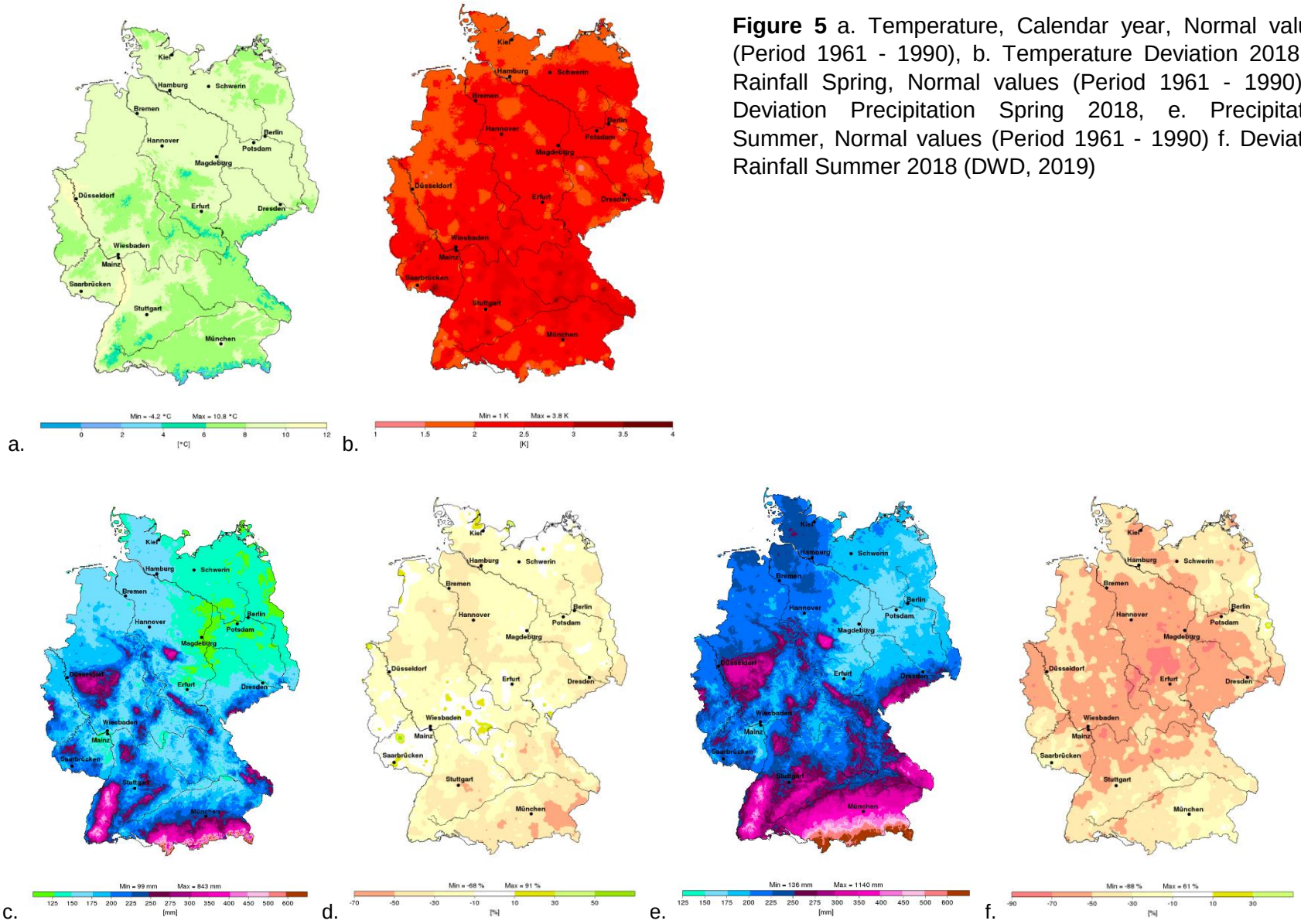


Figure 5 a. Temperature, Calendar year, Normal values (Period 1961 - 1990), b. Temperature Deviation 2018, c. Rainfall Spring, Normal values (Period 1961 - 1990) d. Deviation Precipitation Spring 2018, e. Precipitation Summer, Normal values (Period 1961 - 1990) f. Deviation Rainfall Summer 2018 (DWD, 2019)

3.2 Development of the Maize in the Field Trials

The warm and dry growing season resulted in an overall faster development of the maize plant compared to the average of the years.

The flowering of the genotypes in the field trials took place from the 7th to the 19th of July, respectively 65 to 77 days after planting, 7 days earlier than in average of the years.

After flowering temperatures above 36 ˚C in the last week of July and first week of August resulted in a first harvest on August 8th, three weeks earlier than in average of the years.

The harvest of the maize in the German Bayer Crop Science - Monsanto maize yield trial network started for the silage yield trials on 20th of August and for the grain yield trial harvest at the 14th of September. Both dates also around 3 weeks earlier than in average of the years. **Figure 6** displays the silage maturity across Germany at the 1st October in average of the years 1961-1990 and the deviation from the average 2018. Across all regions in Germany, in contrast to the average of the years at the 1st of October, the silage maturity was out of the optimal harvest range of about 32% to 38% silage dry matter.

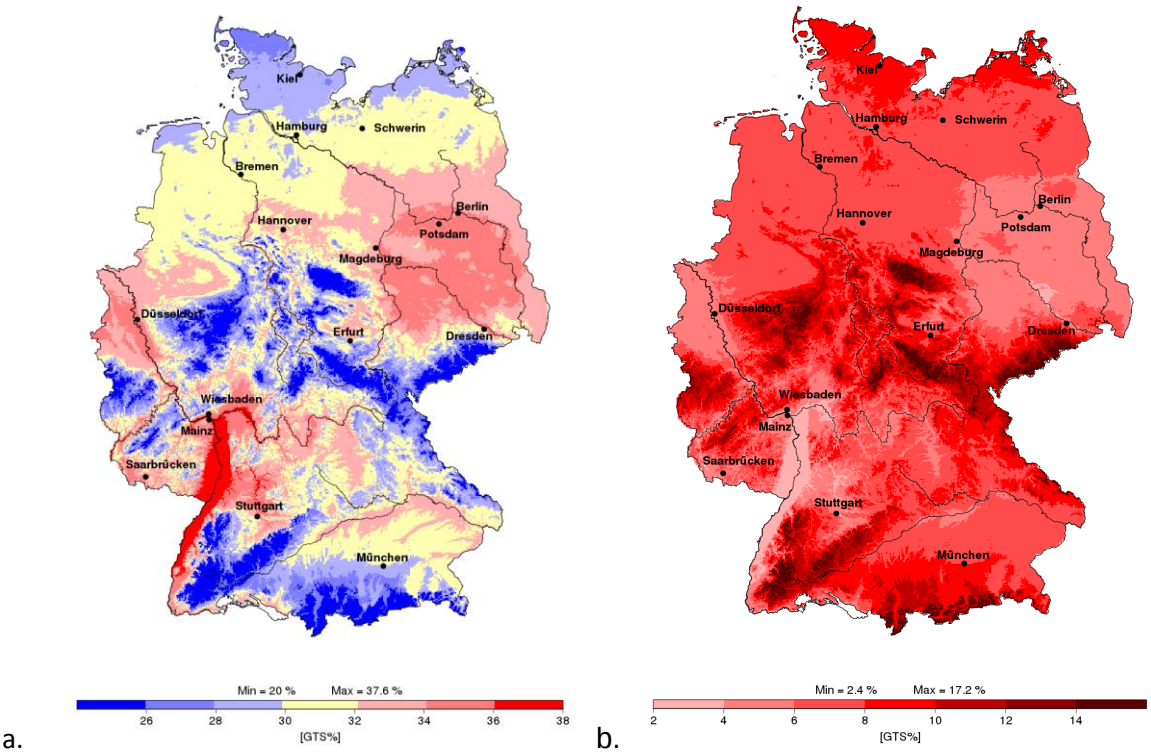


Figure 6 Maize silage maturity (SDM) at 1st October a.) Average of the years 1961-1990 b.) 2018, deviation from the average (DWD, 2019).

3.3 Stalk Rot Disease Development

GSR (*Gibberella zeae*) and FSR (*Fusarium verticillioides)*, the most occurring stalk rot diseases in the North of Europe (Bottalico, 1998), need warm and wet conditions to develop (Manstretta & Rossi, 2016) (Czembor, Stępień, & Waśkiewicz, 2015).

Every year the visual stalk rot rating (SRSR) is possible at single locations during the silage and grain yield trial harvest, mostly in North Rhine Westphalia and Lower Saxony, since there the weather conditions and soils are appropriate for stalk rot diseases. In 2018 the missing precipitation **(Figure 4, Figure 5**), the fast ripening and early harvest of the maize plant resulted in no stalk rot infection in the yield trials across Germany, either silage or grain.

It was planned to monitor the stalk rot disease development in the field trial from beginning of silage maturity to the stage of HM on a weekly basis, but no disease infection was visible in the field trials till the grain yield trials harvest began. The first visual rating (SRSR) and "push test" (STLP) was done at the stage of HM in the experiment.

3.3.1 SRSR and STLP Rating

At Borken site the grain yield trials were harvested on the 25th September with an average moisture content of 25%. On the same day the monitoring of the stalk rot infection in the experiment started with the "push test", captured in STLC, and the visual disease rating of the stalk, captured in SRSR. Because of the advanced physiological stage of the maize plants and the low infection with stalk rot diseases the visual rating SRSR was difficult to conduct. The decision was taken to skip this rating and continue only with the "push test" and the resulting stalk rot percentage (STLP). The first rating was captured in STLP_1, results shown in appendix 1. When comparing the STLP_1 ratings for the hybrids from 2018 and the average of the visual stalk rot rating of the hybrids from the years 2016 and 2017 (SRSR16_17) shown in appendix 1 and **Table 5**, it is obvious that the disease pressure at grain harvest in 2018 is lower than in the average of the years 2016 and 2017. The average and the median in SRSR16_17 is 3, and this is comparable to a STLP rating of about 30%, but the average for the STLP_1 in 2018 is at 3,6% and the median is 0%. These results of the first ratings has led us to the decision to wait with the second harvest of the experiment until the disease infection shows a satisfactory growth. The monitoring of the disease development with a scoring of STLP took place on October 8th (STLP_2) and 16th (STLP_3), results of the ratings are shown in appendix 1 and **Table 5**.

Table 5 Min, max, average and median of the stalk rot disease ratings

	Hybrid				Inbred			
Trait	Min	Max	Average	Median	Min	Max	Average	Median
SRSR16_17	1	5.5	2.9	3				
STLP_1	0	40.0	3.6	0	0	92.9	16.2	8.4
STLP_2	0	58.4	11.2	4.2	0	100	21.7	8.4
STLP_3	0	50.0	9.2	0	0	100	32.0	25
SROTP	0	75.0	16.6	12.5	0	100	47.9	41.7

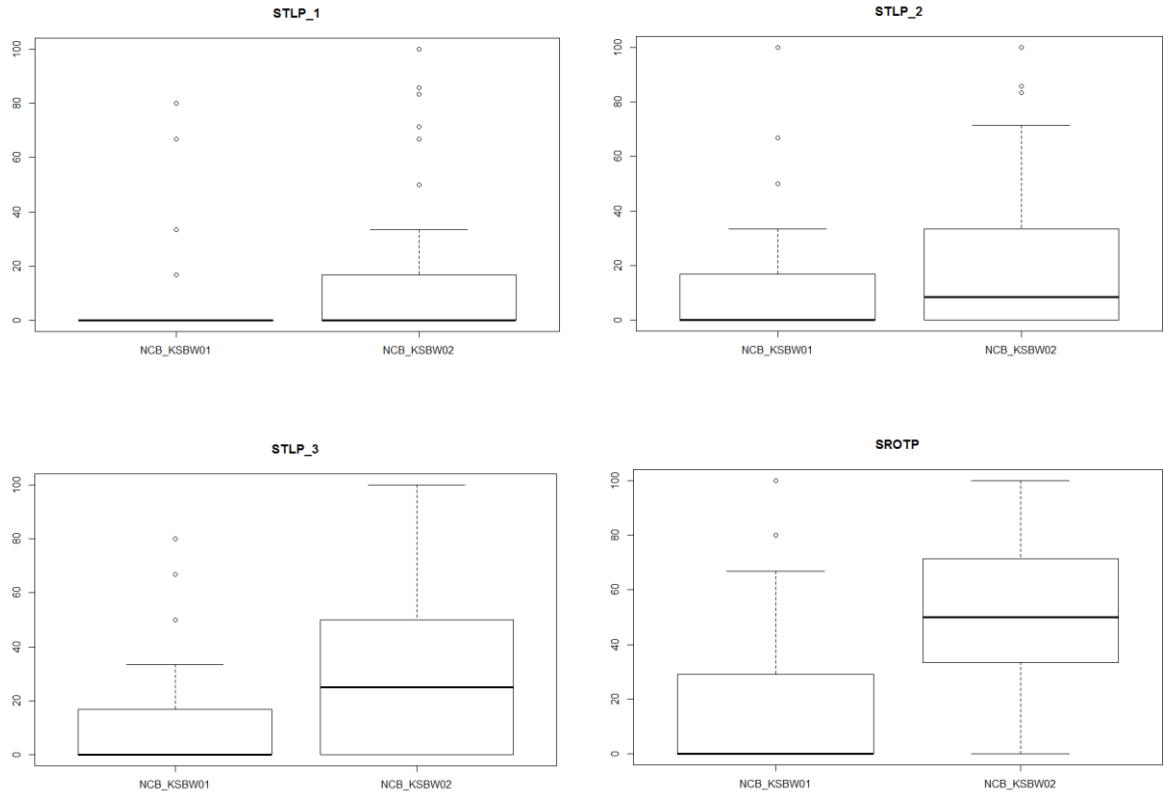


Figure 7 Boxplot showing the variance of the disease monitoring STLP_1, STLP_2, STLP_3 and SROTP in the set of inbred (NCB_KSBW02) and hybrid (NCB_KSBW01).

3.3.2 SROTP Rating

On the 16th of October, three weeks after it was initially planned, the STLP_3 rating showed a sufficient development of the disease infection (**Table 5**, **Figure 7**), especially for the inbred. The average increased from 16,2% for STLP_1 to 32% for STLP_3, same for the median from 8,4% for STLP_1 to 25% for STLP_3. For the hybrids the rating of STLP_2 and STLP_3 was somewhat

difficult, because of the low disease pressure and **Table 5** shows a decrease in median and average. Based on the results for the inbred and the overall physiological maturity of the plants it was decided to harvest the second time. After the harvest the SROTP was rated on the cut stems of the plants. **Figure 8** shows an example of the SROTP rating of INB 16 in both replications. In **Figure 8a.** the stalks are obviously infected with a stalk rot disease; the vascular bundles are destroyed and the SROTP is at 100%. **Figure 8b.** shows no infection in picture one and two as the vascular bundle looked intact, picture three was more in between, but rated as no infection, in picture four to six there was a clear infection, in total SROTP was at 50%.

The variance across all ratings is higher among the inbred lines compared to the hybrids. Comparing the two disease rating methods from the harvest day, there is more variance and a higher level of disease rating with the method SROTP versus STLP_3 (**Table 5, Figure 7**). For the hybrid the median for STLP_3 is at 0% and for SROTP at 12,5%, for the inbred the median is for STLP_3 at 25% and for SROTP at 41.7%.

a.)

b.)

Figure 8 Picture of cut stalks from INB 16, a.) 100% SROTP, “Severe” rating of GSR, *Gibberella zeae,* and FSR, *Fusarium verticilioides* b.) 50% SROTP, upper row picture 1 and 3 clear without infection, picture 2 in-between, bottom row clear infection, “Moderate” rating GSR, *Gibberella zeae.*

3.3.3 Severity Rating from Pathology Laboratory

On the 16th of October the maize plants were rather mature and the evaluation of the STLP_3 and the SROTP was somehow difficult. In mature stalks the moisture level is reduced, and the vascular bundles are dry and reduced. The stalks are in some way “empty”. Both could lead to stalk lodging without any infection with stalk rot diseases and positive ratings of STLP. It is also possible to have 100% SROTP rating and no STLP_3 like for INB 3, INB 16 and HYB 07. In this case the genotype has a very strong stalk rind and the “push test” is not positive. **Figure 9** shows the example of INB 03 with 0% STLP_3 and 83,3% SROTP (**Table 6**), the rind of the stalks is strong and intact.

Figure 9 Picture of cut stalks from INB 16, SROTP rating of 83,3%.

To confirm the ratings of the stalk rot development the decision was taken to sample the roots of some genotypes and sent them to the internal pathology laboratory. The decision which maize genotypes to send to the lab, was based on the results SRSR, STLP (all rating dates) and SROTP analysis. The goal was to have the complete range of ratings in the root samples.
Results from the pathology laboratory showed that the FSR, with the causal agent *Fusarium verticilioides,* and GSR with the causal agent *Gibberella zeae*, were present in sampled roots. The rating (Severity) consists of three different categories “Mild”, “Moderate” and “Severe”. All samples were infected with GSR, ratings from “Mild” to “Moderate”, three samples were infected

with the pathogens GSR and FSR, two from HYB 19 with a “Mild” rating and one from INB 16 with a “Severe” rating (**Figure 8a, Table 6**).

Table 6 displays the severity rating of the pathogen and the STLP and SROTP rating from the field trials. It is obvious that the SROTP is in line with the severity rating of the pathogen. A SROTP rating from 0% to 40% correspond to a “Mild” severity rating, from 50% to 67% to a “Moderate” severity rating and from 71% to 100% to a “Severe” severity rating. The trend for the STLP ratings is the same but not as strong as for the SROTP rating.

Table 6 Genotypes used for field disease evaluation and their respective ratings

Code	Entry#	Rep#	STLP_1	STLP_2	STLP_3	SROTP	Severity	Disease
HYB 07	7	1	0.0	0.0	0.0	100.0	Severe	GSR
		2	33.3	66.7	50.0	50.0	Mild	GSR
HYB 09	9	1	0.0	0.0	16.7	33.3	Mild	GSR
		2	16.7	33.3	33.3	66.7	Moderate	GSR
HYB 19	19	1	0.0	0.0	0.0	20.0	Mild	GSR/FSR
		2	0.0	0.0	0.0	16.7	Mild	GSR/FSR
HYB 46	46	1	0.0	50.0	50.0	40.0	Mild	GSR
		2	0.0	0.0	0.0	0.0	Mild	GSR
INB 03	3	1	0.0	0.0	0.0	83.3	Severe	GSR
		2	0.0	0.0	0.0	100.0	Severe	GSR
INB 11	11	1	16.7	16.7	33.3	33.3	Mild	GSR
		2	33.3	50.0	66.7	66.7	Moderate	GSR
INB 14	14	1	50.0	50.0	66.7	83.3	Severe	GSR
		2	33.3	33.3	33.3	100.0	Severe	GSR
INB 16	16	1	0.0	16.7	0.0	50.0	Moderate	GSR
		2	0.0	0.0	0.0	100.0	Severe	GSR/FSR
INB 25	25	1	66.7	83.3	100.0	100.0	Severe	GSR
		2	83.3	100.0	100.0	100.0	Severe	GSR
INB 31	31	1	33.3	33.3	100.0	83.3	Severe	GSR
		2	71.4	71.4	85.7	71.4	Severe	GSR

3.4 Chlorophyll Content and Cell Wall Components

Table 7 shows the min, max, average and variance values of the chlorophyll content captured by the SPAD meter and the cell wall components of the residual plant measured by the NIRS spectrometer on the grinded samples. A description of the abbreviations is displayed in **Table 3**. All results are shown in appendix 2. The results of the different traits are in line with the results found in literature (Swanckaert, et al., 2017) and past experiments of this type. In general, the

variance is higher for all traits across the inbred compared to the hybrid. Only for the sugar content (SSG) the variance is with 7,7 higher for the hybrid set compared to 5,8 for the inbred set. Also, min and max value for SSG are higher for the inbreds compared to the hybrids. This could be explained by the fact that maturity stage of the inbred was in average more advanced at the first harvest compared to the hybrid. This could also be an explanation of the results for the SPADR2. The variance for the hybrids is at 7,8 with a range from 52,9 to 66,1 while for the inbreds the variance is at 21,7 with a range from 45,5 to 64,0.

Table 7 Min, max, average and variance of chlorophyll content and cell wall components

	Hybrid				**Inbred**			
Trait	Min	Max	Average	Variance	Min	Max	Average	Variance
SPADR2	52.9	66.1	59.5	7.8	45.4	64.0	56.1	21.7
ADF	28.8	35.0	31.2	1.8	23.3	30.0	26.8	2.8
ADL	2.2	3.0	2.5	0.0	1.7	2.6	2.1	0.1
DCW	51.6	60.1	55.8	3.6	53.0	63.6	58.0	6.5
NDF	54.9	65.1	59.4	4.6	48.3	57.8	53.1	6.2
CF	26.8	32.2	29.1	1.3	21.6	27.3	24.9	2.1
DCS	47.9	58.5	54.2	3.9	53.6	65.8	60.2	5.7
DIGND	19.4	27.3	22.8	3.1	14.6	29.3	23.9	11.0
DINAG	35.0	43.4	39.2	2.8	39.0	48.0	43.0	6.0
DMO	47.1	58.7	54.3	4.7	51.8	65.0	60.1	6.9
HCEL	26.1	30.5	28.2	1.2	24.3	28.1	26.3	1.1
NFC	17.4	30.1	24.7	6.5	23.9	34.3	30.2	7.0
OADF	35.5	40.8	37.9	1.2	30.3	37.3	34.0	1.9
ONDF	54.4	65.7	59.1	5.6	49.0	56.8	52.4	6.2
PROS	5.7	8.8	7.3	0.4	7.2	12.5	8.8	1.0
SSG	15.5	27.7	22.1	7.7	20.6	31.6	26.9	5.8

3.5 Genetic Variance

The variance analysis of the effect of the genotype on the chlorophyll content and disease ratings (**Table 8**) and the composition of the residual plant (**Table 9**) showed different results for inbred set compared to hybrid set. The effects of the genetic variance in **Table 8 and 9** are reported as P values.

For the inbred set the variance explained by the genotype is significant (P< 0,05) for all disease ratings (STLP_1, STLP_2, STLP_3 SROTP and Severity) and highly significant (P< 0,001) for the SPADR2. For the hybrid set variance explained by the genotype is significant (P< 0,05) only for

SROTP and not significant (NS) for SPADR2 and the “push test” ratings (STLP_1, STLP_2 and STLP_3) and severity rating from the pathology laboratory (**Table 8**). The results shown in **Table 8** are line with the lower variation of disease development and pressure in the hybrid set displayed in the disease development section of this report (**Table 5 and Figure 7**).

Table 8 Analysis of the effect of the genotype, inbred set versus hybrid set, on chlorophyll content and disease ratings, reported as P values.

Factors	SPADR2	Severity	STLP_1	STLP_2	STLP_3	SROTP
Inbred	< 0.001	0.050	< 0.001	< 0.001	< 0.001	0.002
Hybrid	NS	NS	NS	NS	NS	0.010

NS = Not Significant, P value > 0,05

Table 9 Analysis of the effect of the genotype, inbred set versus hybrid set, on components of residual plant, reported as P values.

	ADF	ADL	CF	DCS	DCW	DIGND	DINAG	DMO	HCEL	NDF	NFC	OADF	ONDF	PROS	SSG
Inbred	< 0.001	0.001	< 0.001	0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.028	< 0.001	0.002	< 0.001	0.001	< 0.001	0.008
Hybrid	NS	NS	NS	0.022	0.003	0.002	NS	0.016	< 0.001	0.003	< 0.001	NS	0.001	0.010	< 0.001

NS = Not Significant, P value > 0,05

The variance explained by the genotype in the inbred set on the composition of the residual plant (**Table 9**) is significant (P<0,05) for all traits and highly significant (P<0,001) for ADL, CF, DCW, DIGND, DINAG, DMO, NDF, OADF and PROS. The effects of the hybrid genotype are significant (P<0,05) for DCS, DCW, DIGND, DMO, NDF, ONDF and PROS and highly significant (P< 0,001) for HCEL, NFC and SSG. No significance genetic variance is shown for the hybrids on the traits ADF, ADL, CF, DINAG and OADF.

The results in **Table 8** show that in the hybrid and the inbred sets for the trait SROTP the genetic variance is significant (P< 0,05) and it could be used to select stalk rot tolerant genotypes in a maize breeding program.

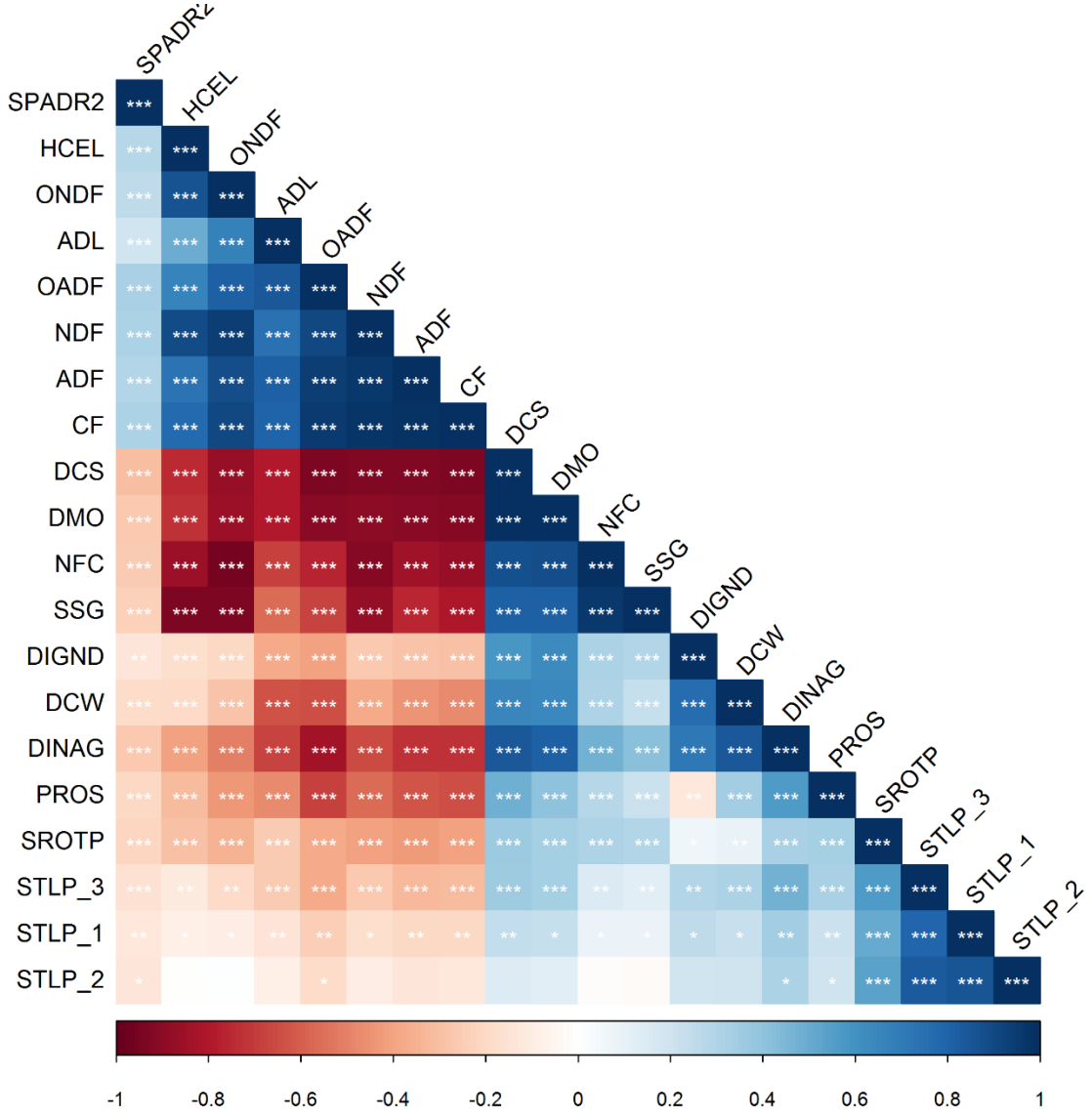
Since there was no significant genetic variance at one of the development stages where it was captured, the “push test” used in the official trials in Germany seems not to be the most reliable method to rate the stalk rot tolerance of hybrids in yield trials.

Because of the highly significant (P<0,001) genetic effects of the traits NFC, SSG and HCEL in the hybrid and the inbred set (**Table 9**) it is of interest to look at the correlation of these traits to the SROTP rating in more details.

3.6 Correlation between Traits

3.6.1 Stalk Rot Ratings, Chlorophyll Content and Cell Wall Components across Inbred and Hybrid Set

To get more insights into the correlation between the traits captured in the experiment correlation plots are displayed in **Figure 10**, the plots is ordered in hierarchical clustering.



Cells that are designated with a * display a significance level of P<0.05, ** for P<0.01 and *** for P<0.001.

Figure 10 Correlation plot for all traits across inbred and hybrid sets with hierarchical clustering order

Because of the wide variance when analyzing the results of the inbred and the hybrid sets together, nearly all correlation are significant (P<0,001, **Figure 10**). Some known strong positive or negative correlation above (-)0,80 between the cell wall composition of the residual plant are shown in **Figure 10**, but this was not part of the study.

Among the disease ratings SROTP shows the highest correlations to some of the cell wall components of the residual plants. The correlations between SROTP and ADL is of -0,43, SROTP and NDF is of -0,41 and SROTP and CF of -0,41, shown in appendix 3. All other correlations between stalk rot ratings and cell wall compositions of the residual plant or the SPADR2 are between - 0,4 and 0,4 shown in appendix 3. This could be an indication that there is little reason to use one of the cell wall components of the residual plant or the SPADR2 as an indirect criterion to select stalk rot tolerant genotypes in a maize breeding program.

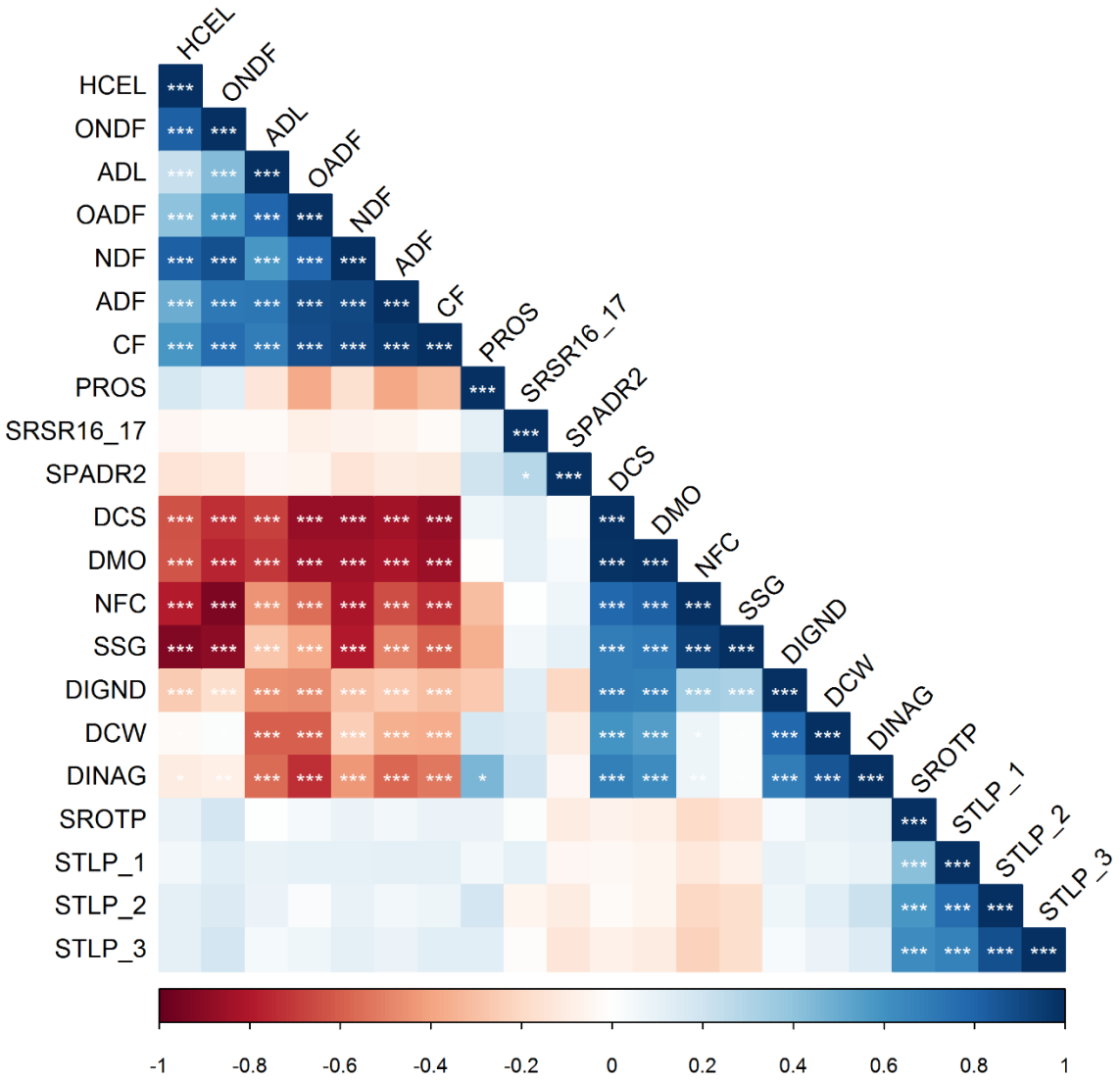
3.6.2 Stalk Rot Ratings, Chlorophyll Content and Cell Wall Components Inbred and hybrid genotypes separately

To reduce phenotyping activities in a maize breeding program, it could be of interest to use the mid parent value of a trait to select hybrid genotypes, The mid parent value of a trait is the average rating of the parents of a hybrid. Also, the genetic variance for all traits was extended by taking hybrid and inbred together in the analysis and the results in **Table 8 and 9** are different between inbred and hybrid genotypes. Because of these reasons it was looked at the correlations for hybrid and inbred separately (**Figure 11 and 12).**

No significant correlation between the SRSR16_17 and any of the stalk rot ratings from 2018 in the hybrid set is detected in the hybrid set, values are between -0,06 and 0,1 as shown in **Table 10**. This is probably affected by the weather condition in 2018 and the resulting low disease pressure especially in the hybrid set.

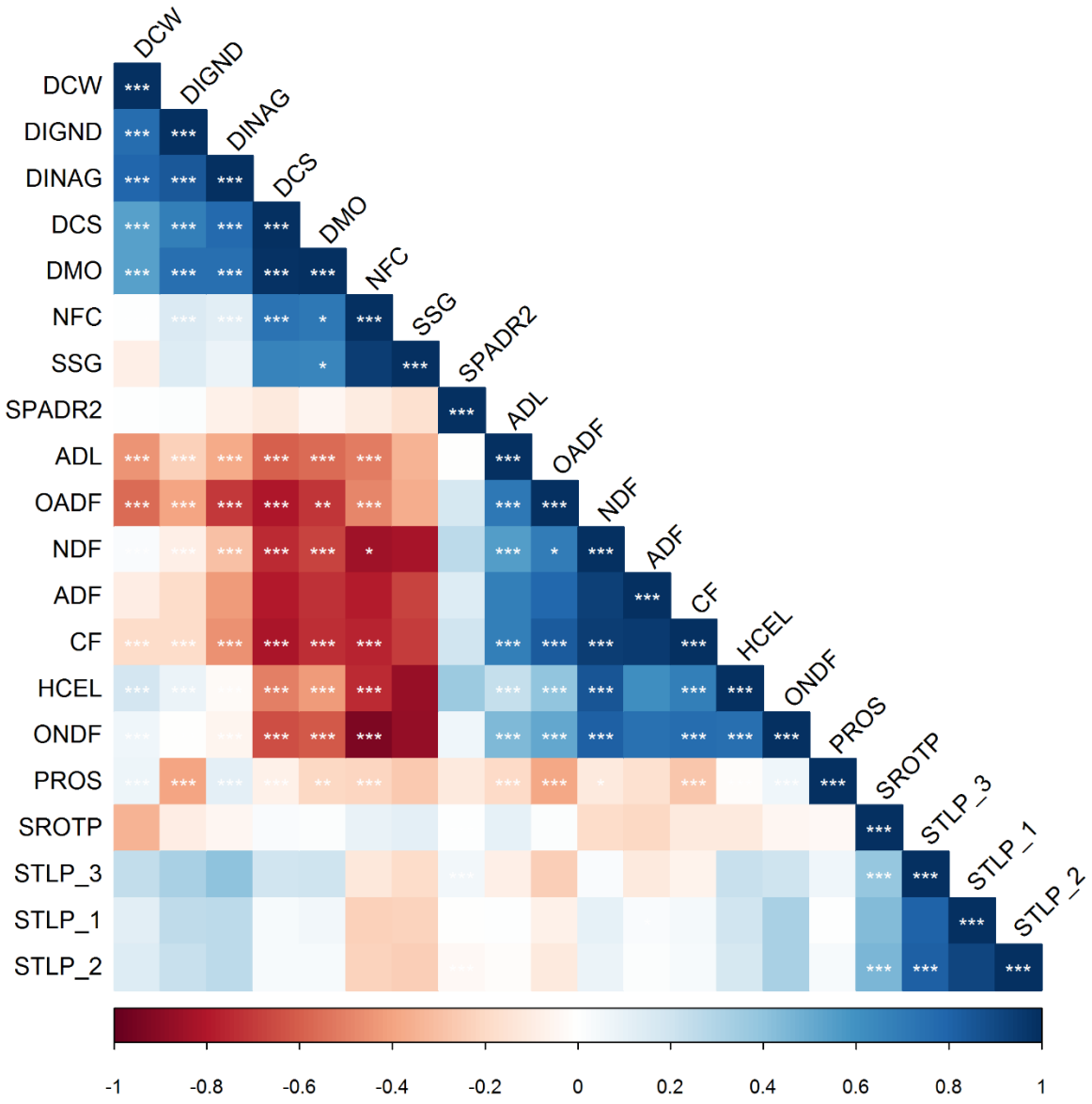
Table 10 Correlation between SRSR_16_17 and stalk rot ratings in the hybrid set

	SRSR16_17	**STLP_1**	**STLP_2**	**STLP_3**	**SROTP**
SRSR16_17	1	0.1	-0.06	-0.02	0.01
STLP_1	0.10	1	0.76	0.67	0.41
STLP_2	-0.06	0.76	1	0.87	0.58
STLP_3	-0.02	0.67	0.87	1	0.62
SROTP	0.01	0.41	0.58	0.62	1



Cells that are designated with a * display a significance level of P<0.05, ** for P<0.01 and *** for P<0.001.

Figure 11 Correlation plot for all traits in the hybrid set with hierarchical clustering order



Cells that are designated with a * display a significance level of P<0.05, ** for P<0.01 and *** for P<0.001.

Figure 12 Correlation plot for all traits in the inbred set with hierarchical clustering order

Correlation data for all traits are shown in appendix 4 (hybrids set) and appendix 5 (inbred set). The correlations between the SPADR2 and the stalk rot ratings range from -0,14 to 0,05 for the hybrids and -0,04 to 0,05 for the inbreds. For the SSG the correlations range from -0,18 to -0,08 for the hybrids and -0,25 to 0,11 for the inbreds. No correlation could be detected for chlorophyll content or sugar content at R2 stage and the stalk rot ratings at HM.

Also, for the other cell wall components no correlation could be detected to the stalk rot ratings in 2018, neither in the inbred nor in the hybrid set (**Figure 11 and 12**). Interesting to see are the correlation cluster among the cell wall components traits in **Figure 11** and **Figure 12**, but this was not part of the study.

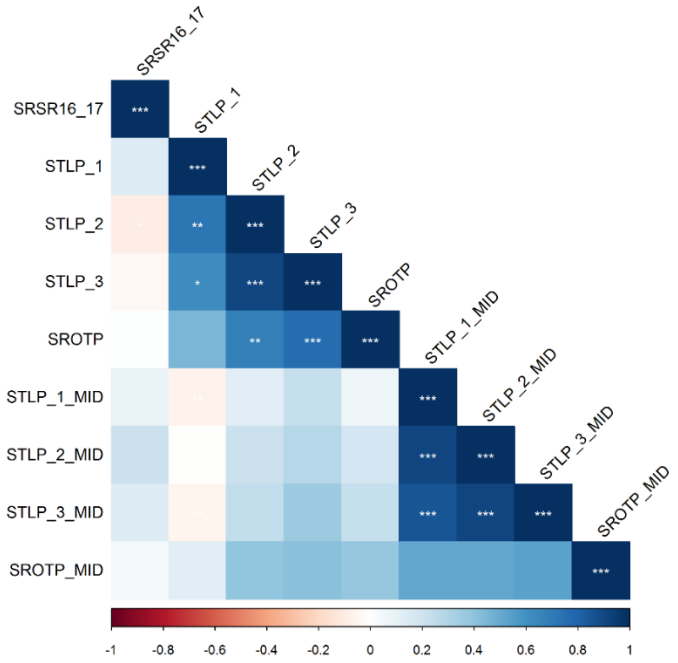
Among the stalk rot ratings, we have relative low correlations between the STLP ratings at different disease development stages and the SROTP, with 0,41 to 0,62 for the hybrids (**Table 10**) and 0,38 to 0,43 for the inbreds (**Table11**). Especially for the STLP_3 and the SROTP from the same day, higher correlation than 0,62 in the hybrid set and 0,38 in the inbred set were expected. This could be caused by the fact, that the plant was rather matured mid of October, because of the rapid translocation of carbohydrates into the kernels during grain fill under drought stress the stalks are “empty” and the “push test” leads to wrong results, because stalks that were not affected by stalk rot disease did break down.

Table 11 Correlation between stalk rot ratings from 2018 in the inbred set

	STLP_1	**STLP_2**	**STLP_3**	**SROTP**
STLP_1	1	0.91	0.8	0.43
STLP_2	0.91	1	0.82	0.45
STLP_3	0.80	0.82	1	0.38
SROTP	0.43	0.45	0.38	1

3.6.3 Stalk Rot Ratings in the Hybrid Set and the Mid Parent Value

The effects of the genotypes in the inbred and the hybrid set display significant effects (P<0,05) for the trait SROTP in both sets (**Table 8**). Because of this it was looked at the correlation between the mid parent value for SROTP and the hybrid value of SROTP. **Figure 13** shows the correlation between the mid parent value of all stalk rot ratings and the corresponding hybrid value and to the average SRSR rating of the hybrids from 2016 and 2017. Mid parent values are signed with “_MID”.



Cells that are designated with a * display a significance level of P<0.05, ** for P<0.01 and *** for P<0.001.

Figure 13 Correlation between the Stalk Rot Rating in the hybrid set and the Mid Parent value

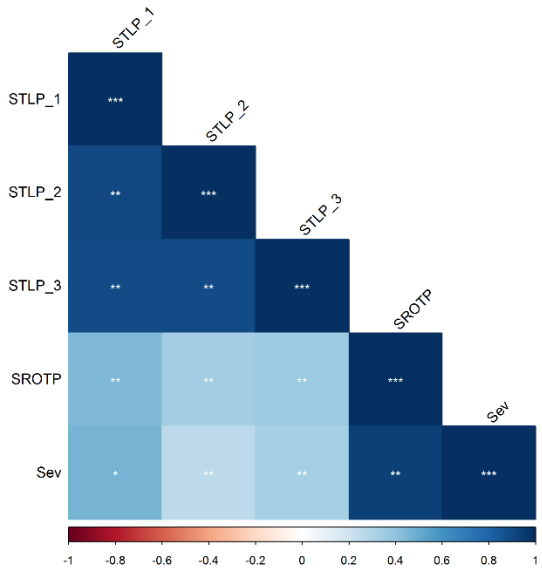
Comparing to the correlation between the hybrid stalk rot ratings from 2018 and the SRSR16_17, the correlations are higher for the mid parent values and the SRSR16_17, even if they are with a range from 0,04 – 0,21 not high and not significant (P>0,05), appendix 6.
Based on this data with a not significant (P>0,05) correlation between SROTP and SROTP_MID of 0,38 (Appendix 6, **Figure 13**) it will not be possible to use the mid parent value to select stalk rot tolerant hybrid. However, 2018 was a year with low disease pressure, somehow difficult disease ratings and one location of the experiment was discarded. Taking all this into account, it could be of interest to explore the selection opportunity on mid parent value for stalk rot tolerance in hybrid in future research.

3.6.4 Stalk Rot Rating and Severity Rating

To get more insight in the relationship between the different stalk rot ratings in the field and the stalk rot severity rating (Sev) from the pathology laboratory a correlation plot was performed, displayed in **Figure 14**. There is a significant (P<0,01) high correlation of 0,93 (Appendix 7)

between SROTP and Sev. For the rating resulting of the “push test” the correlation is lower with a range between 0,26 and 0,46. This could also be an indication that the “push test” is not the right method to discover the stalk rot tolerance of maize.

Looking at the results it must be taken into consideration that the number of samples sent to the laboratory was very low, only 20, and the selection of the sample was not random but based on the SROTP to have the whole range from 0% to 100% SROTP. Future research is needed to find the most reliable phenotyping methods for stalk rot disease tolerance.



Cells that are designated with a * display a significance level of P<0.05, ** for P<0.01 and *** for P<0.001.

Figure 14 Correlation between the stalk rot rating and the severity rating from the pathology laboratory

4 Conclusion

Overall no correlation between the chlorophyll content, sugar content or other cell wall components measured in this study and the stalk rot tolerance could be found. Therefore, we consider of limited interest the use of one of the traits tested herein as an indirect selection criterion for stalk rot tolerance in maize breeding programs.

However, 2018 the weather condition was extreme, and the stalk rot disease pressure was rather low, which makes the disease ratings somehow difficult.

The “push test” used in the official trials in Germany seems not be the most reliable method to test the stalk rot tolerance of hybrids in yield trials. The relative low correlation among the stalk rot rating captured with the “push test” and the ratings on the cut stems or coming from the pathology laboratory needs further research, which of the phenotyping method will be the best to use in the selection for stalk rot tolerant hybrids in a maize breeding program. A significant high correlation of STROP and Sev does indicate that it would be the better trait to use.

Even if the correlation was low with the data captured in this study it might be of further interest to explore if it is possible to use the mid parent value of the disease rating on the cut stems to select stalk rot tolerant hybrids in maize breeding programs.

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Appendix

Appendix 1: Stalk Rot Ratings across Genotypes

Code	SRSR16_17	STLP_1	STLP_2	STLP_3	SROTP
HYB 01	5	0	0	0	0
HYB 02	4.8	0	0	0	0
HYB 03	4.6	0	0	0	8.4
HYB 04	4.8	0	0	0	0
HYB 05	2.6	0	0	0	0
HYB 06	1.7	0	0	0	33.4
HYB 07	3.6	16.7	33.4	25	75
HYB 08	5.1	0	8.4	16.7	41.7
HYB 09	3.9	8.4	16.7	25	50
HYB 10	2	0	0	0	0
HYB 11	1.6	8.4	16.7	0	0
HYB 12	3	0	0	0	12.5
HYB 13	1.3	0	8.4	0	0
HYB 14	1.3	0	0	0	0
HYB 15	3.9	0	0	0	0
HYB 16	5.3	16.7	25	16.7	18.4
HYB 17	5	8.4	16.7	16.7	16.7
HYB 18	4.8	0	0	0	0
HYB 19	1.6	0	0	0	18.4
HYB 20	1.1	0	8.4	0	0
HYB 21	1.2	0	0	0	0
HYB 22	3.2	0	0	0	0
HYB 23	2.4	0	8.4	8.4	0
HYB 24	1.3	0	0	0	0
HYB 25	1	16.7	25	25	26.7
HYB 26	1	0	0	0	0
HYB 27	4.6	33.4	33.4	16.7	16.7
HYB 28	3.2	0	0	0	30
HYB 29	1.2	0	0	0	0
HYB 30	2.2	0	0	0	26.7
HYB 31	1.2	16.7	41.7	50	50
HYB 32	1.2	0	16.7	16.7	25
HYB 33	4.2	0	0	0	12.5
HYB 34	3	0	8.4	0	0
HYB 35	2.5	8.4	33.4	25	33.4
HYB 36	3.2	0	8.4	0	10

HYB 37	3.5	40	58.4	48.4	50
HYB 38	3.5	0	0	8.4	8.4
HYB 39	3.5	0	16.7	16.7	33.3
HYB 40	5.5	0	10	18.4	25
HYB 41	4	0	0	0	20.9
HYB 42	1.2	0	41.7	33.4	40
HYB 43	1.2	0	0	0	22.5
HYB 44	3.2	0	16.7	16.7	8.4
HYB 45	5.2	0	0	0	0
HYB 46	2.2	0	25	25	20
HYB 47	1.2	0	41.7	33.3	45
HYB 48	2.5	0	16.7	0	16.7
INB 01		8.4	16.7	41.7	25
INB 02		0	8.4	8.4	50
INB 03		0	0	0	91.7
INB 04		8.4	16.7	8.4	0
INB 05		8.4	8.4	16.7	33.4
INB 06		8.4	25	50	25
INB 07		0	0	33.4	8.4
INB 08		0	8.4	0	75
INB 09		0	0	0	41.7
INB 10		8.4	8.4	8.4	25
INB 11		25	33.4	50	50
INB 12		0	0	0	0
INB 13		33.4	25	50	35.4
INB 14		41.7	41.7	50	91.7
INB 15		0	0	8.4	25
INB 16		0	8.4	0	75
INB 17		25	41.7	66.7	75
INB 18		0	0	0	41.7
INB 19		0	0	25	66.7
INB 20		0	0	0	40
INB 21		0	0	16.7	33.3
INB 22		58.4	100	91.7	66.7
INB 23		92.9	92.9	92.9	75.7
INB 24		0	36.7	46.7	71.7
INB 25		75	91.7	100	100
INB 26		8.4	8.4	25	58.4
INB 27		16.7	0	16.7	16.7
INB 28		25	33.4	33.4	33.3
INB 29		0	0	33.4	50
INB 30		8.4	16.7	25	25
INB 31		52.4	52.4	92.9	77.4

Appendix 2: Chlorophyll Content and Cell Wall Components across Genotypes

Code	SPADR2	ADF	ADL	DCW	NDF	CF	DCS	DIGND	DINAG	DMO	HCEL	NFC	OADF	ONDF	PROS	SSG
HYB 01	61.6	29.3	2.2	58.0	56.1	27.2	57.8	24.8	41.3	58.3	26.9	28.2	36.6	56.2	7.2	26.1
HYB 02	63.8	32.1	2.7	55.8	59.8	29.8	54.0	23.6	39.2	54.0	27.8	24.3	38.8	59.5	7.0	23.1
HYB 03	60.5	33.4	3.0	52.1	63.5	31.2	50.6	20.6	36.1	51.1	30.2	22.7	40.0	61.4	6.6	19.2
HYB 04	63.2	32.4	2.7	53.6	61.0	29.6	52.6	21.6	36.6	53.2	28.6	25.3	38.5	59.3	6.7	22.2
HYB 05	59.9	32.0	2.6	56.3	60.2	29.0	54.3	23.5	38.5	54.7	28.3	25.6	38.0	59.4	7.3	22.9
HYB 06	58.6	29.5	2.5	54.5	56.0	27.5	56.2	22.3	38.3	56.6	26.5	29.0	36.5	54.6	7.2	26.3
HYB 07	56.6	32.8	2.8	55.1	61.3	31.0	51.7	21.2	37.8	51.8	28.5	22.4	39.2	60.2	6.9	20.7
HYB 08	59.1	30.9	2.3	60.1	58.3	28.2	57.5	27.3	41.8	57.6	27.4	27.1	36.7	58.5	6.8	24.8
HYB 09	59.9	30.4	2.3	59.6	56.8	28.4	57.4	26.8	41.5	58.1	26.4	27.3	37.1	57.3	6.5	25.9
HYB 10	57.3	31.9	2.6	55.2	60.6	29.8	53.9	22.4	38.7	54.3	28.8	24.8	37.9	58.4	7.6	21.3
HYB 11	56.0	34.0	2.7	57.1	62.8	31.1	52.7	24.3	38.4	52.4	28.9	23.2	39.5	61.6	6.7	21.1
HYB 12	59.3	34.2	2.8	56.9	64.5	31.2	52.3	24.1	38.5	52.6	30.3	22.5	39.7	62.3	6.5	19.1
HYB 13	59.3	32.0	2.6	54.8	59.3	29.2	54.0	22.1	37.4	54.3	27.3	26.6	38.2	57.7	6.9	24.2
HYB 14	57.5	31.0	2.5	56.8	59.1	29.1	54.5	22.7	39.7	54.3	28.1	24.5	36.9	58.9	7.6	21.5
HYB 15	55.3	32.4	2.7	58.3	59.9	29.6	55.6	26.1	40.1	55.7	27.6	25.8	38.3	58.7	6.5	24.2
HYB 16	66.1	30.6	2.3	54.7	59.4	29.1	53.5	20.8	38.6	53.9	28.8	24.4	37.4	58.1	8.2	20.9
HYB 17	64.4	31.2	2.5	57.0	59.3	29.4	54.6	23.4	40.3	54.7	28.2	24.1	38.0	60.0	7.7	21.9
HYB 18	60.6	29.7	2.4	55.4	56.0	27.8	56.6	23.3	39.2	56.9	26.4	28.6	37.7	55.5	7.1	26.8
HYB 19	61.0	29.8	2.6	51.7	56.5	27.4	54.9	20.0	36.0	55.5	26.7	29.5	38.1	54.4	7.3	27.3
HYB 20	57.4	31.5	2.4	58.1	61.2	29.3	54.6	24.9	40.3	54.4	29.7	24.0	37.6	61.3	6.7	19.8
HYB 21	59.3	31.9	2.4	55.7	59.4	29.5	54.0	23.5	37.4	54.7	27.5	26.5	38.7	58.1	5.7	24.7
HYB 22	62.4	33.5	2.8	51.6	62.3	31.2	50.7	20.0	35.0	50.7	28.8	24.1	40.3	59.3	6.7	21.7
HYB 23	65.3	31.0	2.5	54.9	58.7	28.7	54.2	21.8	37.8	54.6	27.7	26.4	38.0	57.6	7.0	23.7
HYB 24	55.4	30.2	2.5	55.2	57.3	28.2	56.3	24.0	39.1	56.5	27.0	28.3	37.6	56.0	6.6	25.5
HYB 25	52.9	31.5	2.3	58.0	61.1	29.5	54.9	25.7	40.8	55.3	29.6	23.9	37.9	60.8	6.9	20.3
HYB 26	58.2	30.6	2.4	54.2	57.5	28.0	54.9	22.0	37.7	55.3	27.0	27.7	37.6	56.7	7.1	25.0

HYB 27	60.7	28.8	2.5	56.4	54.9	26.8	58.5	25.0	40.5	58.7	26.1	30.1	36.2	54.4	6.9	27.7
HYB 28	60.3	31.1	2.6	53.3	58.9	28.8	54.4	22.6	37.8	54.9	27.8	26.6	37.9	57.7	6.7	24.2
HYB 29	58.3	30.1	2.4	56.8	57.4	28.3	56.5	24.5	40.5	56.6	27.3	26.8	36.7	56.8	7.1	24.2
HYB 30	59.5	31.1	2.6	57.0	60.3	29.4	52.2	20.9	39.8	51.7	29.3	20.7	38.1	63.0	8.4	18.3
HYB 31	56.5	35.0	3.0	53.4	65.1	32.2	47.9	19.4	36.9	47.1	30.1	17.4	40.8	65.7	7.7	15.5
HYB 32	59.5	29.9	2.3	55.7	58.2	28.0	54.4	21.1	39.5	54.0	28.3	24.6	37.3	58.8	7.8	22.1
HYB 33	58.8	30.3	2.3	58.0	59.5	28.4	54.1	22.4	41.3	53.8	29.2	21.9	37.2	62.0	8.8	19.1
HYB 34	62.2	30.3	2.4	58.0	60.8	28.3	54.2	22.6	41.2	54.0	30.5	22.2	36.2	61.3	8.5	16.0
HYB 35	57.6	31.5	2.7	55.4	59.9	29.3	53.9	23.2	39.3	53.7	28.3	24.1	39.1	60.2	7.2	22.2
HYB 36	61.8	31.9	2.8	56.2	60.5	29.8	52.4	22.2	40.3	52.1	28.6	20.2	38.9	62.4	8.2	18.7
HYB 37	64.0	32.3	2.7	54.9	60.5	30.1	52.5	21.7	38.9	52.0	28.3	22.3	38.8	60.8	7.7	20.2
HYB 38	60.4	30.6	2.4	56.3	58.3	29.1	55.1	23.3	41.0	54.5	27.7	23.9	37.3	59.2	7.6	22.1
HYB 39	56.3	32.2	2.8	54.5	60.7	30.2	52.7	22.0	39.1	52.1	28.5	22.3	38.4	60.5	7.2	20.4
HYB 40	56.9	29.7	2.2	59.2	57.9	28.1	56.7	25.2	43.4	56.6	28.3	23.5	35.5	59.5	8.5	20.7
HYB 41	56.3	31.7	2.7	54.9	61.8	30.0	51.6	20.4	38.2	50.7	30.1	21.7	39.0	62.0	7.8	18.8
HYB 42	60.6	29.6	2.5	56.0	58.1	27.4	55.3	22.1	40.0	55.6	28.4	25.6	37.0	58.8	8.1	21.8
HYB 43	61.8	30.3	2.6	53.7	58.3	28.5	53.9	21.6	39.0	54.0	28.0	24.4	38.2	59.2	8.0	21.7
HYB 44	59.8	29.5	2.3	56.0	56.5	27.9	56.0	22.4	39.7	56.6	27.1	27.2	36.3	55.2	7.5	24.4
HYB 45	58.6	30.6	2.7	54.4	59.5	28.5	54.0	21.2	39.2	53.7	28.8	24.2	37.5	58.9	8.0	20.7
HYB 46	58.8	30.7	2.4	54.9	60.1	29.4	53.6	22.5	39.6	53.9	29.3	23.2	38.3	60.6	7.3	20.1
HYB 47	55.4	30.1	2.5	55.5	59.2	28.7	54.5	22.5	40.9	54.3	29.1	23.0	37.4	60.0	8.1	19.6
HYB 48	62.5	30.8	2.6	57.1	59.2	28.9	55.3	24.6	41.5	55.2	28.4	23.6	37.2	60.9	7.5	21.3
INB 01	61.6	26.3	2.0	56.4	52.1	24.7	60.4	22.5	41.0	60.7	25.9	32.8	34.4	49.6	7.9	29.6
INB 02	58.0	29.3	2.6	53.7	55.7	27.1	56.8	21.4	39.0	56.6	26.4	29.3	37.3	54.2	7.7	27.1
INB 03	58.8	27.5	2.3	54.3	53.6	25.8	57.6	19.2	39.2	57.3	26.0	30.3	35.6	51.0	8.4	28.1
INB 04	52.4	26.1	1.8	62.4	51.0	23.7	63.5	27.9	46.1	63.4	24.9	32.3	32.9	51.2	9.1	29.2
INB 05	52.5	26.8	1.8	57.5	53.1	25.0	59.0	20.9	39.1	59.0	26.3	32.8	34.3	50.3	8.4	28.0
INB 06	61.8	28.3	2.4	60.7	56.4	26.7	59.9	26.7	44.8	60.2	28.1	27.3	34.8	54.8	8.6	24.2
INB 07	56.0	27.0	2.1	61.0	54.6	24.4	61.3	26.8	44.9	61.5	27.6	29.9	33.1	52.2	9.1	25.9
INB 08	53.4	28.7	2.5	55.7	56.0	27.2	57.4	24.0	41.6	57.2	27.3	27.3	36.3	56.8	7.7	24.9
INB 09	56.0	26.9	2.3	56.2	53.1	25.1	60.3	24.5	42.6	60.8	26.2	30.9	35.0	52.2	8.3	28.1

INB 10	47.7	25.3	2.0	58.7	49.6	23.4	63.4	26.9	44.5	64.0	24.4	34.1	32.8	49.5	8.3	31.6
INB 11	58.2	28.3	2.3	59.9	55.0	26.6	60.2	26.8	42.9	60.3	26.8	30.2	34.6	53.3	7.2	26.6
INB 12	55.5	28.6	2.0	63.6	56.6	26.1	61.2	29.3	46.6	61.3	28.1	27.4	33.4	54.3	8.8	23.7
INB 13	61.7	27.9	2.1	60.8	55.6	26.1	60.9	28.0	44.0	62.1	27.7	30.1	34.1	52.0	7.7	26.4
INB 14	63.0	27.9	2.4	55.6	54.8	25.7	59.5	24.0	41.8	60.3	26.9	30.5	35.6	51.0	8.4	27.3
INB 15	58.4	26.2	1.9	58.4	51.4	23.9	62.3	25.4	43.8	62.1	25.2	32.9	33.4	50.7	8.3	29.4
INB 16	53.9	25.1	1.8	55.7	49.4	23.4	60.2	19.7	39.7	59.8	24.3	34.1	33.8	49.0	8.7	31.4
INB 17	45.4	23.3	1.7	62.3	48.3	21.6	65.8	27.5	48.0	65.0	25.0	34.2	30.3	49.0	9.9	29.5
INB 18	45.5	26.6	2.4	53.0	52.1	24.7	57.2	17.0	39.7	56.1	25.6	29.1	35.0	52.3	10.4	26.2
INB 19	56.0	24.4	1.9	56.3	49.5	23.0	62.2	23.2	43.6	61.5	25.1	32.9	32.8	50.3	9.3	29.3
INB 20	58.0	26.4	2.2	55.9	51.5	24.3	60.0	22.1	40.9	60.0	25.1	32.3	34.5	50.9	8.5	29.6
INB 21	54.4	26.1	2.0	57.9	52.5	25.1	60.8	24.7	43.1	61.1	26.3	31.0	33.3	51.0	8.3	27.2
INB 22	50.8	26.9	2.1	59.8	54.1	25.1	59.9	25.8	45.3	59.6	27.3	26.6	33.3	56.7	9.1	23.7
INB 23	59.7	26.4	1.8	60.3	53.7	24.6	61.3	27.7	46.0	61.1	27.4	28.3	33.5	56.7	9.1	25.2
INB 24	64.0	23.5	1.8	58.4	49.5	22.5	63.9	24.8	45.0	63.5	26.0	34.3	31.9	49.0	9.3	28.5
INB 25	54.8	26.7	2.1	55.5	52.8	24.9	59.6	23.5	42.8	59.9	26.1	29.4	34.2	53.4	8.9	26.2
INB 26	61.8	23.8	1.7	57.7	49.8	22.4	61.7	21.1	44.5	61.3	26.0	30.9	32.2	49.9	10.3	26.9
INB 27	58.4	27.7	2.2	58.7	54.6	25.6	58.4	24.0	43.7	58.7	27.0	26.2	34.4	55.8	9.2	24.1
INB 28	56.0	29.9	2.2	59.5	56.8	27.3	56.9	22.5	41.4	56.0	26.9	26.6	36.0	56.1	8.7	24.4
INB 29	54.9	27.4	2.2	58.8	54.0	25.5	60.8	24.3	44.3	60.5	26.6	29.5	34.5	53.3	9.4	26.2
INB 30	59.3	30.0	2.3	57.1	57.8	27.0	53.6	14.6	39.1	51.8	27.8	23.9	34.1	56.5	12.5	20.6
INB 31	52.5	25.6	2.35	57.3	52.1	24.7	61.5	26.0	45.6	61.2	26.5	29.4	33.5	53.5	8.6	26.4

Appendix 3: Correlation between all Traits across Inbred and Hybrid Set

	STLP_1	STLP_2	STLP_3	SROTP	SPADR2	ADF	ADL	DCW	NDF	CF	DCS	DIGND	DINAG	DMO	HCEL	NFC	OADF	ONDF	PROS	SSG
STLP_1	1	0.85	0.79	0.5	-0.13	-0.21	-0.15	0.22	-0.18	-0.21	0.24	0.25	0.34	0.23	-0.09	0.08	-0.25	-0.1	0.21	0.08
STLP_2	0.85	1	0.83	0.53	-0.14	-0.14	-0.09	0.2	-0.1	-0.13	0.15	0.19	0.31	0.13	-0.01	-0.02	-0.19	0	0.2	-0.03
STLP_3	0.79	0.83	1	0.57	-0.16	-0.33	-0.26	0.31	-0.27	-0.32	0.35	0.29	0.47	0.33	-0.12	0.16	-0.38	-0.2	0.32	0.13
SROTP	0.5	0.53	0.57	1	-0.22	-0.43	-0.26	0.09	-0.41	-0.41	0.36	0.06	0.32	0.33	-0.3	0.3	-0.38	-0.34	0.33	0.29
SPADR2	-0.13	-0.14	-0.16	-0.22	1	0.29	0.19	-0.19	0.31	0.31	-0.31	-0.14	-0.27	-0.27	0.28	-0.26	0.32	0.25	-0.21	-0.24
ADF	-0.21	-0.14	-0.33	-0.43	0.29	1	0.81	-0.45	0.96	0.98	-0.92	-0.29	-0.73	-0.9	0.73	-0.85	0.94	0.88	-0.63	-0.76
ADL	-0.15	-0.09	-0.26	-0.26	0.19	0.81	1	-0.64	0.74	0.8	-0.79	-0.38	-0.67	-0.79	0.49	-0.68	0.84	0.67	-0.48	-0.56
DCW	0.22	0.2	0.31	0.09	-0.19	-0.45	-0.64	1	-0.38	-0.48	0.65	0.76	0.84	0.64	-0.2	0.3	-0.65	-0.29	0.34	0.23
NDF	-0.18	-0.1	-0.27	-0.41	0.31	0.96	0.74	-0.38	1	0.97	-0.92	-0.27	-0.66	-0.89	0.89	-0.91	0.9	0.94	-0.56	-0.88
CF	-0.21	-0.13	-0.32	-0.41	0.31	0.98	0.8	-0.48	0.97	1	-0.94	-0.3	-0.73	-0.91	0.77	-0.87	0.95	0.9	-0.65	-0.8
DCS	0.24	0.15	0.35	0.36	-0.31	-0.92	-0.79	0.65	-0.92	-0.94	1	0.58	0.84	0.99	-0.75	0.87	-0.93	-0.87	0.48	0.82
DIGND	0.25	0.19	0.29	0.06	-0.14	-0.29	-0.38	0.76	-0.27	-0.3	0.58	1	0.71	0.63	-0.18	0.31	-0.41	-0.21	-0.13	0.29
DINAG	0.34	0.31	0.47	0.32	-0.27	-0.73	-0.67	0.84	-0.66	-0.73	0.84	0.71	1	0.82	-0.41	0.47	-0.84	-0.51	0.56	0.41
DMO	0.23	0.13	0.33	0.33	-0.27	-0.9	-0.79	0.64	-0.89	-0.91	0.99	0.63	0.82	1	-0.73	0.88	-0.9	-0.86	0.4	0.82
HCEL	-0.09	-0.01	-0.12	-0.3	0.28	0.73	0.49	-0.2	0.89	0.77	-0.75	-0.18	-0.41	-0.73	1	-0.86	0.66	0.86	-0.32	-0.93
NFC	0.08	-0.02	0.16	0.3	-0.26	-0.85	-0.68	0.3	-0.91	-0.87	0.87	0.31	0.47	0.88	-0.86	1	-0.77	-0.96	0.28	0.96
OADF	-0.25	-0.19	-0.38	-0.38	0.32	0.94	0.84	-0.65	0.9	0.95	-0.93	-0.41	-0.84	-0.9	0.66	-0.77	1	0.82	-0.68	-0.67
ONDF	-0.1	0	-0.2	-0.34	0.25	0.88	0.67	-0.29	0.94	0.9	-0.87	-0.21	-0.51	-0.86	0.86	-0.96	0.82	1	-0.44	-0.93
PROS	0.21	0.2	0.32	0.33	-0.21	-0.63	-0.48	0.34	-0.56	-0.65	0.48	-0.13	0.56	0.4	-0.32	0.28	-0.68	-0.44	1	0.21
SSG	0.08	-0.03	0.13	0.29	-0.24	-0.76	-0.56	0.23	-0.88	-0.8	0.82	0.29	0.41	0.82	-0.93	0.96	-0.67	-0.93	0.21	1

Appendix 4: Correlation between all Traits in the Hybrid Set

	SRSR16_17	STLP_1	STLP_2	STLP_3	SROTP	SPADR2	ADF	ADL	DCW	NDF	CF	DCS	DIGND	DINAG	DMO	HCEL	NFC	OADF	ONDF	PROS	SSG
SRSR16_17	1	0.1	-0.06	-0.02	0.01	0.28	-0.06	-0.03	0.15	-0.07	-0.03	0.11	0.12	0.18	0.1	-0.05	0	-0.09	-0.03	0.11	0.05
STLP_1	0.1	1	0.76	0.67	0.41	-0.05	0.11	0.09	0.06	0.1	0.11	-0.04	0.09	0.09	-0.06	0.05	-0.13	0.1	0.12	0.05	-0.08
STLP_2	-0.06	0.76	1	0.87	0.58	-0.11	0.05	0.08	0.12	0.09	0.07	-0.03	0.06	0.21	-0.06	0.11	-0.21	0.02	0.16	0.18	-0.18
STLP_3	-0.02	0.67	0.87	1	0.62	-0.14	0.08	0.04	0.08	0.11	0.12	-0.08	0.03	0.15	-0.11	0.11	-0.23	0.06	0.18	0.13	-0.18
SROTP	0.01	0.41	0.58	0.62	1	-0.11	0.05	0	0.09	0.08	0.08	-0.07	0.03	0.11	-0.09	0.09	-0.19	0.04	0.18	0.08	-0.15
SPADR2	0.28	-0.05	-0.11	-0.14	-0.11	1	-0.11	-0.05	-0.11	-0.15	-0.12	0.01	-0.21	-0.05	0.03	-0.15	0.06	-0.06	-0.13	0.2	0.1
ADF	-0.06	0.11	0.05	0.08	0.05	-0.11	1	0.72	-0.36	0.9	0.96	-0.84	-0.27	-0.58	-0.82	0.49	-0.65	0.89	0.71	-0.39	-0.5
ADL	-0.03	0.09	0.08	0.04	0	-0.05	0.72	1	-0.6	0.58	0.69	-0.68	-0.46	-0.56	-0.71	0.22	-0.45	0.79	0.43	-0.14	-0.28
DCW	0.15	0.06	0.12	0.08	0.09	-0.11	-0.36	-0.6	1	-0.25	-0.37	0.59	0.78	0.85	0.55	-0.03	0.07	-0.6	0.01	0.17	0.01
NDF	-0.07	0.1	0.09	0.11	0.08	-0.15	0.9	0.58	-0.25	1	0.91	-0.87	-0.3	-0.44	-0.85	0.82	-0.81	0.79	0.87	-0.17	-0.79
CF	-0.03	0.11	0.07	0.12	0.08	-0.12	0.96	0.69	-0.37	0.91	1	-0.88	-0.31	-0.53	-0.86	0.58	-0.74	0.88	0.76	-0.32	-0.6
DCS	0.11	-0.04	-0.03	-0.08	-0.07	0.01	-0.84	-0.68	0.59	-0.87	-0.88	1	0.69	0.68	0.98	-0.64	0.78	-0.87	-0.74	0.06	0.69
DIGND	0.12	0.09	0.06	0.03	0.03	-0.21	-0.27	-0.46	0.78	-0.3	-0.31	0.69	1	0.69	0.68	-0.26	0.34	-0.47	-0.18	-0.27	0.32
DINAG	0.18	0.09	0.21	0.15	0.11	-0.05	-0.58	-0.56	0.85	-0.44	-0.53	0.68	0.69	1	0.62	-0.11	0.07	-0.74	-0.09	0.45	0.01
DMO	0.1	-0.06	-0.06	-0.11	-0.09	0.03	-0.82	-0.71	0.55	-0.85	-0.86	0.98	0.68	0.62	1	-0.63	0.81	-0.85	-0.77	-0.01	0.71
HCEL	-0.05	0.05	0.11	0.11	0.09	-0.15	0.49	0.22	-0.03	0.82	0.58	-0.64	-0.26	-0.11	-0.63	1	-0.78	0.4	0.82	0.17	-0.92
NFC	0	-0.13	-0.21	-0.23	-0.19	0.06	-0.65	-0.45	0.07	-0.81	-0.74	0.78	0.34	0.07	0.81	-0.78	1	-0.55	-0.94	-0.31	0.93
OADF	-0.09	0.1	0.02	0.06	0.04	-0.06	0.89	0.79	-0.6	0.79	0.88	-0.87	-0.47	-0.74	-0.85	0.4	-0.55	1	0.59	-0.38	-0.38
ONDF	-0.03	0.12	0.16	0.18	0.18	-0.13	0.71	0.43	0.01	0.87	0.76	-0.74	-0.18	-0.09	-0.77	0.82	-0.94	0.59	1	0.12	-0.89
PROS	0.11	0.05	0.18	0.13	0.08	0.2	-0.39	-0.14	0.17	-0.17	-0.32	0.06	-0.27	0.45	-0.01	0.17	-0.31	-0.38	0.12	1	-0.36
SSG	0.05	-0.08	-0.18	-0.18	-0.15	0.1	-0.5	-0.28	0.01	-0.79	-0.6	0.69	0.32	0.01	0.71	-0.92	0.93	-0.38	-0.89	-0.36	1

Appendix 5: Correlation between all Traits in the Inbred Set

	STLP_1	STLP_2	STLP_3	SROTP	SPADR2	ADF	ADL	DCW	NDF	CF	DCS	DIGND	DINAG	DMO	HCEL	NFC	OADF	ONDF	PROS	SSG
STLP_1	1	0.91	0.8	0.43	-0.01	0.02	0	0.13	0.1	0.04	0.03	0.25	0.26	0.05	0.19	-0.24	-0.08	0.32	0	-0.23
STLP_2	0.91	1	0.82	0.45	-0.04	0.01	-0.02	0.14	0.08	0.03	0.03	0.22	0.26	0.03	0.17	-0.23	-0.12	0.32	0.03	-0.25
STLP_3	0.8	0.82	1	0.38	0.05	-0.12	-0.09	0.24	0.02	-0.07	0.19	0.32	0.4	0.2	0.22	-0.13	-0.25	0.2	0.06	-0.19
SROTP	0.43	0.45	0.38	1	0	-0.21	0.1	-0.34	-0.19	-0.12	0.02	-0.1	-0.05	0.01	-0.12	0.09	0.01	-0.05	-0.04	0.11
SPADR2	-0.01	-0.04	0.05	0	1	0.15	-0.01	0	0.26	0.19	-0.12	0.01	-0.08	-0.05	0.36	-0.11	0.17	0.06	-0.12	-0.17
ADF	0.02	0.01	-0.12	-0.21	0.15	1	0.68	-0.1	0.93	0.95	-0.79	-0.19	-0.43	-0.73	0.61	-0.78	0.78	0.74	-0.17	-0.67
ADL	0	-0.02	-0.09	0.1	-0.01	0.68	1	-0.45	0.55	0.68	-0.6	-0.24	-0.38	-0.56	0.23	-0.52	0.71	0.44	-0.2	-0.35
DCW	0.13	0.14	0.24	-0.34	0	-0.1	-0.45	1	0.02	-0.19	0.54	0.76	0.79	0.54	0.18	0	-0.56	0.06	0.07	-0.09
NDF	0.1	0.08	0.02	-0.19	0.26	0.93	0.55	0.02	1	0.94	-0.75	-0.09	-0.3	-0.67	0.85	-0.85	0.69	0.82	-0.12	-0.83
CF	0.04	0.03	-0.07	-0.12	0.19	0.95	0.68	-0.19	0.94	1	-0.82	-0.19	-0.46	-0.74	0.68	-0.77	0.83	0.76	-0.28	-0.7
DCS	0.03	0.03	0.19	0.02	-0.12	-0.79	-0.6	0.54	-0.75	-0.82	1	0.68	0.77	0.98	-0.51	0.73	-0.8	-0.65	-0.08	0.66
DIGND	0.25	0.22	0.32	-0.1	0.01	-0.19	-0.24	0.76	-0.09	-0.19	0.68	1	0.84	0.75	0.08	0.16	-0.4	-0.01	-0.4	0.14
DINAG	0.26	0.26	0.4	-0.05	-0.08	-0.43	-0.38	0.79	-0.3	-0.46	0.77	0.84	1	0.75	-0.03	0.13	-0.71	-0.06	0.11	0.08
DMO	0.05	0.03	0.2	0.01	-0.05	-0.73	-0.56	0.54	-0.67	-0.74	0.98	0.75	0.75	1	-0.43	0.71	-0.72	-0.62	-0.21	0.65
HCEL	0.19	0.17	0.22	-0.12	0.36	0.61	0.23	0.18	0.85	0.68	-0.51	0.08	-0.03	-0.43	1	-0.75	0.39	0.75	-0.02	-0.87
NFC	-0.24	-0.23	-0.13	0.09	-0.11	-0.78	-0.52	0	-0.85	-0.77	0.73	0.16	0.13	0.71	-0.75	1	-0.49	-0.93	-0.22	0.94
OADF	-0.08	-0.12	-0.25	0.01	0.17	0.78	0.71	-0.56	0.69	0.83	-0.8	-0.4	-0.71	-0.72	0.39	-0.49	1	0.51	-0.39	-0.37
ONDF	0.32	0.32	0.2	-0.05	0.06	0.74	0.44	0.06	0.82	0.76	-0.65	-0.01	-0.06	-0.62	0.75	-0.93	0.51	1	0.06	-0.88
PROS	0	0.03	0.06	-0.04	-0.12	-0.17	-0.2	0.07	-0.12	-0.28	-0.08	-0.4	0.11	-0.21	-0.02	-0.22	-0.39	0.06	1	-0.27
SSG	-0.23	-0.25	-0.19	0.11	-0.17	-0.67	-0.35	-0.09	-0.83	-0.7	0.66	0.14	0.08	0.65	-0.87	0.94	-0.37	-0.88	-0.27	1

Appendix 6: Correlation between the Stalk Rot Rating in the Hybrid Set and the Mid Parent value

	SRSR16_17	STLP_1	STLP_2	STLP_3	SROTP	STLP_1_MID	STLP_2_MID	STLP_3_MID	SROTP_MID
SRSR16_17	1	0.14	-0.10	-0.04	0	0.09	0.21	0.15	0.04
STLP_1	0.14	1	0.72	0.62	0.45	-0.07	-0.01	-0.06	0.12
STLP_2	-0.10	0.72	1	0.91	0.68	0.13	0.21	0.24	0.39
STLP_3	-0.04	0.62	0.91	1	0.76	0.23	0.28	0.36	0.41
SROTP	0	0.45	0.68	0.76	1	0.07	0.18	0.23	0.38
STLP_1_MID	0.09	-0.07	0.13	0.23	0.07	1	0.91	0.85	0.51
STLP_2_MID	0.21	-0.01	0.21	0.28	0.18	0.91	1	0.91	0.51
STLP_3_MID	0.15	-0.06	0.24	0.36	0.23	0.85	0.91	1	0.53
SROTP_MID	0.04	0.12	0.39	0.41	0.38	0.51	0.51	0.53	1

Appendix 7: Correlation between the Stalk Rot Rating and the Severity Rating

	STLP_1	STLP_2	STLP_3	SROTP	Sev
STLP_1	1	0.9	0.89	0.44	0.46
STLP_2	0.9	1	0.89	0.34	0.26
STLP_3	0.89	0.89	1	0.36	0.33
SROTP	0.44	0.34	0.36	1	0.93
Sev	0.46	0.26	0.33	0.93	1