Norwegian University
of Life Sciences

Master's Thesis 2023 60 ECTS
Faculty of Bioscience, department of plant sciences

The use of polytunnels to study the effect of drought stress and increased temperature on spring wheat.

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1. Abstract

An increase in wheat production will be vital to feed a growing population. Heat and drought are already causing great losses in yield, and climate change is expected to damage production in the future. Breeding for drought tolerance in wheat is therefore essential to ensure food security. To create drought conditions in this trial, two polytunnels were utilized. 16 genotypes from Norway, Sweden, Lithuania, Latvia, and Estonia were grown in polytunnels and exposed to two different temperature and irrigation treatments. The temperature treatment aimed to keep one tunnel ambient and for the temperature to increase in the other. For irrigation, it was attempted to decrease the grain yield by 50%. There were also aspirations to identify varieties tolerant to drought stress. This was the pilot season for this trial, and the first season the polytunnels were utilized. Therefore, several issues occurred during the season. These included elevated humidity due to poor ventilation and sub-par results due to a delay in achieving sufficient drought stress. However, the results from the ambient tunnel were satisfactory, and some interesting observations were made. The replicates in the ambient tunnel exposed to drought experienced a 30% reduction in yield, with the variety 013-01 being the best performing under both irrigation treatments. The treatments significantly affected all agronomic traits, and there was a significant difference between the genotypes. However, the interaction between genotype and treatment was only significant for plant height. When the varieties in the polytunnels were compared to those grown outside at Vollebekk in 2022, the majority ranked similarly regardless of treatment. The exception was DS-720-3-DH which ranked noticeably lower when exposed to drought as opposed to the NOBALwheat trial and control replicate, indicating genotype by environment interactions. These results suggest that the trial could be repeated to gain knowledge about drought tolerance in wheat. In future replications of the trial, however, it would be beneficial to improve the management of the tunnels to create better drought conditions and improve ventilation. High throughput phenotyping should also be included to gain further insight into the stress responses of the plants.

2. Introduction

With the threat of climate change, there is expected more extreme weather, increased temperatures, and more extended periods without precipitation (Tatar et al., 2016). Water is one of the most crucial factors limiting grain yield (GY) and plant productivity in agriculture (Stallmann et al., 2018). Wheat is one of the world's most important food crops; therefore, longer periods of drought have detrimental effects on food production. Studying the impact of drought stress on wheat is essential to ensure food security in the future.

Wheat production will need to increase to meet the growing population's dietary needs. Conventional breeding methods have led to an annual increase in production of 1% on average (Reynolds & Langridge, 2016). To meet the demand, wheat production will have to increase by an estimated 40% by 2050. In addition, climate change and higher temperatures are estimated to cause a 6% reduction in yield for each degree Celsius increase, while drought is already causing staggering losses in grain yield each year (Tricker et al., 2018).

In Norway, the climate is generally too cold for wheat production, and wheat is therefore mainly grown in the southeastern parts of the country. Both spring wheat and winter wheat are grown in Norway, with both being used for human consumption if the grain is of high enough quality. The lower-quality grain is mainly used for animal feed. (https://graminor.no/plant-breeding/cereals/wheat/?lang=en). With the expectations of climate change and increased temperatures, larger areas could become eligible for wheat production in the future, and it could become an increasingly important cultivar.

Drought tolerance is a trait that will be increasingly important in the future. Drought tolerance refers to a plant's ability to withstand drought stress and sustain physiological activity despite a lack of available water. It is a complex trait regulated by many genes and involves several different mechanisms. It can be divided into two categories: drought avoidance and drought resistance. Drought avoidance refers to mechanisms that allow the plant to maintain high water status despite drought, such as increasing root growth to uphold water absorption. Whereas drought resistance refers to the ability to maintain cell turgor pressure and continue metabolism despite low water status in the cells, for example by synthesis of osmolytes (Meshram, 2022).

When breeding for drought tolerance, selections are either done in areas with naturally occurring drought or by creating controlled or managed environments. Selection is based on how the varieties perform, and agricultural traits and other characteristics are evaluated to determine how the genotypes tolerate drought. Some characteristics that are of interest include final yield, leaf water retention, photosynthetic rate, and root development (https://agriinfo.in/measurement-of-drought-resistance-in-plant-breeding-2128/). Methods of conducting drought trials consist of blocking rain from reaching the fields in wet areas or irrigating the control plots in dry areas. In this trial, polytunnels will be utilized in combination with drip irrigation to create control and drought replicates under two different temperatures.

2.1 General Effects of drought and heat stress

Water deficit, or the lack of available water, occurs in most natural and agricultural habitats. Lack of water affects many physiological processes in plants. The primary effect is a reduction in water potential, hydraulic resistance, and cell dehydration. The secondary effects include reduced cell expansion, reduced cellular and metabolic activity, closure of stomata, photosynthetic inhibition, and altered carbon partitioning. Loss of water potential causes reduced turgor pressure, leading to volume reduction. On a molecular level, water deficit can cause destabilization of membranes and proteins, which leads to production of reactive oxygen species and higher concentration of ions, which leads to ion toxicity (Taiz et al,. 2018).

How the plants are affected by water deficit depends on the duration of the drought, its severity, and at what point during the growth stage it occurs. The types of droughts are divided into terminal and intermittent drought. Terminal drought refers to drought where the water availability decreases progressively during the season, while intermittent drought is caused by one or more intervals of poor irrigation during the season (Neumann, 2008). Terminal drought occurring during late stages of the growth season will coincide with the plants' flowering and grain filling stages and can lead to severe losses in yield or complete crop failure (Thungo, 2020).

Elevated temperatures and heat stress affect the plants in several ways. Changes in heat affect plant growth and development, leading to faster life cycle completion. Processes like water and nutrient uptake are affected by temperature increases, and it influences plants' photosynthetic capacity (El Sabagh et al., 2019). All these processes are essential to maintain productivity and achieve high yields. At the molecular level, elevated temperatures affect various cellular processes, such as cell metabolism and protein synthesis. Elevated temperature suppresses the development of normal cellular proteins, while simultaneously inducing the synthesis of heat shock proteins, rubisco activase, chloroplast glyceraldehyde 3-phosphate dehydrogenase, and chloroplast protein synthesis elongation factor (Prasad et al., 2008).

Heat and drought rarely occur separately, making it difficult to separate which responses are driven by which stressors. Moreover, some responses are antagonistic, and heat and drought share some common mechanisms. Some regions that produce the most wheat are arid or semi-arid areas and already need ways to alleviate drought stress in the crops. Climate change will also disproportionately affect these areas, making crop production more challenging. In some areas of the world with cooler climates, an increase in temperature may be beneficial, but it will not compensate for the negative impacts in other regions (Zhang et al., 2018).

Some responses are common for all environmental stresses, such as the formation of reactive oxygen species (ROS). ROS are generated in the chloroplast, mitochondria and in the cytoplasm upon exposure to heat and drought stresses, and cause membrane damage (El Sabagh et al., 2019). Heat shock proteins are another response to stressful environments. The combination of high temperature and drought has a negative, additive impact on plant phenology and physiology, i.e., growth, chlorophyll content, leaf photosynthesis, grain number, spikelet fertility, grain filling duration, and grain yield (Tricker et al., 2018).

2.2 Drought stress and gas exchange

The activity of the stomata in leaves is one way for plants to alter their physiology in response to abiotic stresses. The stomata are epidermal pores that link intercellular spaces with the surrounding environment, with the ability to open or close by the movements of two surrounding guard cells (Negi, 2014). Transpiration, the movement of water through the plants, happens through the stomata. Approximately 97% of all water absorbed is lost through transpiration. The gradient in vapor pressure between the leaves and the atmosphere drives the transpiration, with the loss being greater in warm and dry areas compared to cooler and more humid climates (Broughton, 2022). In response to a lack of water availability, the stomata will close as a measure to reduce transpiration rates and preserve water. However, this will also reduce the influx of CO_2 into the cells, limiting the plant's photosynthetic activity (Negi, 2014). This causes a trade-off between maintaining turgor pressure in the cells and having access to sufficient concentrations of CO_2 to uphold photosynthetic rates. Monitoring the stomatal conductance of crops and comparing this to their overall performance could be of interest to breeders. This can be used to screen for genotypes that preserve water while maintaining high yield and could be implemented in further breeding programs for drought tolerance.

Measuring the stomatal gas exchange can be challenging. However, the University of Tartu recently developed a technology that makes measuring gas exchange values directly in the field possible. This system, KaRal, is a combination of porometers and photosynthesis systems which are currently available. Porometers are systems that utilize sensors in a measuring head to measure humidity on the leaf, where these values are used to calculate transpiration (E) and stomatal conductance(G_s). Photosynthesis systems, however, measures H_2O and CO_2 concentration from the leaves using an Infrared Gas-analyzer (IRGA). This system can be used to calculate transpiration and stomatal conductance, but also the CO_2 uptake of the leaf (A_{net}).

The custom-made instrument KaRaL is something between the porometers and photosynthesis systems. The device features a photosynthetically active radiation (PAR) sensor, and sensors for both leaf temperature and ambient temperature. When a leaf is about to be measured, it is clamped with the measurement cuvette, and the air surrounding the leaf is pumped into a special type of bag that absorbs neither water vapor nor carbon dioxide, from which the H_2O and CO_2 concentrations are measured, and values stored as reference. Once a leaf is clamped to the measuring cuvette, the air in the bag is pumped through the cuvette and again measured with the IRGA. The flow rate and total volume of the gas tubes and the cuvette is tailored such that it is possible to obtain a reliable sample value, and for each "reference bag" volume of air, the program then calculates E, G_s and A_{net}. Each cycle measures 20 leaves in total, outputting the average E, G_s and A_{net} as well as PAR, leaf, and ambient temperature for the cycle/plot. Implementing the KaRal system when selecting for drought tolerance could prove valuable.

2.3 Strategies for conducting drought trials.

There are several strategies when conducting drought trials as a part of breeding programs. Direct selection is possible in arid or semi-arid environments, where drought occurs naturally, and irrigation systems can be used to create the control conditions. However, in areas where drought occurs sporadically, other steps must be taken to create the drought conditions needed for selection. In such cases, dedicated infrastructures are built to create drought conditions by, for instance, preventing precipitation from reaching the soil. Rainout shelters are commonly used for this purpose. (Langridge & Reynolds, 2021). These structures can be movable or static. The mobile shelters are usually built on rails, and either moved manually or in response to rain sensors that activate a drive system that drags the shelter over the field. The static rainout shelters are more akin to greenhouses with permanently closed roofs but can be adjusted to uphold ventilation. These require irrigation as the roof cannot be opened (Blum, 2000).

Movable rainout shelters with rain monitors that close in response to precipitation minimize unintended shelter effects in the field, as they are only closed for short periods. These are more vulnerable to the environment, and the need for sensors and electricity makes them expensive. The static shelters are more suited for long-term studies due to their robustness. However, permanently closing the roofs could have unintended effects on the microclimate in the field. Even if the walls can be adjusted to allow ventilation, closed roof changes temperature, humidity, and the amount of photosynthetically active radiation that permeates and reaches the canopy. The need for irrigation systems is another downside, as it makes it more expensive and moves the trial further away from natural conditions (Kundel et al., 2018).

In this thesis, 16 wheat varieties will be exposed to drought stress in polytunnels. Collected data on targeted agronomic traits will be used to evaluate and compare their performance. These traits include maturity, protein content, yield, height, and stomatal gas exchange, which are traits known to have physiological responses to drought (Wang et al., 2016). Since drought and elevated temperatures often occur simultaneously (Prasad et al., 2011), the effects of drought in ambient and elevated temperatures will be monitored. The performance of the polytunnels will also be reviewed to study if they are suitable as a tool that can be utilized when researching crops' responses to abiotic stresses.

The thesis will aim to evaluate the execution of the drought trial. The focus will be on the polytunnels and their performance in drought stress trials. Their performance will be evaluated based on the performance of the varieties grown in them, compared to the same genotypes being grown in an ordinary outside field trial. To what degree the intended stress is achieved, and other factors will also be considered. The experience gained will be used to improve future experiments.

2.4 Research questions:

- Study the effects of irrigation and temperature treatments on agricultural traits.
- Compare the performance of the genotypes under well-watered and drought conditions in ambient and elevated temperature regimes.
- Evaluate the performance of the polytunnels and how they can be better utilized in future experiments.

3. Materials and methods

3. 1 Field trial material and design

A collection of sixteen spring wheat varieties sourced from the NOBALwheat project (Breeding toolbox for sustainable food system of the Nordic Baltic region, 2020-2023) was used in the trial. The wheat varieties originate from Norway, Sweden, Lithuania, Latvia, and Estonia. This collection represents significant phenotypic variation (based on previous field trial results, data not shown) and encompasses historical and recent spring varieties alongside lines chosen based on their extreme phenotypes. Due to its high variation in many traits, the collection was deemed highly relevant for evaluating the trait responses to the environment.

The experiment was arranged as a randomized incomplete block design (block size of 4) with two replicates of each watering treatment as sections in each tunnel (temperature treatment) and the varieties tested on field trial plots within each watering section. Due to the availability of only two tunnels, temperature treatments were not replicated. The collection was replicated 8 times across temperature and water treatments (section environmental experiment conditions). Each collection replicate was surrounded with border plots (variety Bastian) from all four sides to eliminate border effects and to buffer water treatments (Figure 1).

Table 1: List of names and line codes for the varieties included in the trial.

Variety	*Line*	*Country*
013-01	NW158	Latvia
013-074	NW167	Latvia
876	NW121	Estonia
990-2	NW134	Estonia
Caress	NW63	Sweden
DS-17-16-DH	NW252	Lithuania
DS-638-5-DH	NW262	Lithuania
DS-655-7-DH	NW265	Lithuania
DS-720-3-DH	NW274	Lithuania
F-013-032	NW154	Latvia
Zombi	NW58	Norway
Betong	NW64	Norway
Hiie	NW79	Estonia
ROBIJS	NW137	Latvia
Runar	NW33	Norway
Voore	NW105	Estonia

3.2 Environmental experiment conditions

The experiment aimed to test two crucial environmental factors for plants: water availability and temperature. One of two polytunnels (ambient) was used as a temperature control treatment, closely mimicking the temperature outside. Another tunnel (elevated) was used as an elevated temperature treatment, with attempts to increase its temperature by around 5°C relative to the ambient tunnel. Within each tunnel, two watering treatments were present: control (with relative soil water content >80%) and drought (relative soil water content < 30%). Each watering treatment replicate was separated by a double border plot column/row to mitigate the influence of soil water content gradients between the treatments.

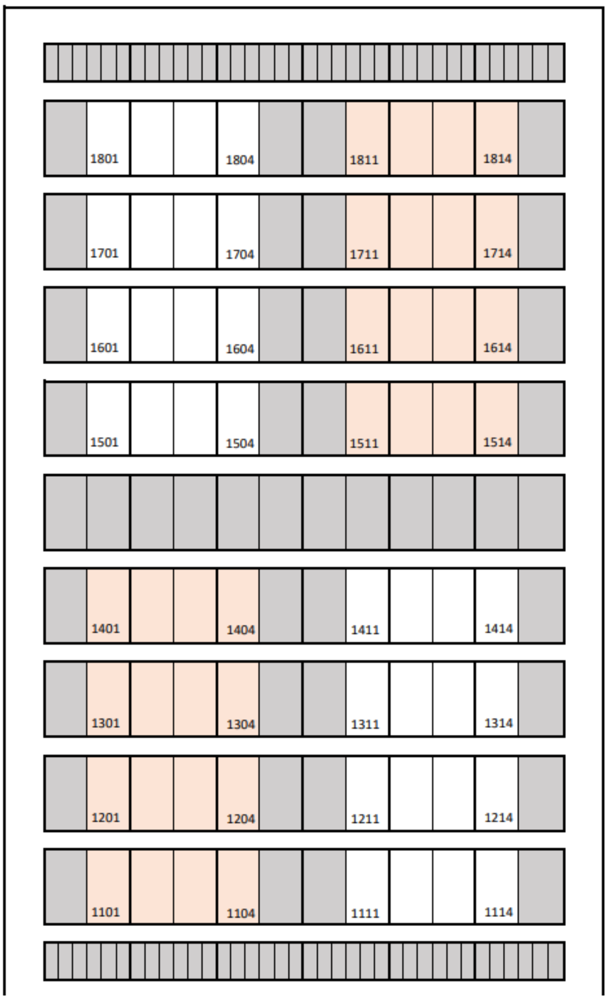

Figure 1: Field map of the ambient tunnel. Red plots are well-irrigated, white are drought, and grey are border plots. The plots are sowed as D-plots and are 2 meters long and 75 cm wide.

3.3 Irrigation system

The watering system consisted of the following elements: a headboard including main valves and the steering unit, large main pipes, and smaller secondary pipes (hereafter referred to as T-pipes). Each large main pipe independently fed water to one experimental treatment zone and each experimental plot was watered with three T-pipes to achieve even watering. Figure 2 visualizes how the pipes were distributed in the field. The T-pipes provide drip irrigation in a 20 cm circumference per hole, with 25 cm between each hole. The pipes were arranged to create an overlap between the drips, to water a larger area. The irrigation was controlled remotely through the Hydrawise app (https://www.hydrawise.com/) by regulating the length and frequencies of the watering periods.

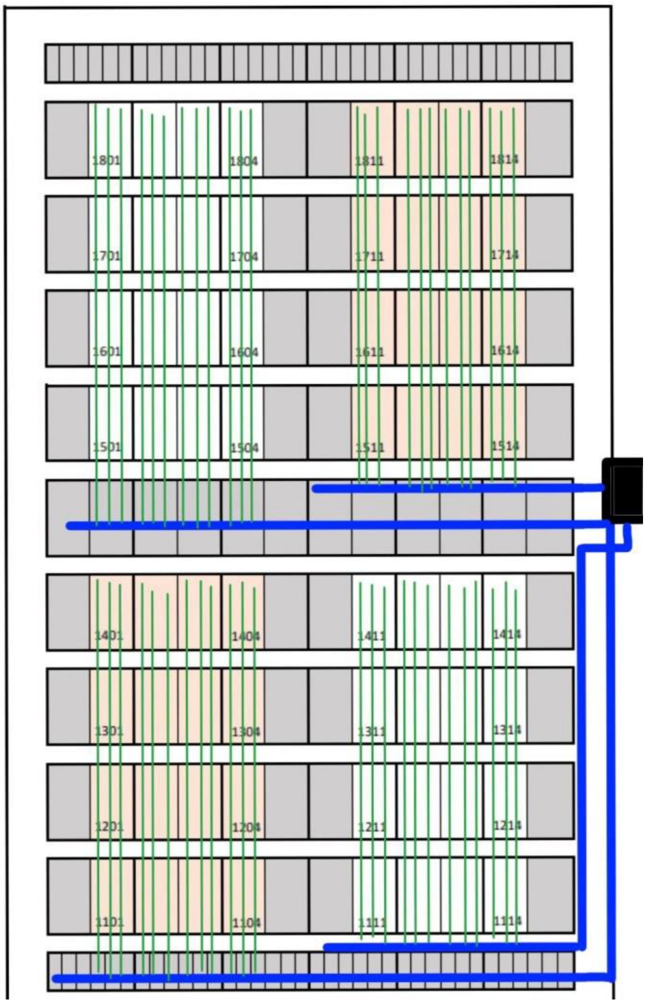

Figure 2: Approximation of placement of water pipes in the field. The blue lines represent the pipes from the headboard, and the green lines represent-pipes laid in the field.

3.4 Management

The field was sowed on May 27, 2022, followed by irrigation system installation. The first watering was on June 4. Initially, all zones were irrigated continuously for approximately 36 hours to thoroughly soak the field to promote germination. After this, the irrigation was put on hiatus until June 23, before the watering was regulated to achieve the wanted conditions in the field. The well-watered zones got 120 minutes of watering daily, while the dry zones got 30 minutes a day. This proved to be too much water in the dry zones, and the soil moisture was too high to induce drought stress. Therefore, the watering in the dry zones was stopped completely. The soil was sufficiently irrigated in the control zones, and the watering was put on halt in these zones as well from June 29 to July 15. By August 4, the drought zones in the ambient tunnel were sufficiently dry. Reactive watering was applied in an attempt to achieve a 50% reduction in yield. In the elevated tunnel, the soil still had sufficient soil moisture, so no changes were made to the watering regime. Due to the high humidity in the elevated tunnel, it was decided to lift the walls on the long side, the same way as in the ambient tunnel, to increase airflow and decrease humidity. Based on water flow rates through the irrigation system, it was estimated that each hour of irrigation equals 0.1mm of rainfall.

3.5 Sensors

The polytunnels utilized two different sensors: Soil scout sensors and EasyGrowth sensors. The Soil scout sensors are provided by Agdir (https://soilscout.com/solution/wireless-soil-moisture-sensor), and they monitor soil properties including moisture, temperature, salinity, and water potential. The EasyGrowth (https://soilscout.com/solution/wireless-soil-moisture-sensor) sensors monitor air temperature, humidity, and soil temperature with hourly resolution.

The soil scout sensors were installed in the ground at 8 locations in each tunnel (2 in each treatment replicate). At each location, two sensors were placed, one at 30 cm depth and one at 15 cm depth, 16 sensors in total. As different varieties may have variant root architecture (which influences their water uptake and confounds with soil moisture reads), soil scout sensors were placed in plots with two varieties only, 013-01 and Hiie), chosen based on the maximum achieved spatial reading resolution. EasyGrowth sensors were placed in the border plots in 6 locations in each tunnel, in the upper, middle, and lower sections. There were also two easygrowth sensors placed outside the tunnels to get reference values.

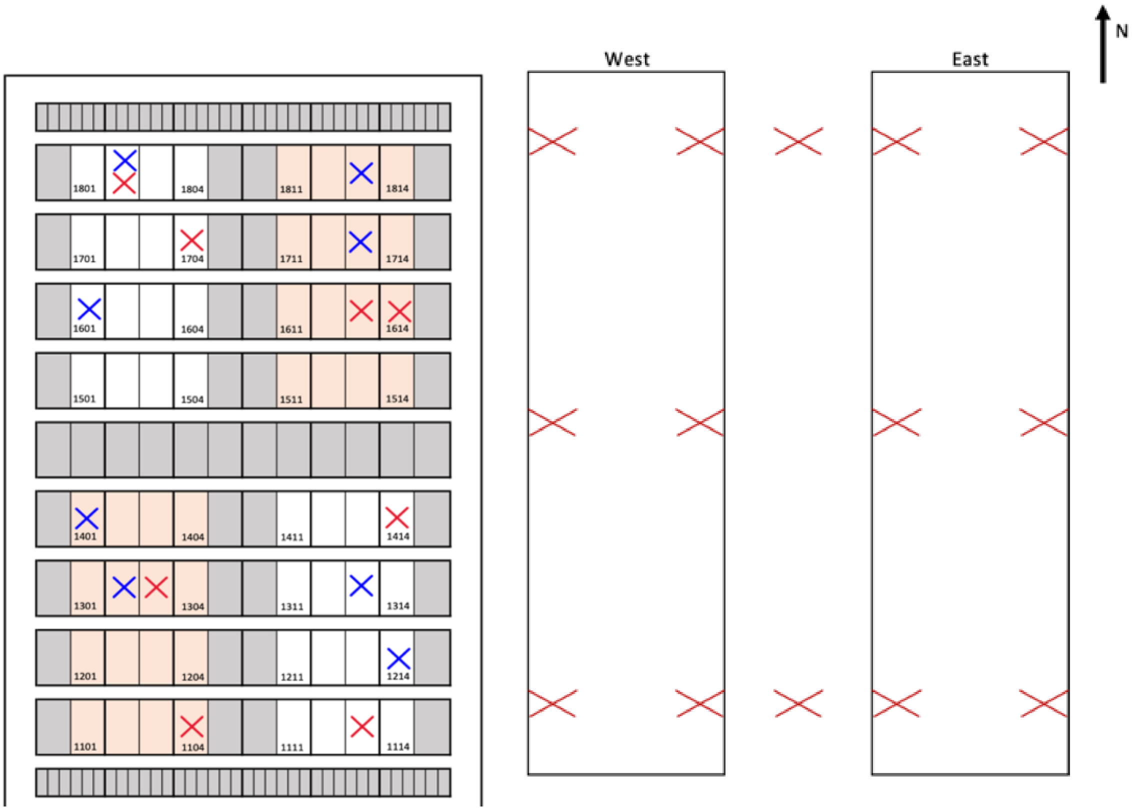

Figure 3: Map of Agdir and EasyGrowth sensors. Agdir to the left, blue marks show the sensor placement in the ambient tunnel, and red marks show the sensor placement in the elevated tunnel. EasyGrowth to the right, red marks show the placement of the sensors in both tunnels and outside the tunnels.

3.6 Control of atmosphere.

The atmosphere was regulated manually. By closing the walls or roof, the temperature would increase, while opening the walls or roofs would decrease the temperature. Closed walls/roof would also reduce the air flow and increase the humidity in the tunnels. The Reactive regulation of the atmosphere was based on sensor data and the weather forecast.

In the ambient tunnel, the short end walls were entirely open, and the long side walls were lifted approximately 1 meter to allow airflow. The roof was opened during the daytime when there were people in the vicinity to close them in case of rain, but they were kept closed at night. In the elevated tunnel, the short end walls were closed entirely during the season. At the beginning of the season, the long side walls were also kept closed, but they were lifted during the trial to allow airflow. They were raised approximately 1 meter, the same as in the ambient tunnel. The roof was opened during fieldwork to reduce temperature and humidity, especially when doing gas exchange measurements. The roof was also opened in both tunnels sporadically to let out birds.

3.7 Data collection

3.7.1 Grain yield:

The grain was harvested on September 15 using mainly a plot harvester. Plots with excessive lodging were collected using a sickle to prevent the mixing of the different varieties before being put into the harvester. The grain was dried in drying cabinets and then weighed to assess grain yield (GY) before it was cleaned.

3.7.1.1 Grain dimensions

Marvin proline seed analyzer (Marvitech GmbH, Germany) was used to measure thousand-grain weight (TGW) and kernel size, including kernel area, length, and width.

3.7.2 Grain protein content:

Grain protein content (GPC) was determined by near infrared reflectance spectroscopy on full kernels using Perten Inframatic 9200 spectrometer (Perten Instruments AB)

3.7.3 Height:

The height was measured manually on July 30 using a ruler from the soil surface to the base of the heads. Three measurements were taken for each plot by estimating a mean plant height (PH) for each measurement. These were used to calculate the average height for each plot.

3.7.4 Days to heading:

It was collected heading data by recording the day when approximately 50% of plants in a plot had their heads fully emerged. This was done daily from 12. July to 22. July. The same person did the assessment of the days to heading (DH) for the entire season, to minimize errors and biases. The dates were calculated into days since sowing.

3.7.5 Days to maturity:

Days to maturity (DM) was assessed by recording the day when approximately 50% of plants had reached the maturity stage, which in final terms means that the stems are discolored and the grain ripe. This was estimated based on visual assessment, and the dates were calculated into days since sowing.

3.7.6 Lodging

Lodging was estimated by assessing the percentage of the plots that had been lodged.

3.7.7 Gas exchange and stomatal conductance

Gas exchange was measured using the KaRal system, an instrument developed and lent to us by the research group of Hannes Kollist at the University of Tartu. Measurements were done weekly in each tunnel for 5 weeks. For each plot, 20 leaves were measured. The measurements were done on 5 selected varieties. Three Norwegian varieties were included in the measurements: Runar, Zombi, and Betong, as well as the Swedish variety Caress and the Estonian breeding line DS-720-3-DH.

3.7.8 NOBALwheat data:
Access was given to include data from the NOBALwheat field trial that was conducted at Vollebekk during the field season of 2022. This trial included the same 16 varieties as in the polytunnels. By including this data, the performance of the varieties in polytunnels can be compared to how they performed in a standard field trial.

3.8 Statistical analysis of the field trials

To assess the significance of variance components for a trait (genotype and environmental treatments), ANOVA (analysis of variance) was conducted using mixed model (1):

$$P_{ijkl} = \mu + g_i + l_j + g_i \times l_j + R_k + R{:}B_{kl} + e_{ijkl} \quad (1)$$

Where P_{ijkl} is the phenotype (trait) value for genotype g_i under watering treatment l_j in replicate R_k and block B_l. Small letters denote fixed effects, capitalized letters denote random effects and "×" denotes interaction of effects, and ":" denotes nesting of effects. μ is the general mean and e denotes the error, $IID(0, \sigma_e^2)$.

As each temperature treatment (tunnel) was replicated only once, ANOVA was conducted for each tunnel separately.

For each trait, means (LSmeans) were calculated with respect to genotypic and environmental treatment effects based on model (1).

The LSmeans were calculated using packages "lme4" and "lmerTEST" and custom scripts in R, version 4.2.1.

Broad-sense heritability (H^2) was used to assess data quality (replicability), calculated for using equation (4):

$$H^2 = \frac{\sigma_G^2}{\sigma_G^2 + \sigma_e^2} \quad (4)$$

where σ_g^2 is the genotypic variance and σ_e^2 is the error variance of a trait.

Variance components for equation (4) were estimated using package "lme4" using a fully random model (5):

$$P_i = G_i + e_i \quad (5)$$

where P_i is the phenotype (trait) value of genotype G_i and e_i is the error term, $IID(0, \sigma_e^2)$.

Heritability estimates were calculated separately for every environmental treatment.

Correlation coefficients were calculated using Pearson's correlation formula.

$$r = \frac{\sum(x - m_x)(y - m_y)}{\sqrt{\sum(x - m_x)^2 \sum(y - m_y)^2}}$$

Where x and y are two vectors of length n, and m_x and m_y correspond to the means of x and y, respectively.

Principle component analysis was performed using the means per genotype for the traits under different irrigation treatments in the ambient tunnel.

Heatmaps were used to visually present plot values for each treatment.

4. Results

4.1 Climatic conditions

4.1.1Air temperature- EasyGrowth sensors:

The temperature in the tunnels was higher than outside, on average 0.6 and 1.9°C warmer for ambient and elevated tunnels, respectively. During the day, the ambient tunnel was 0.9°C warmer, and the elevated was 3.1°C warmer. Figure 4 shows the temperature development for one week in August. It shows how the temperature increased when the sun rose in the morning and decreased after sunset. It also shows the difference in temperature development between the two tunnels. In the elevated, the temperature increased more rapidly and to higher temperatures than in the ambient. The differences were smaller during the night, and both tunnels cooled off rapidly after sunset.

Table 2: Average temperature measurements for outside, the elevated tunnel and the ambient tunnel. Daytime: between 8 AM and 6 PM, Nighttime: between 6 PM to 8 AM

Tunnel	T_{avg} **°C**	**Daytime** T_{avg} **°C**	**Nighttime** T_{avg} **°C**
Outside	17.4	21.2	14.2
Ambient	18.1	22.1	14.7
Elevated	19.3	24.3	15.1

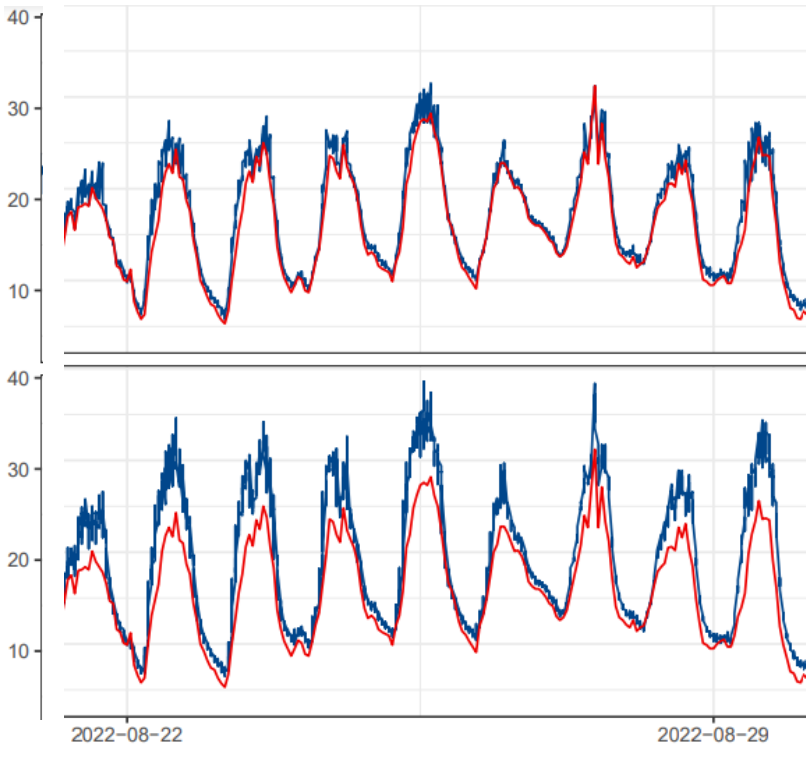

Figure 4: Air temperature sensor readings during one week in August for ambient (top) and elevated (bottom) tunnels. Red – ambient temperature, blue – temperature inside.

4.1.2 Humidity- easygrowth sensors

The easygrowth sensors also monitored humidity. The table below shows how the average relative humidity (RH) was nearly identical for the elevated tunnel and outside reference value. The ambient, however, had a lower average RH. However, the elevated tunnel was noticeably elevated for the daytime relative humidity, with an RH of 88.4% compared to 72.0% and 76.0% for the outside environment and ambient tunnel, respectively. At night, the differences were smaller, with the outside environment and the ambient tunnel having an RH of 96.1% and the elevated tunnel 99.0%. Figure 5 shows how the humidity developed during a week in August. It shows how the ambient tunnel had lower RH values than the elevated, especially during the daytime.

Table 3: Average relative humidity measurements for outside and the elevated and ambient tunnel. Daytime: between 8 AM and 6 PM, Nighttime: between 6 PM to 8 AM

Tunnel	***Rh%***	***Daytime Rh%***	***Nighttime Rh%***
Outside	94.1	72.0	96.1
Ambient	86.9	76.0	96.1
Elevated	94.1	88.4	99.0

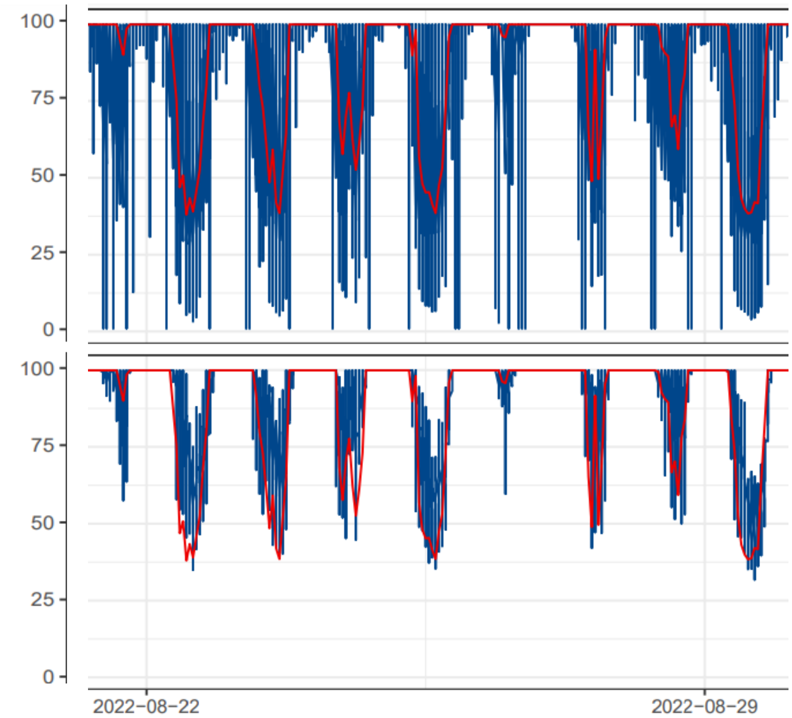

Figure 5: Air humidity sensor readings during one week in August for ambient (top) and elevated (bottom) tunnels. Red – ambient humidity, blue – humidity inside. Daytime: between 8 AM and 6 PM, Nighttime: between 6 PM to 8 AM. Full plot available as an additional resource.

4.1.3 Soil moisture- agdir sensors

With the number of sensors included in this trial, and as the field season was several months long, the amount of data collected by the Agdir sensors was immense. Therefore, it was opted to include a snapshot of one sensor for each irrigation treatment in the two tunnels. They show the general development of the water balance in the soil during the season from the installment of the sensors until harvest in the fall. The other sensors show a similar development, at both 15cm and 30 cm depth. The sensors included are all at 15 cm depth. Complete data is available upon request.

Ambient tunnel:

The graphs show the water balance of the soil for the irrigation treatments in the ambient tunnel. It shows the period after installation before irrigation started and then the heavy watering at the beginning of the season. For the dry section, the water balance descends steadily throughout the season after the initial soaking, becoming sufficiently dry at the beginning of July and dropping to significantly low levels. The water balance fell somewhat in the watered sections, but the irrigation maintained it at acceptable levels. Periods with heavier irrigation can be seen by the tops in the graph.

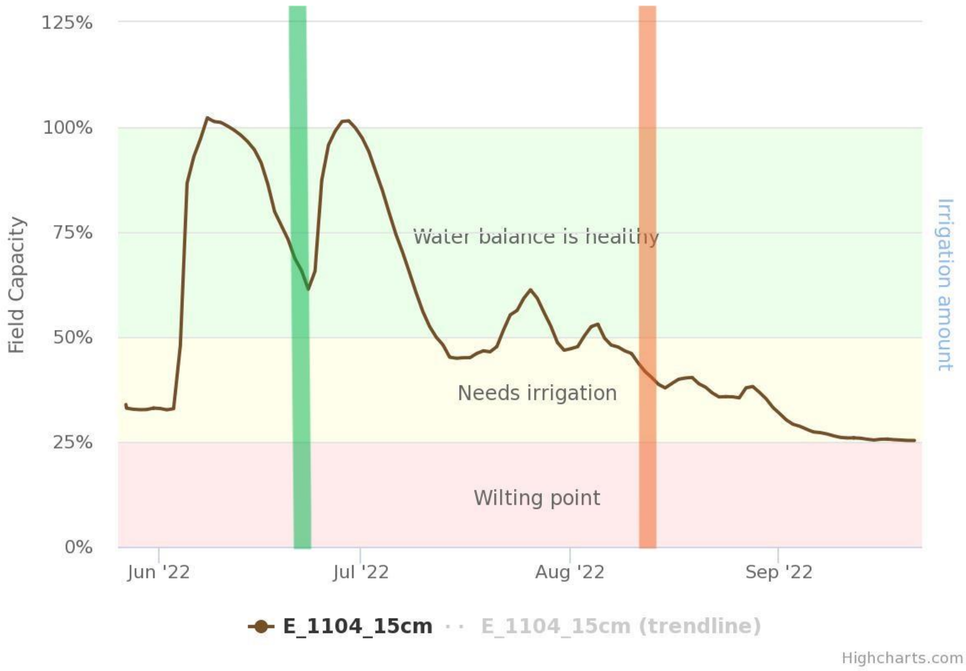

Figure 6: Water balance recorded by one sensor in a watered section of the field in the ambient tunnel. The green marking highlights the approximate timepoint for heading date, the red marking indicates the approximate timepoint for maturity.

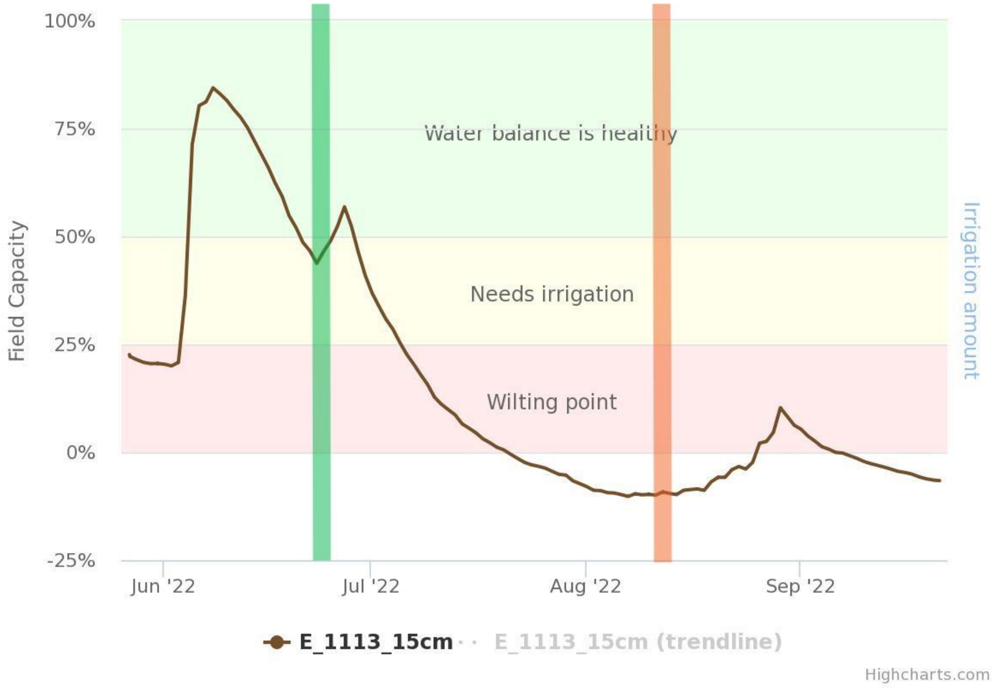

Figure 7: Water balance recorded by one sensor in a dry section of the field in the ambient tunnel. The green marking highlights the approximate timepoint for heading date, the red marking indicates the approximate timepoint for maturity.

Elevated tunnel:

The graphs show the water balance of the soil for the irrigation treatments in the elevated tunnel. Equal to the ambient tunnel, the graphs show the period before the first watering and the heavy irrigation that followed early in the season. In the watered section, the water balance stayed saturated during the season, except for some dips, similar to the ambient tunnel. However, the high water balance was maintained until the end of the season. In the dry section, the water balance decreased steadily but less quickly or severely than in the ambient tunnel, and the final water balance at the end of the season was higher than in the ambient tunnel.

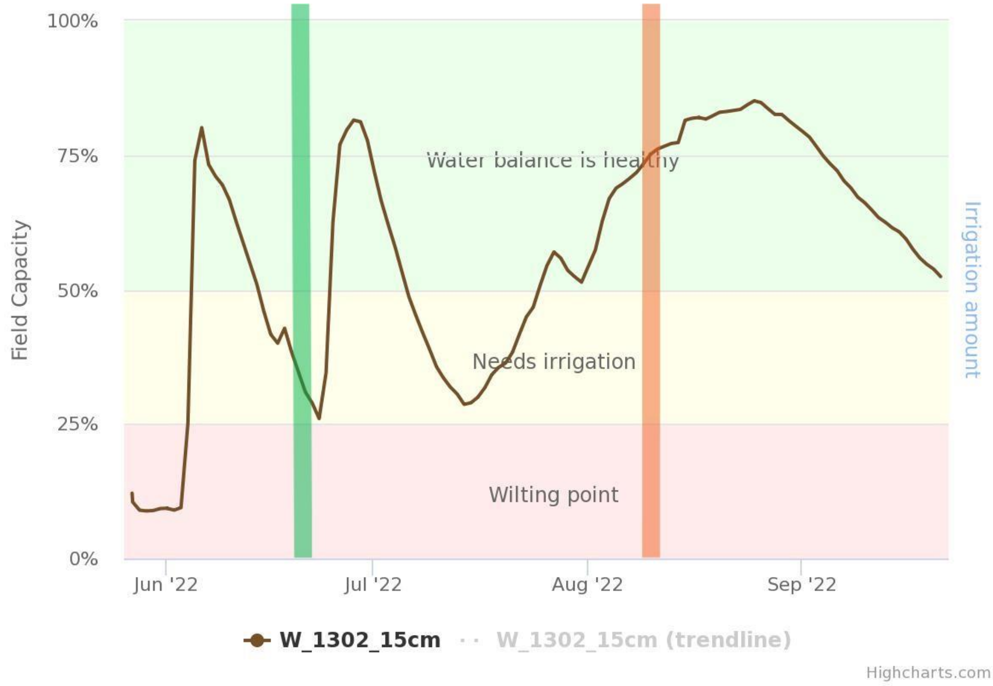

Figure 8: water balance recorded by one sensor in a watered section of the field in the elevated tunnel. The green marking highlights the approximate timepoint for heading date, the red marking indicates the approximate timepoint for maturity.

Figure 9: water balance recorded by one sensor in a dry section of the field in the elevated tunnel. The green marking highlights approximate timepoint for heading date, the red marking indicates the approximate timepoint for maturity.

4.2 The Experiment

4.2.1 Heritability

The broad sense heritability for the traits is mostly higher for the drought treatments than for the control. The exceptions are the heading date in both tunnels and plant height in the ambient tunnel, where the heritability is higher for the well-watered replicates. The values vary between the ambient and elevated tunnel.

Table 4: Broad sense heritability calculations for the measured traits under different irrigation treatments in both tunnels.

	Ambient tunnel		***Elevated tunnel***	
Trait	**Drought**	**Control**	**Drought**	**Control**
GY	41.1%	8%	49.9%	24.1%
GPC	51.8%	17.7%	42.5%	30.5%
PH	86%	89.3%	84.6%	68.3%
DH	82.4%	95.3%	90.6%	91.6%
DM	92.1%	53.4%	75.2%	73.8%

4.2.2 Grain yield

Descriptive statistics

The effect of drought was greater in the ambient tunnel than in the elevated tunnel. The sections exposed to drought experienced a 30% and 8% reduction in yield in the ambient and elevated tunnels, respectively. As seen in table 5, the average grain yield in the ambient tunnel for the control treatment was 537g per m^2, and the drought treatment was 374g per m^2. In the elevated tunnel, the average grain yield for the control was 451g per m^2, and for drought was 416g per m^2.

The heatmap (figure 11) shows the grain yield value for each plot as they were distributed in the tunnels. The boxplot (figure 10) shows the averages from the different treatments and the grain yield distribution. It is possible to observe differences between the treatments in both tunnels, with the ambient drought replicate showing a sizable reduction in yield compared to the control replicate. While the difference between the irrigation treatments is smaller in the elevated tunnel. The effects of the irrigation treatments are also visible in the plot values. The watered zones in the ambient tunnel show higher yield values than the dry zones. The differences are less apparent in the elevated tunnel but are still visible. The heatmaps also make it possible to identify outliers. One can see that the plots 1613 and 1104 have a noticeably higher yield than other plots, both being the variety 013-01, while the plot 2213 (Runar) stands out by having less yield. The boxplot shows two outliers in the elevated drought replicate, with Runar being the low-performing and 013-01 being the high performing.

ANOVA on mixed model:

The interactions of variety and irrigation on grain yield are presented in table 6. There is a significant difference between the lines in both tunnels, with the ambient tunnel having a lower value than the elevated. Irrigation was also significant, with the p-value value being less than 0.001 for both tunnels. The interactions between line and irrigation did not significantly affect the grain yield in either tunnel.

Table 5: Average grain yield for different irrigation treatments in both tunnels

Mean GY_g_m2	***Ambient***	***Elevated***
Control	537	451
Drought	374	416

Table 6: p-values from anova analysis on grain yield.

Pr(>F) GY_g_m2	***Ambient***	***Elevated***
Line	<0.001***	0.003**
Irrigation	<0.001***	<0.001***
Line*Irrigation	0.659 ns	0.884 ns

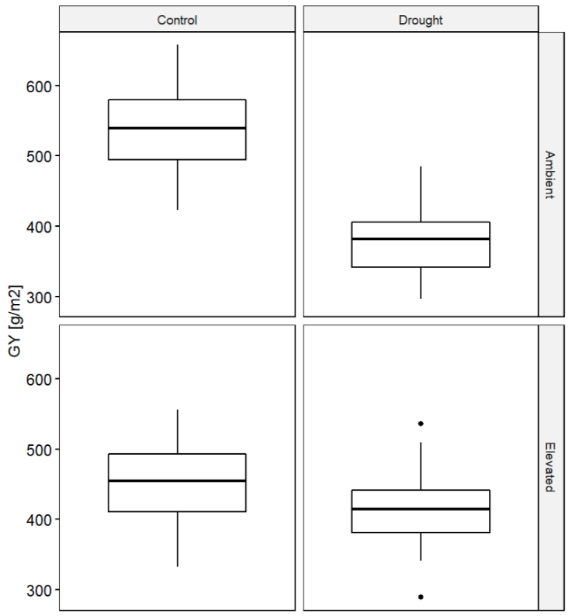

Figure 10: Boxplot showing the distribution of grain yield for the irrigation treatments under different temperatures.

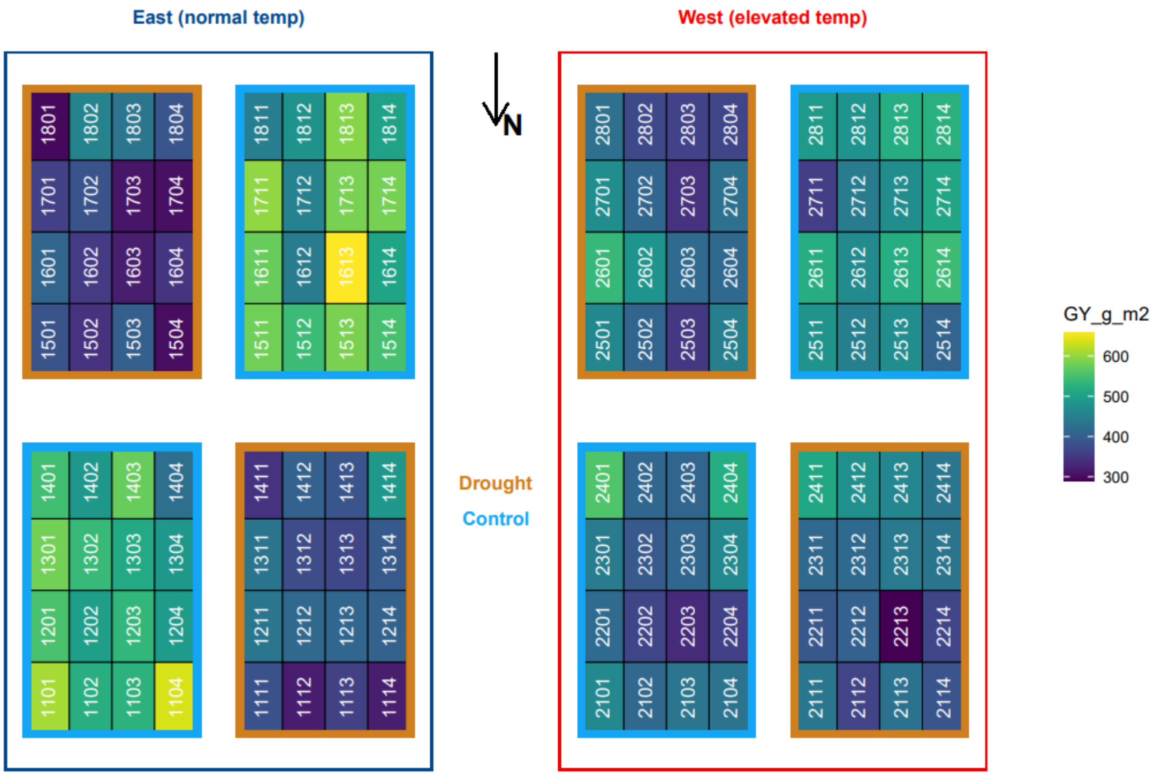

Figure 11: Grain yield for each plot. Orange border around the replicates with drought and a blue border around the well-irrigated replicates.

Ranking of grain yield

In the ambient tunnel, 013-01 is the best-performing variety in drought and control conditions. 990-2 had the least reduction when exposed to drought; though it was the worst performing in control, it had the second highest yield during drought. In the elevated tunnel, 013-01 is still the highest-performing variety under control conditions. When exposed to drought, however, DS-655-7-DH has the highest yield. This is a substantial change from the ambient tunnel, where the DS-655-7-DH ranked as number 8 and 11 in control and drought, respectively. The differences between the drought and control are more minor in the elevated tunnel. There were differences in yield for each variety under different temperatures.

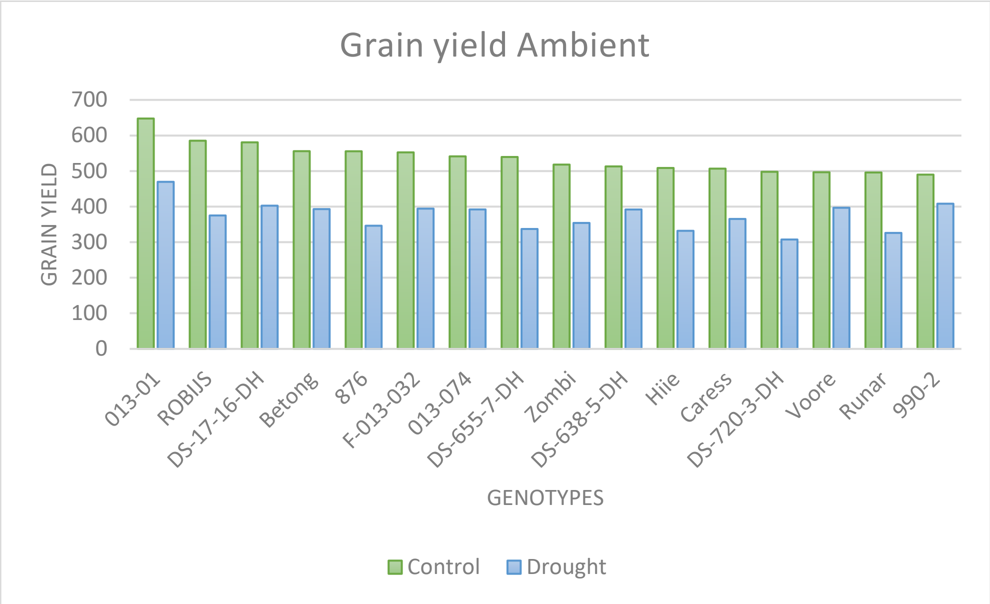

Figure 12: Ranking and comparison of grain yield for each variety in control and dry conditions in the ambient tunnel.

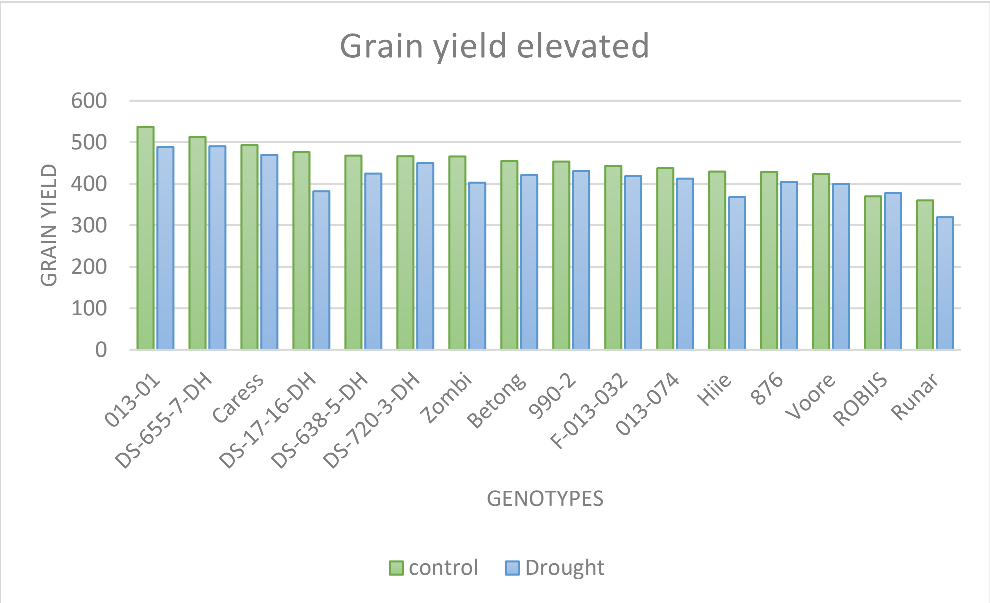

Figure 13: Ranking and comparison of grain yield for each variety in control and dry conditions in the elevated tunnel.

4.2.2.1 Seed parameters

The boxplots show the distribution of different seed parameters in both tunnels for both irrigation treatments. The measurements include thousand-grain weight (figure 14), kernel area (figure 15), kernel width (figure 16), and kernel length (figure 17). TGW was reduced under drought, more so in the ambient tunnel than in the elevated tunnel. The same can be said for kernel area and kernel width, as they both are noticeably reduced under drought in both tunnels. Kernel length however, was less affected by the drought treatments, and the differences between the two tunnels are lesser in comparison to the other seed parameters.

4.2.2.2 ANOVA and correlation analysis for seed parameters

The ANOVA analysis (table 7) for the effects of drought on the different seed parameters shows that thousand grain weight, kernel area and kernel width were significantly affected by drought in the ambient tunnel, width kernel width being the most significantly affected. The effect of drought on kernel length, however, was not significant. In the elevated tunnel, only the kernel width was significantly affected by the drought, while the other traits were not.

The correlations analysis (table 8) for the traits shows there was a significant correlation between the kernel width, kernel area, and thousand-grain weight, and the final grain yield. The kernel width has the highest coefficient (0.44), while the kernel area and thousand-grain weight had coefficients of 0.29 and 0.28, respectively, and also show a correlation with the grain yield. The kernel length had the lowest coefficient with 0.093, and it had no significant effect on grain yield. Table 9 presents the correlation between grain yield and the seed dimensions when the different treatments are considered. The table shows how there is a negative correlation between grain yield and TGW, area, and length for the ambient drought replicates. In the elevated tunnel, however, there are strong positive correlations between grain yield and the same components for both irrigation treatments. The table also shows the significance of the correlations, where only the TGW and width in the elevated control replicates, and width in the elevated drought replicates, had significant correlations with grain yield.

Table 7: Anova analysis for the effects of drought on seed parameters in the ambient and elevated tunnel

Pr(>F) Seed parameters	***Ambient***	***Elevated***
TGW	0.016 *	0.24^{ns}
Area	0.008 **	0.154^{ns}
Length	0.163^{ns}	0.268^{ns}
Width	<0.001***	0.025 *

Table 8: Correlation coefficient between grain yield and seed parameters independently from treatment

Seed parameters	***Correlation with GY***	***p-value***
TGW	0.288	<0.001 ***
Area	0.279	0.0014**
Length	0.093	0.2941
Width	0.439	<0.001***

Table 9: Correlation coefficients between grain yield and seed parameters under the different temperature and irrigation treatments

Temperature	*Irrigation*	*TGW*	*Area*	*Width*	*Length*
Ambient	**Control**	0.049^{ns}	0.094^{ns}	0.066^{ns}	0.073^{ns}
Ambient	**Drought**	-0.259^{ns}	-0.223^{ns}	0.014^{ns}	-0.280^{ns}
Elevated	**Control**	0.412*	0.304^{ns}	0.497**	0.133^{ns}
Elevated	**Drought**	0.331^{ns}	0.259^{ns}	0.390*	0.072^{ns}

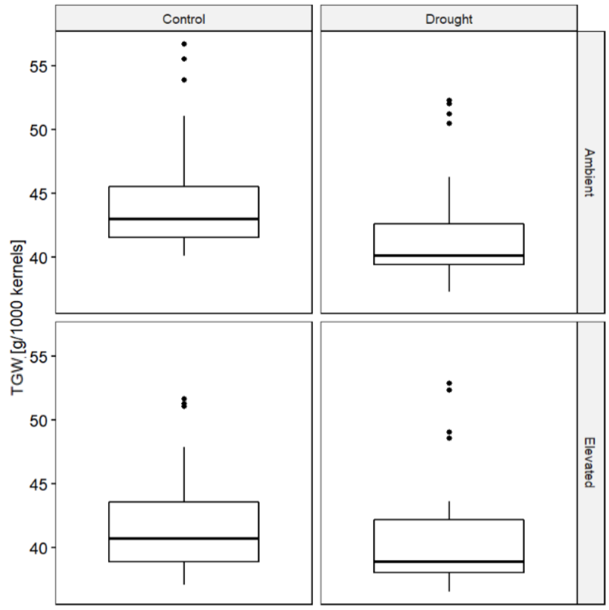

Figure 14: Boxplot showing the distribution of thousand-grain weight for both tunnels under different irrigation treatments.

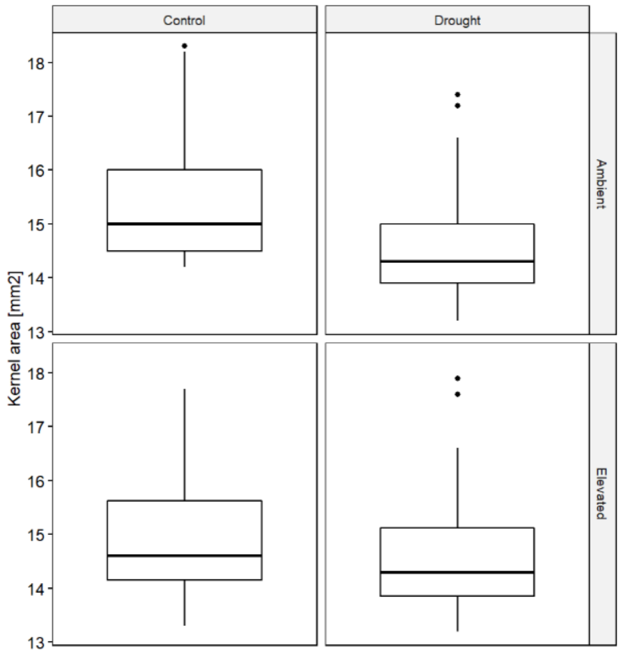

Figure 15:Boxplot showing the distribution of kernel area for both tunnels under different irrigation treatments.

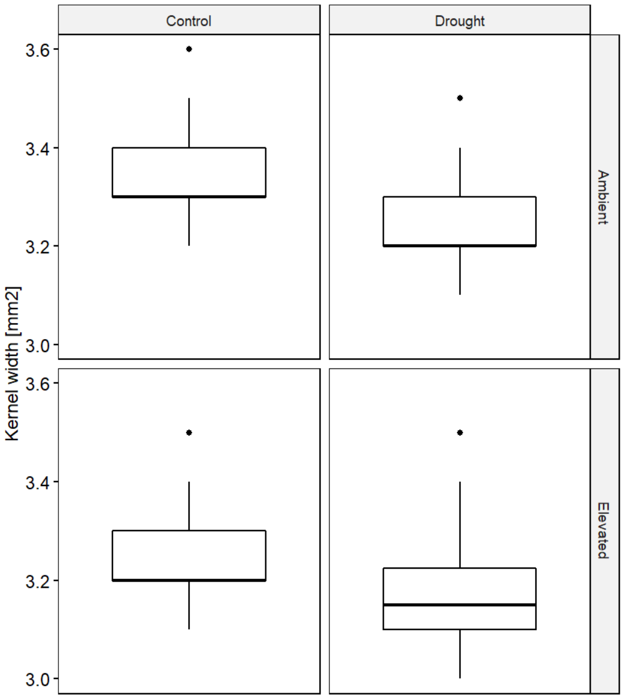

Figure 16: Boxplot showing the distribution of kernel width for both tunnels under different irrigation treatments.

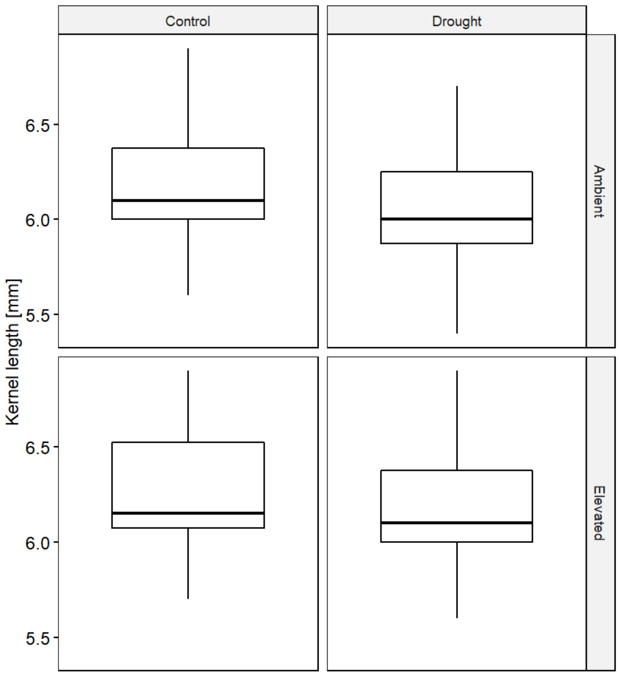

Figure 17: Boxplot showing distribution of kernel length for both tunnels under different irrigation treatments.

4.2.3 Grain protein content

Descriptive statistics:

The grain protein content is measured in percent, and the results are presented in table 10. The differences are not substantial in the ambient tunnel, with the average GPC being 11.9% and 11.43% in the control and drought zones, respectively. The effect is more apparent in the elevated tunnel, where there is a sizeable increase in GPC for the control replicate compared to the drought replicate. The drought zones had 11.87% protein on average, while the control had 13.43%. This is visible in the heatmap (figure 18), which shows more considerable differences in protein in the elevated tunnel, and that the watered zones have higher values than the dry. The ambient tunnel is more monotone, however, with little visible difference between the irrigation treatments.

ANOVA for mixed model:

The grain protein content was affected significantly by both the variety and the irrigation treatments, as seen in table 11. The p-value is below 0.001 for both the control and drought replicates in both tunnels. The interaction between line and irrigation, however, had no significant effect on protein content in either temperature treatment.

Table 10: Average grain protein content for different irrigation treatments in both tunnels.

Mean GPC%	***Ambient***	***Elevated***
Control	11.9	13.43
Drought	11.43	11.87

Table 11: p-values from anova analysis on grain protein content.

Pr(>F) GPC%	***Ambient***	***Elevated***
Line	<0.001***	<0.001***
Irrigation	<0.001***	<0.001***
Line*irrigation	0.078^{ns}	0.421^{ns}

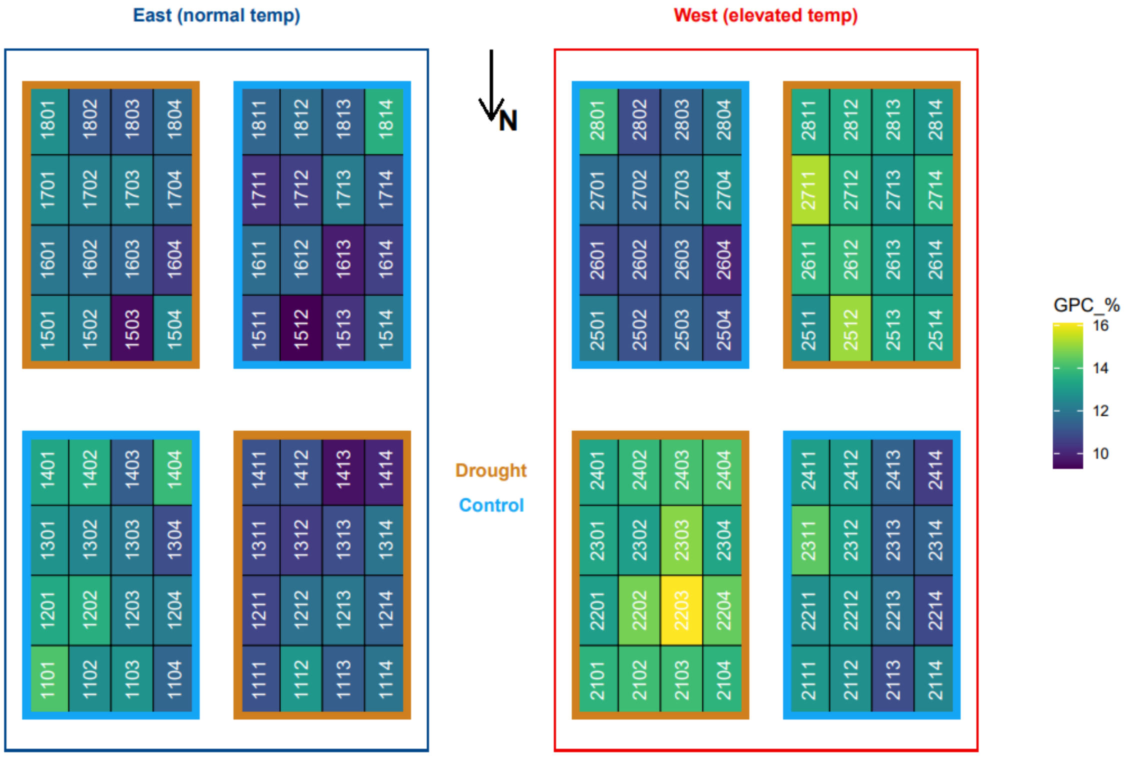

Figure 18: grain protein content for each plot. Orange border around the replicates with drought and a blue border around the well-irrigated replicates.

Ranking of grain protein content

The variety 876 has the highest grain protein content in both tunnels for the well-irrigated replicates. When exposed to drought, however, the GPC is reduced. The variety 876 is the highest ranking for the control replicate in both tunnels, DS-655-7-DH is the highest-ranking variety in the drought replicate in the ambient tunnel, and it has the smallest value differences between the irrigation treatments. However, the differences in GPC between the replicates are minor in the ambient tunnel. In the elevated tunnel, GPC is on average greater than in the ambient, and the difference between each variety for the different irrigation treatments is more substantial. For the drought replicate, DS-720-3-DH has the highest grain protein content.

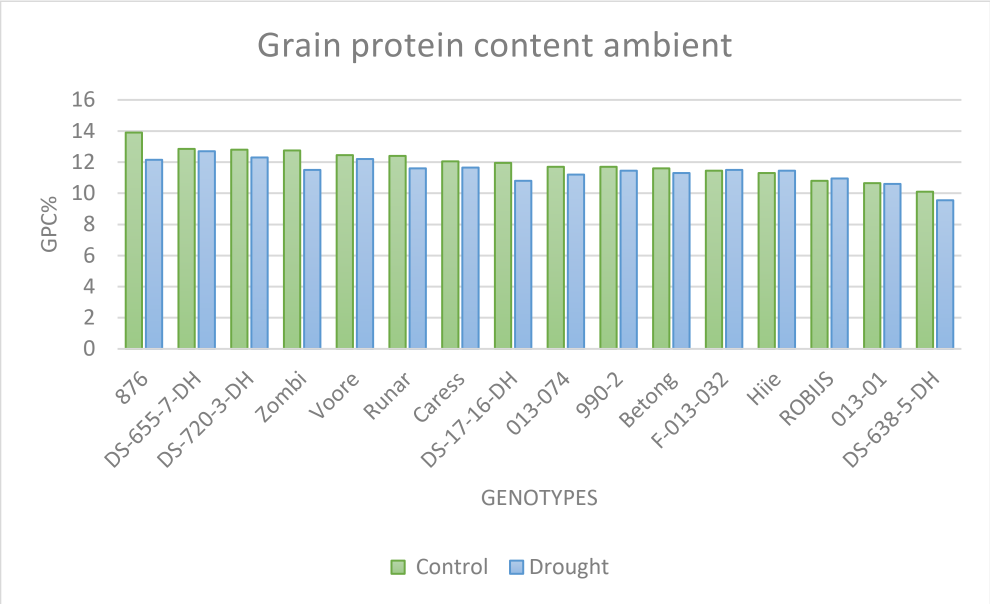

Figure 19: Comparison of protein content for each variety in the different watering treatments in the ambient tunnel.

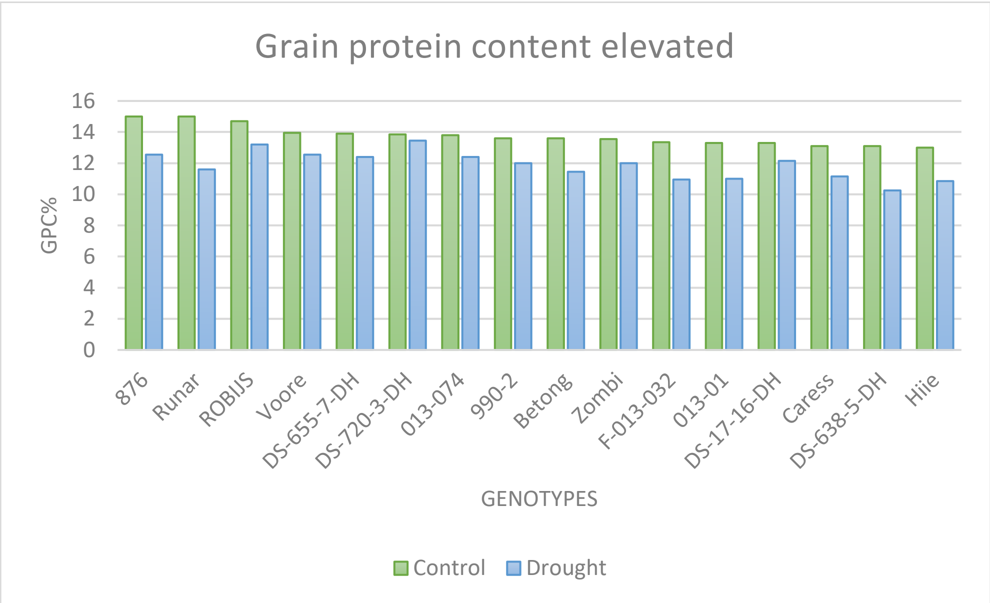

Figure 20: Comparison of protein content for each variety in the different watering treatments in the elevated tunnel.

4.2.4 Plant height

Descriptive statistics:

The difference in plant height when exposed to the different treatments are presented in table 12. It shows that the difference was slight in the elevated tunnel, with the average height being 95.2cm for the control group and 95.9 for the drought. For the ambient tunnel, however, the difference was greater. Here the control group had an average height of 98.1cm, while the drought group was 92.3cm on average. In the ambient tunnel, the control group grew taller than in the elevated while simultaneously showing more negative effects from the drought treatment. The differences between the irrigation treatments are hardly visible in the heatmap (figure 21), as there is only a slight difference in colour for the drought replicate compared to the watered. However, the heatmaps show sizable variations between genotypes, and outliers can be identified. The plots 1313, 1502, and 2513, all of which are the variety Zombi, stand out as growing shorter than average, while 1202 (DS-655-7-DH) is noticeably taller.

ANOVA for mixed model

There is a significant difference between the varieties. Irrigation had a significant effect in the ambient tunnel but not in the elevated. The interaction between line and irrigation in the elevated tunnel was insignificant, with a p-value of 0.446. In the ambient tunnel, however, the p-value was 0.047, indicating significant interactions between the variety and the irrigation treatment and their effect on plant height in the ambient tunnel.

Table 12: Average plant height for the different irrigation treatments in both tunnels.

PH_cm	***Ambient***	***Elevated***
Control	98.06	95.16
Drought	92.3	95.90

Table 13: p-value for ANOVA analysis on plant height.

Pr(>F) PH_cm	***Ambient***	***Elevated***
Line	<0.001***	<0.001***
Irrigation	<0.001***	0.268^{ns}
Line*irrigation	0.047*	0.446^{ns}

Heat map

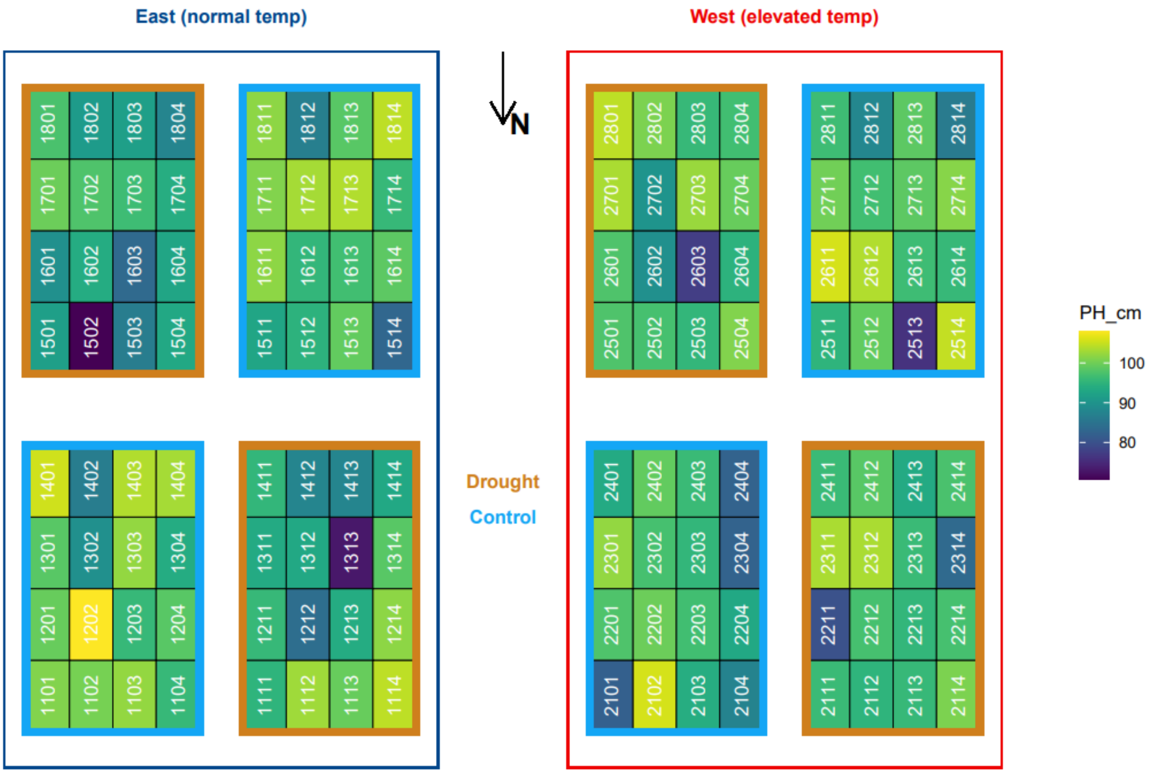

Figure 21: Plant height for each plot. Orange border around the replicates with drought and a blue border around the well-irrigated replicates.

Ranking of plant height

The ranking of the varieties shows the height reduction that occurred in the ambient tunnel for the drought replicates. DS-655-7-Dh grew the tallest, while Zombi grew the shortest in both tunnels, and Zombi also had the largest height reduction due to drought in the ambient tunnel. The genotype DS-720-3-DH is the least affected by the drought, with only a 1.5cm reduction in height for the dry replicate compared to the control. For the elevated tunnel, there are minimal differences between the irrigation treatments, with some varieties (DS-655-7-DH and Caress) growing taller in the drought replicate. Here the DS-720-3-DH genotype grew the tallest.

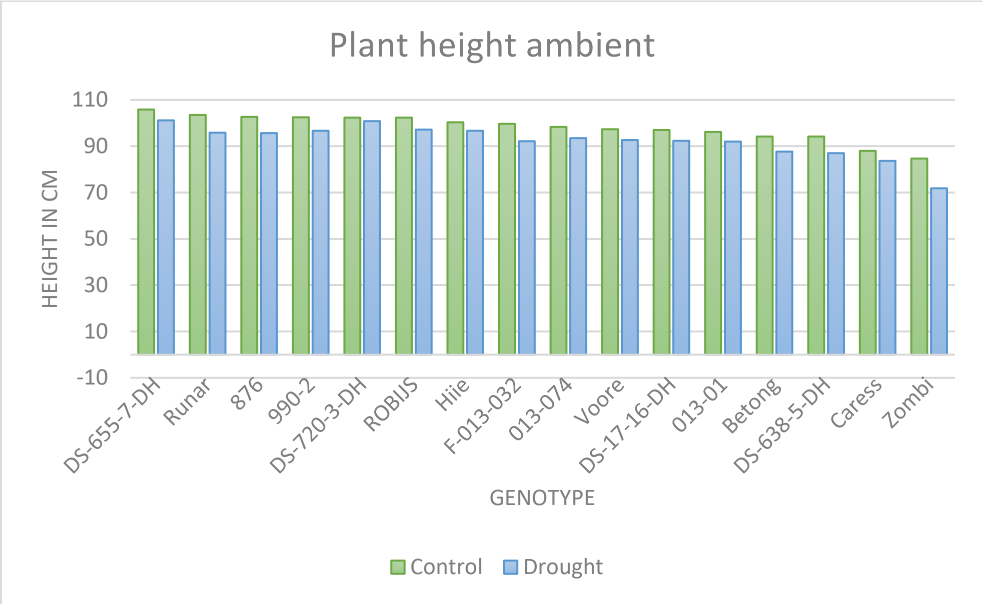

Figure 22: Figure comparing plant height for each variety in the different watering treatments in the ambient tunnel.

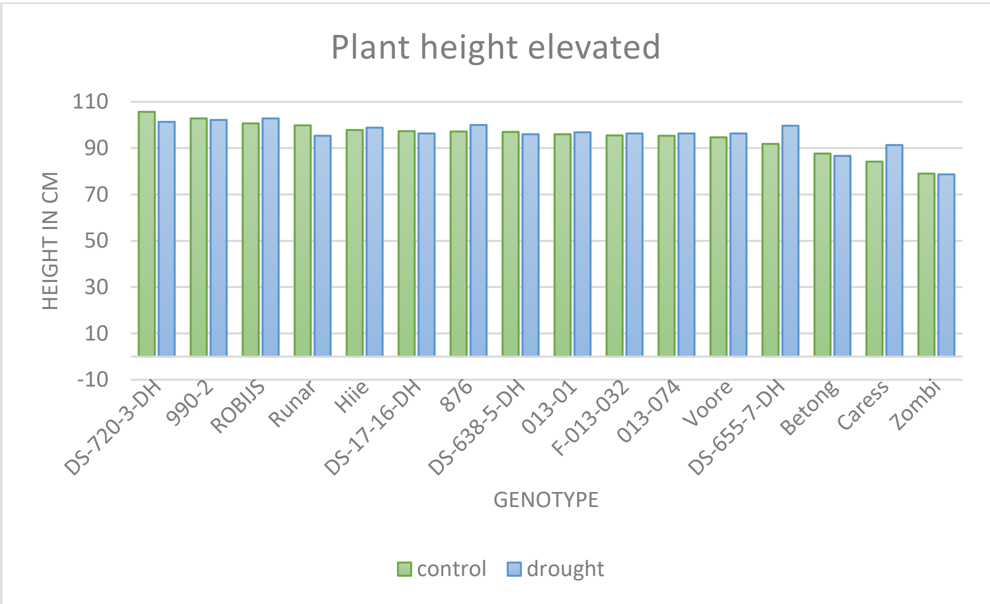

Figure 23: Figure comparing plant height for each variety in the different watering treatments in the elevated tunnel.

4.2.5 Days to heading.

Descriptive statistics:

There is a minimal difference in the average time it took for the varieties to reach the heading stage in the two tunnels. However, the irrigation treatment had an effect, and there is a difference between the control and drought replicates in both tunnels. In the ambient tunnel, the control took 49.2 days to head, and the drought took 47.8 days, as seen in table 14. In the elevated tunnel, the control took 48.4 days, and the drought took 47.9 days. The heatmap (figure 24) shows similar differences between the drought and the watered in each tunnel, though there is a slightly greater difference in the ambient. Several plots are possible to identify as outliers based on the heatmap, especially in the ambient tunnel. The plot numbers 1304, 1503, 1512, and 1413 are all the genotype DS-638-5-DH, which is the greatest outlier in all replicates for the ambient tunnel as it reached the heading stage noticeably later than the other genotypes.

ANOVA for mixed model:

The p-value shows that both line and irrigation significantly affected the heading date in both tunnels. The impact of irrigation is more significant in the elevated tunnel, with a p-value of 0.007 compared to less than 0.001 in the ambient tunnel. The interactions between line and irrigation, however, had no significant effect in either tunnel.

Table 14: Average days to heading for different irrigation treatments in both tunnels.

Mean DH_dss	***Ambient***	***Elevated***
Control	49.19	48.38
Drought	47.78	47.9

Table 15:p-values for ANOVA analysis on days to heading.

Pr(>F) DH_dss	***Ambient***	***Elevated***
Line	<0.001***	<0.001***
Irrigation	<0.001***	0.007**
Line*Irrigation	0.377^{ns}	0.504^{ns}

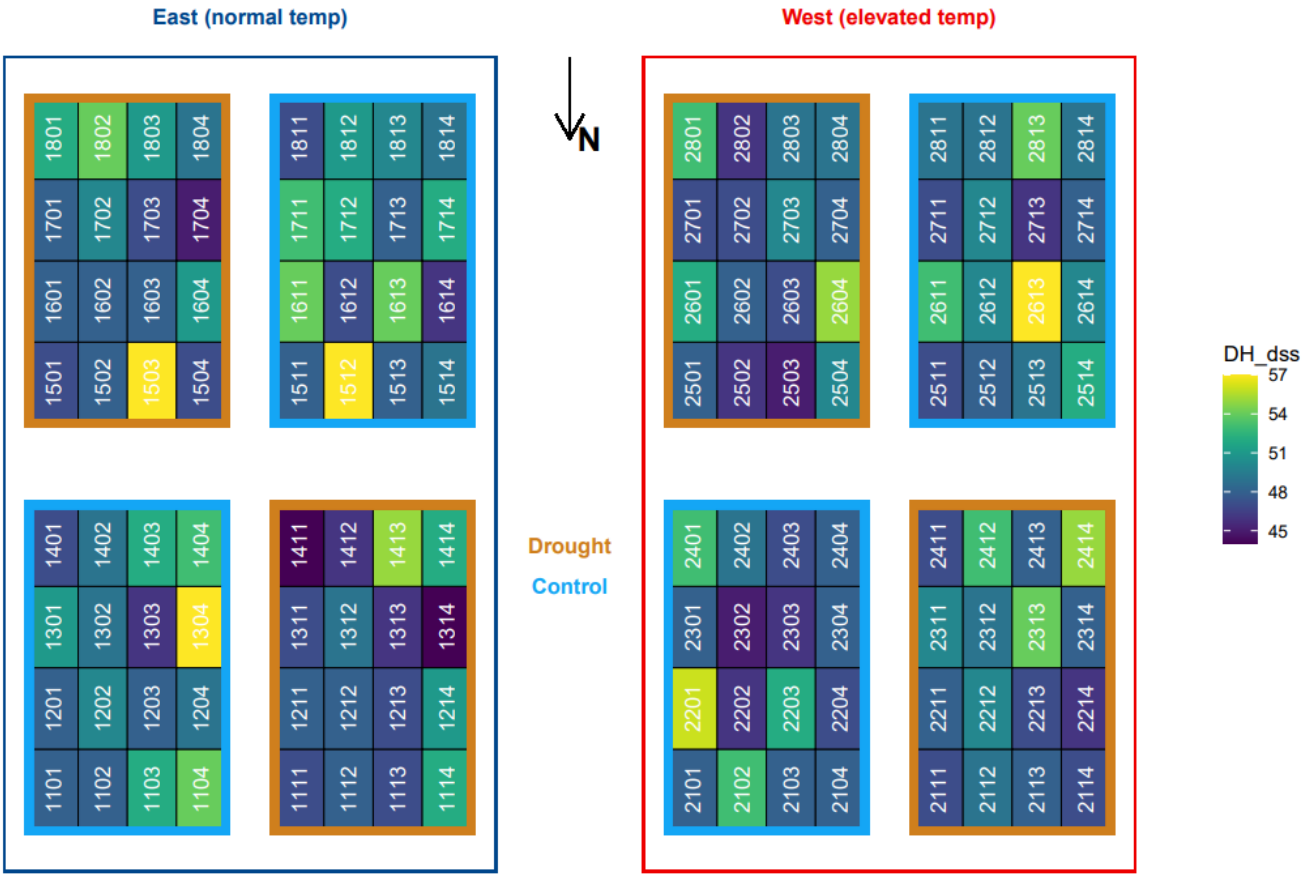

Figure 24: heading date for each plot. Orange border around the replicates with drought and a blue border around the well-irrigated replicates.

Ranking of days to heading

The ranking of the varieties shows minimal differences in days to heading between the different irrigation treatments, especially in the elevated tunnel. In the ambient tunnel, there is a slight difference between the different replicates, with the drought-treatments on average reaching the heading stage earlier than the well-irrigated. The ranking makes it possible to observe variation between the genotypes, and the time they took to reach the heading stage. DS-638-5-DH took the longest amount of time to reach heading in both tunnels, while the varieties Runar and Hiie spent the least amount of time.

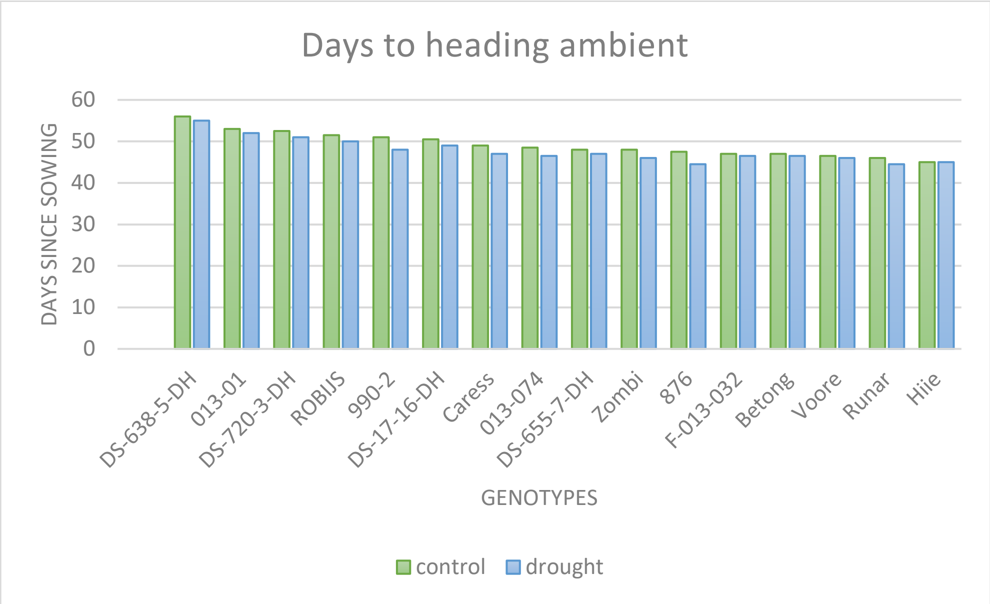

Figure 25: Figure comparing days to heading for each variety in the different watering treatments in the ambient tunnel.

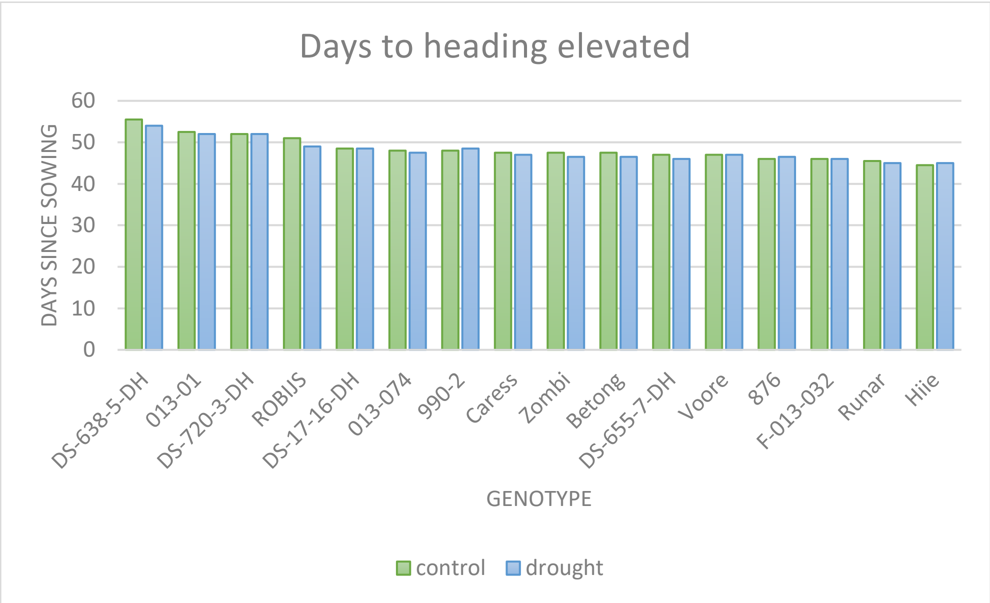

Figure 26: Figure comparing days to heading for each variety in the different watering treatments in the elevated tunnel.

4.2.6 Days to maturity.

Descriptive statistics:

For the control group in the ambient and elevated tunnel, the average time it took to reach maturity was 92 and 91 days, respectively, as seen in table 16. The difference in the drought treatments between the tunnels was more apparent. The average time it took for the varieties in the drought replicates to reach maturity was 84.2 days for the ambient tunnel compared to 86.2 days in the elevated tunnel. In both tunnels, the water deficit made the wheat mature earlier. The heatmap (figure 27) shows the spatial distribution of the varieties in the field. The difference between the irrigation is possible to observe in the ambient tunnel, while in the elevated tunnel, the difference is less noticeable but still present.

ANOVA for mixed model

The ANOVA analysis shows significant differences between varieties, with the p-value being smaller than 0.001 for the genotypes in both tunnels. Irrigation also had a significant effect in both tunnels, while the interaction between irrigation and varieties was close to significant for the ambient temperature (p=0.068) but not for the elevated temperature (p=0.176).

Table 16: Average days to maturity for the different treatments in both tunnels.

Maturity_Dss	***Ambient***	***Elevated***
Control	*92.68*	*91.69*
Drought	*84.28*	*86.16*

Table 17: p-values from ANOVA analysis on days to heading.

Pr(>F) DM_dss	***Ambient***	***Elevated***
Line	<0.001***	<0.001***
Irrigation	<0.001***	<0.001***
Line*Irrigation	0.068^{ns}	0.176^{ns}

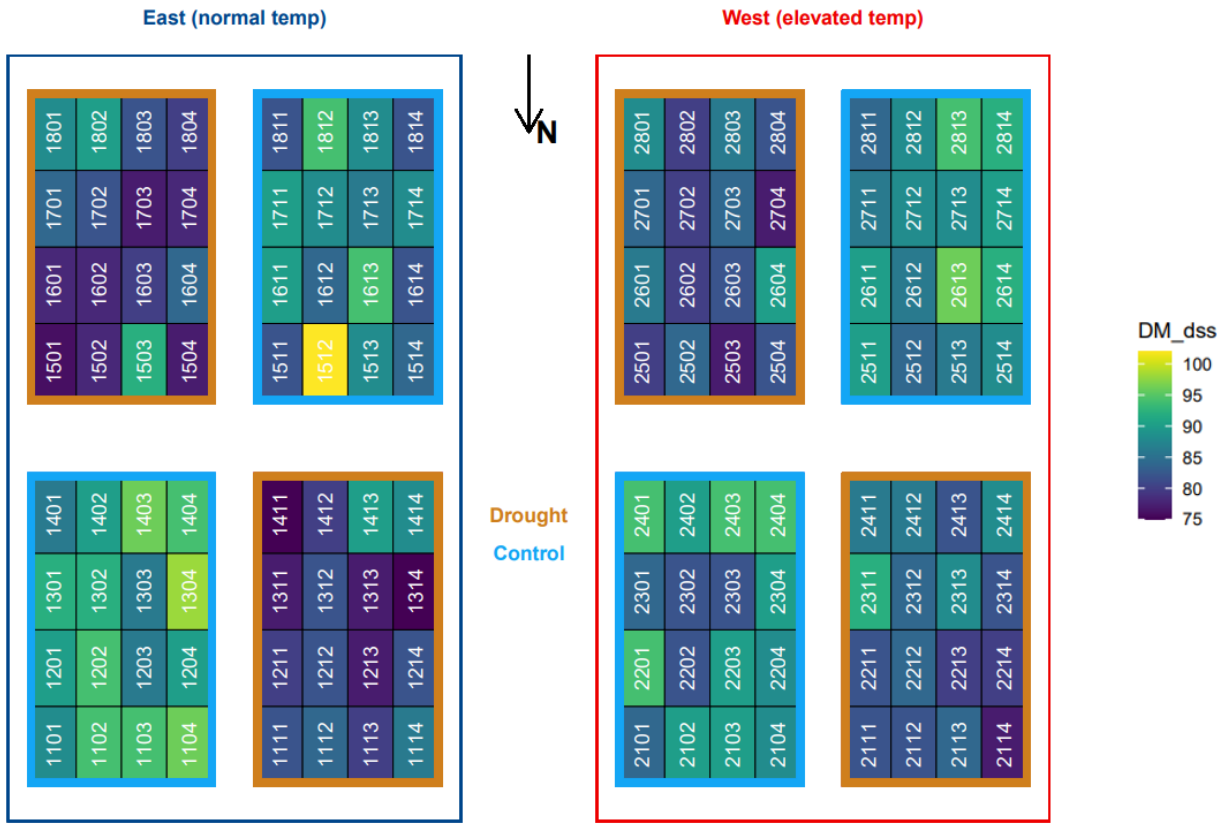

Figure 27: Maturity for each plot. Orange border around the replicates with drought and a blue border around the well-irrigated replicates.

Ranking of days to maturity

The ranking of the days to maturity for the different genotypes presents the differences between the irrigation treatments in each tunnel. In the ambient tunnel, there are apparent differences between the replicates exposed to drought and the control, with the drought-treated replicates maturing earlier than the control. In the elevated tunnel, the differences are smaller, but still noticeable. The differences in the time it took to reach maturity for the different genotypes can be observed in both tunnels, with Runar, Hiie, and 876 being among the fastest to reach maturity in both tunnels, while DS-638-5-DH took the longest time independently from both irrigation and temperature treatment.

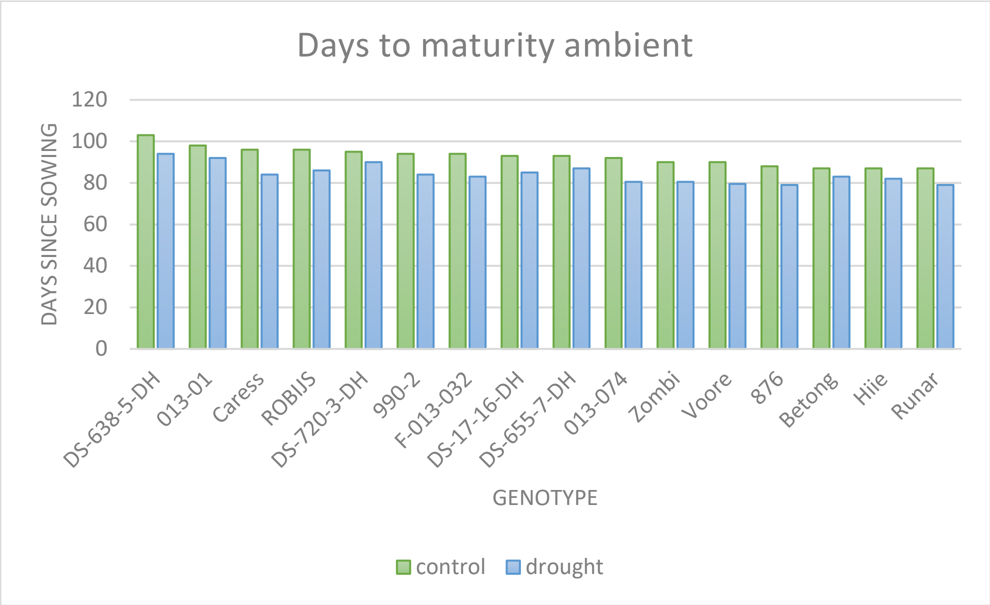

Figure 28: Figure comparing days to maturity for each variety in the different watering treatments in the ambient tunnel.

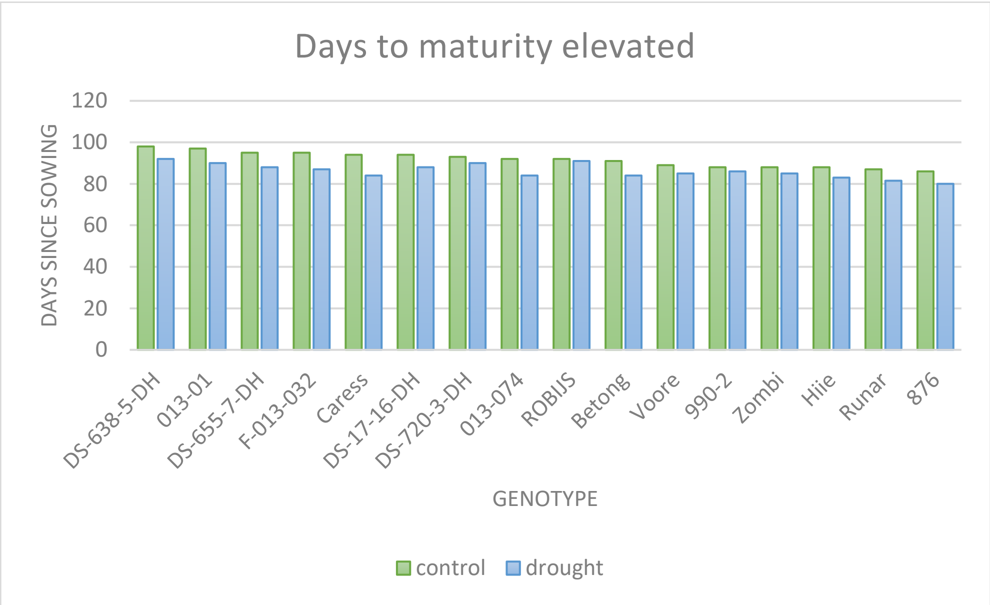

Figure 29: Figure comparing days to maturity for each variety in the different watering treatments in the elevated tunnel.

4.2.7 Lodging

Descriptive statistics:

Table 18 shows that there is a noticeable difference in the severity of the lodging between the two tunnels and the different watering regimes. The ambient drought section had the least lodging of the treatments, with an average of 19.8%. The control section in the same tunnel had more lodging, however, with a value of 36.9%. In the elevated tunnels, the drought section had an average of 59.2%, while the watered section had the highest amount of lodging, with 85.8%. The difference between the tunnels is apparent in the heatmap (figure 30). Here one can see the higher amounts of lodging in the elevated tunnel and spatial effects in the field. The sections closest to the short wall in the ambient tunnel experienced noticeably less lodging compared to the plots closer to the tunnel's center, while in the elevated tunnel, the field was more evenly affected by the lodging.

ANOVA for mixed model:

The P-values show that both line and irrigation significantly affected the amount of lodging in the elevated tunnel. In the ambient tunnel, irrigation significantly affected lodging, with a p-value of 0.009, while the effect of the lines was insignificant. The interaction between the line and irrigation had no significant effect in either tunnel.

Table 18: Average percentage of lodging that occurred under different irrigation treatments in both tunnels.

Lodging%	***Ambient***	***Elevated***
Control	36.86	85.84
Drought	19.84	59.2

Table 19: p-values for the anova analysis on the lodging.

Pr(>F) Lodging	***Ambient***	***Elevated***
Line	0.14^{ns}	<0.001***
Irrigation	0.009**	<0.001***
Line*Irrigation	0.737^{ns}	0.089^{ns}

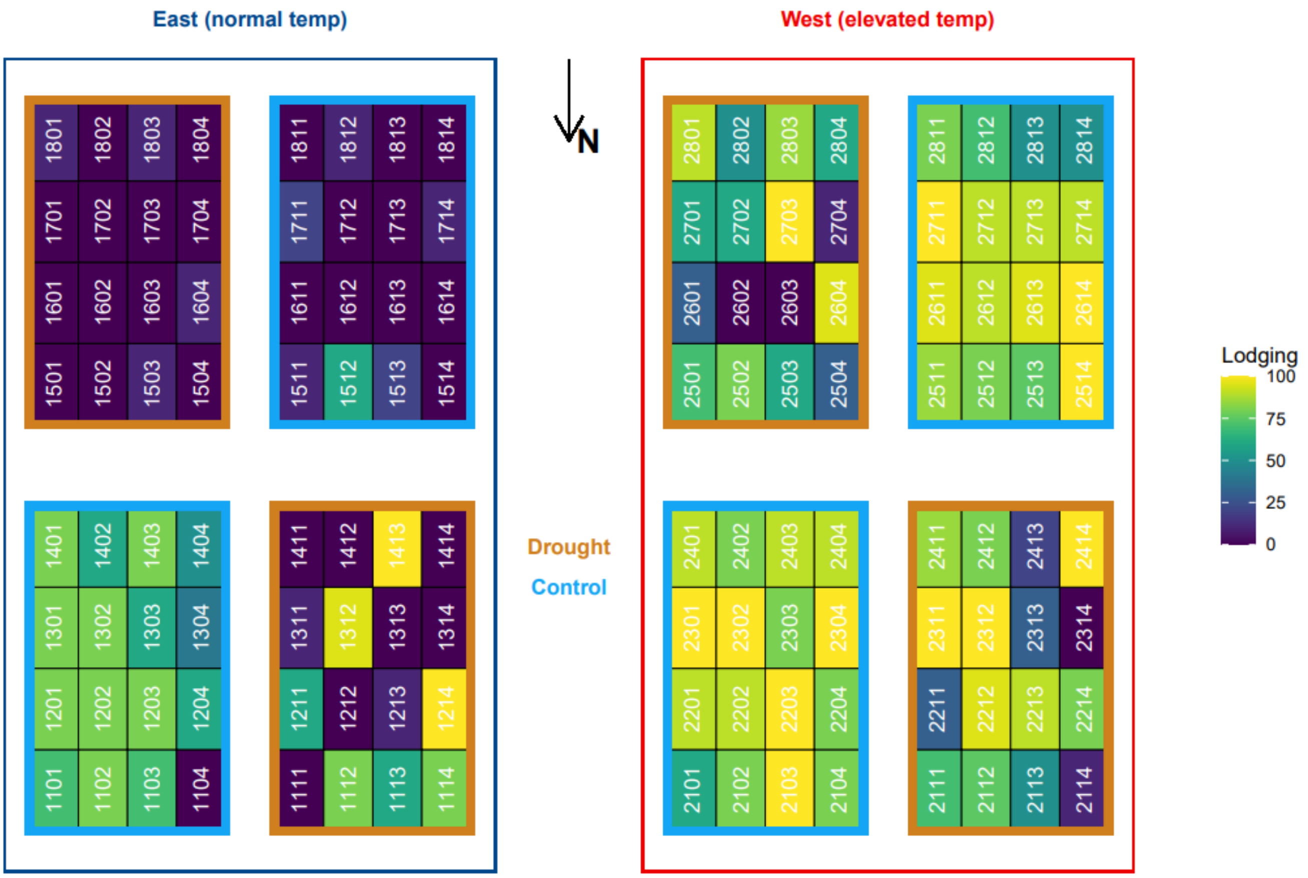

Figure 30: Percentage of lodging for each plot. Orange border around the replicates with drought and a blue border around the well-irrigated replicates.

4.2.8 Correlation matrix

In the ambient control treatment, the highest positive correlation coefficient is between lodging and grain protein content (0.51), while there is a slight negative correlation between days to heading and grain protein content (-0.38). For the ambient drought treatment, there is a positive correlation between days to heading and days to maturity (0.63). Moreover, there is a negative correlation between grain protein content and grain yield (-0.41) and grain protein content and days to heading (-0.45).

In the elevated tunnel, the control treatment shows a negative correlation between grain yield and grain protein content (-0.64). There is a positive correlation between the heading date and maturity (0.62), and days to maturity and grain yield (0.45). There are several positive correlations between traits for the drought replicate in the elevated tunnel. The greatest being between the heading date and days to maturity (0.73). The correlation between lodging and maturity (0.49) and lodging and plant height (0.52) is also noticeable.

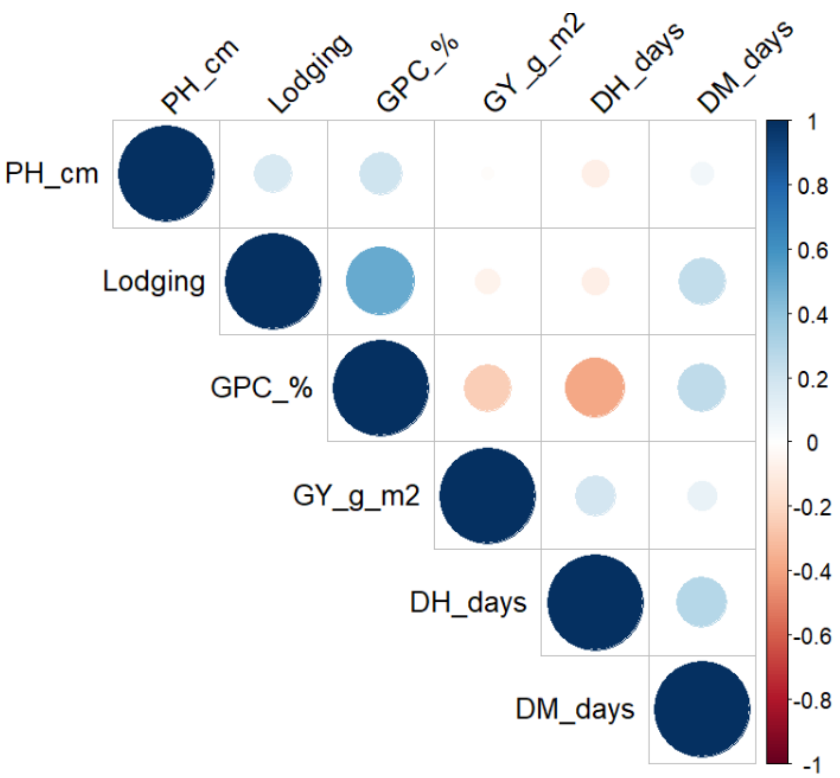

Figure 31: Correlation matrix between traits under ambient control treatment.

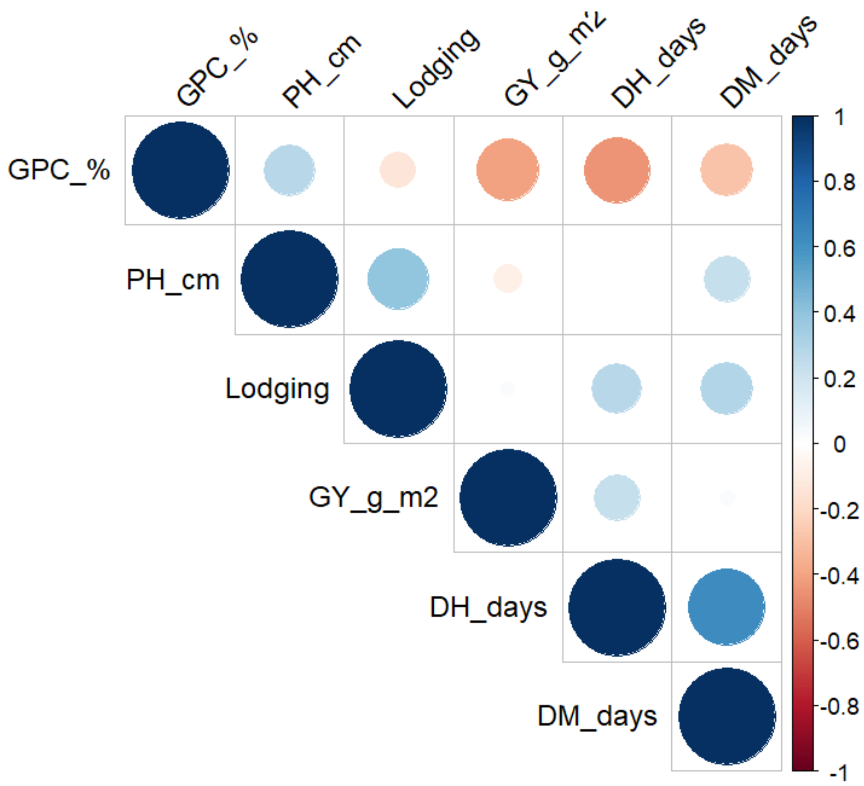

Figure 32: Correlation matrix between traits under ambient drought treatment.

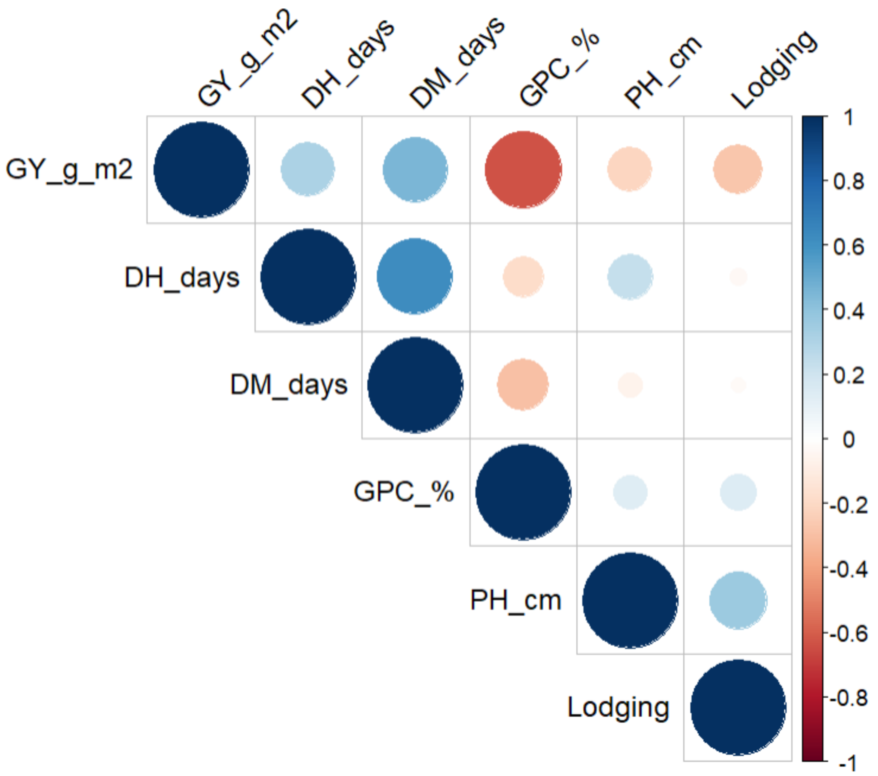

Figure 33: Correlation matrix between traits under elevated control treatment.

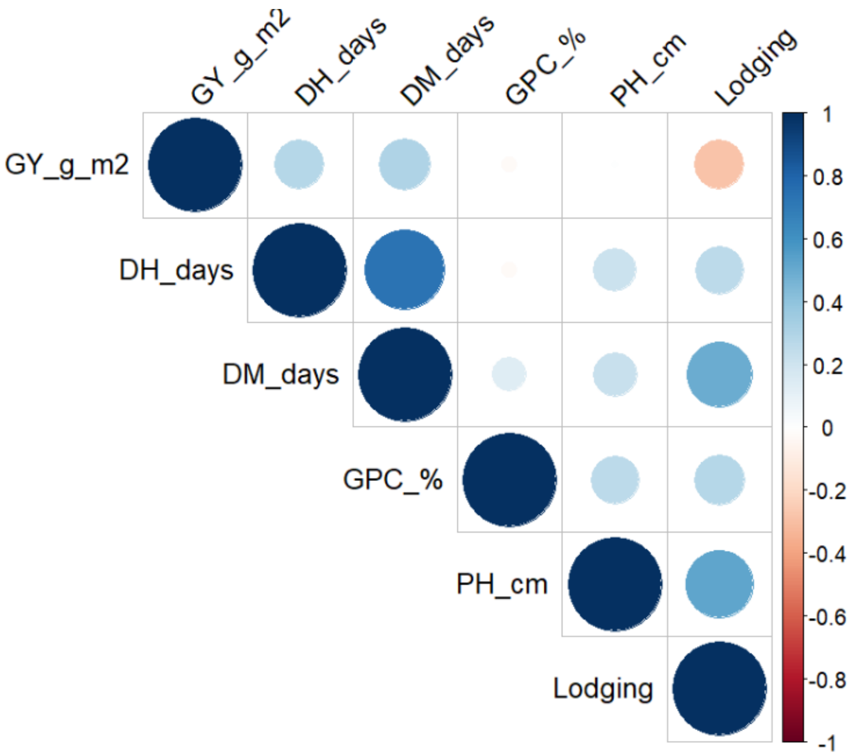

Figure 34: Correlation matrix between traits under elevated drought treatment.

4.2.9 Principal component analysis

The principal component analysis shows the variance for the different treatments. The two first principal components represent about 75% of the total variance in the dataset. The two irrigation treatments are distinct in the principal component analysis biplot (Figure 35), separated by mainly principal component 1 (PC1, capturing 45.5% of variance). The main contributors to PC1 are GY and DM. PC2 captures 30.4% of explained variance. The main contributors to PC2 are GPC, PH, and DH. The plot also shows how much the irrigation treatments affect the lines. The distance between the lines for drought and control treatments on the axis of the different traits displays to what degree they are affected. Short distance implicates a small effect, and long distance implicates a large effect.

Lines showing a small effect of irrigation treatments on yield are NW262, NW158, and NW265. Lines with a large effect of the treatment, however, are NW63, NW58, and NW154. For grain protein content, the lines NW265, NW154, and NW134 are only slightly affected, while NW274, NW262, and NW121 show a large effect of the drought. Regarding plant height, the lines showing minor differences when exposed to drought are NW134 and NW154, while NW274 and NW58 show a large effect. Regarding maturity, line NW262 shows a slight difference, while NW63 and NW58 show a substantial difference between the treatments. NW262 also shows a small difference for heading date along with NW158 and NW252. While NW63 shows a large difference for days to heading.

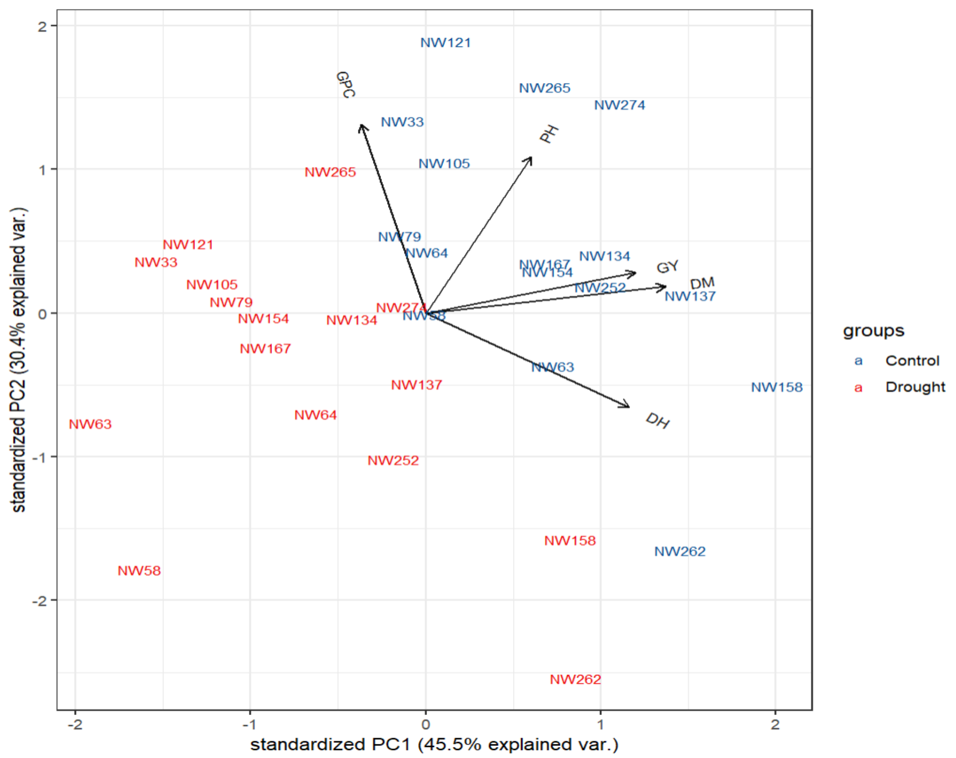

Figure 35: PCA biplot showing variance for the measured traits for different varieties under different irrigation treatments in the ambient tunnel.

4.2.10 NOBALWheat comparison

Figure 36 compares the final grain yield of the 16 varieties grown in the control and drought replicate and the ambient tunnel, and outdoors at Vollebekk in 2022. The figure visualizes how the different varieties rank and how the order of the varieties differs between the three trials. One can see how the yield is reduced in the tunnels, more so under drought than in the control. Most of the varieties rank similarly in the different trials. NW274 (DS-720-3-DH) and NW265 (DS-655-7-DH), however, rank noticeably differently in the three trials. Both land around the middle in the control, perform better outdoors, and fall to the bottom in the drought. The yield for the NW274 and NW265 goes from 618.6 and 631.5 outdoors to 281.6 and 328.5 in the drought, respectively. NW158 and NW033 are on the other end of the spectrum and are very stable, performing similarly in all trials, with NW158 (013-01) performing the best and NW33 (Runar) performing the worst.

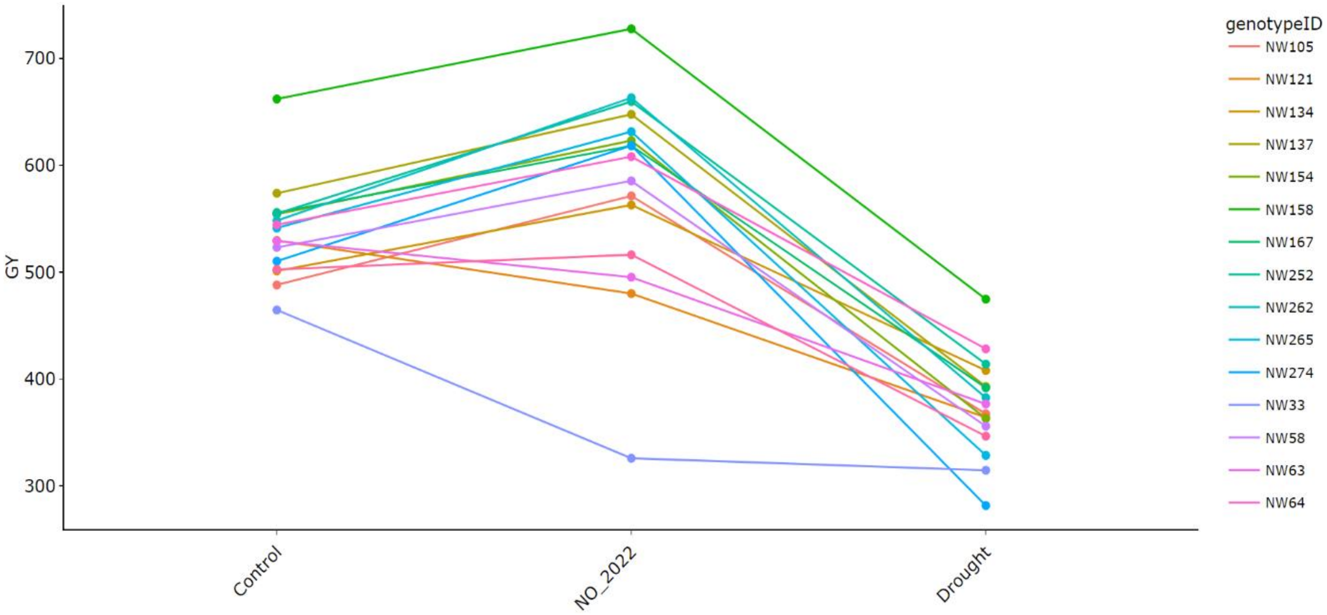

Figure 36: Comparison of the varieties and their final grain yield when grown under different treatments in the polytunnels and outside at Vollebekk.

4.2.11 Gas exchange

ANOVA

ANOVA analysis on the effect of the treatments on the KaRal data (Table 20) shows a significant difference between the genotypes, and that watering significantly affected the gas exchange. The interaction between watering and genotypes, however, is shown to be insignificant.

Table 20: Significance of the different treatments and the interactions between the treatments on gas exchange values.

Type III Analysis of Variance Table with Satterthwaite's Method			
Genotype	*******		
Watering	*******		
Genotype x Watering	**ns**		

Least square means

Table 21 presents least-square means for the genotypes, as well as each variety, gas exchange values, and the calculated p-value. The table shows the average gas exchange values for the five varieties without specifying irrigation or temperature treatments. There is a noticeable variation between the genotypes. DS-720-3-DH had the highest gas exchange value at 514.85, followed by Betong, which had 472.61. Zombi had 426.75, Caress had 394.45, and Runar had the lowest value with 365.15. All the p-values show significance.

Table 22 shows the average gas exchange values for the different replicates with the same temperature and irrigation treatment and the calculated p-values. The elevated watered treatment had the highest average gas exchange value of 665.92. The drought treatment in the elevated tunnel, however, had 442.88. For the ambient tunnel, the values were 433.41 and 196.84 in the watered and drought treatment, respectively. There is a noticeable difference between the different treatments, with the ambient drought having significantly reduced gas exchange, compared to the watered counterparts and the drought treatment in the elevated tunnel.

Table 21: Least square means calculations for the varieties and their average gas exchange values. Calculated based on five time points; measured weekly from July 18 to August 18.

Genotype	G_s	***Std. Error***	***Df***	***T value***	***Lower***	***Upper***	***Pr(<t)***
Caress	394.45	47.18	12.00	8.36	291.64	497.25	2.39E-06
DS-720-3-DH	514.85	47.28	12.09	10.89	411.93	617.76	1.32E-07
Zombi	426.76	46.97	11.79	9.08	324.21	529.30	1.14E-06
Betong	472.61	47.18	12.00	10.02	369.80	575.41	3.51E-07
Runar	365.15	47.79	12.62	7.64	261.59	468.71	4.42E-06

Table 22: Average gas exchange values for the different replicates when exposed to different irrigation and temperature treatments. Calculated based on five time points; measured weekly from July 18 to August 18.

Temp	***Watering***	G_s	***Std. Error***	***Df***	***T value***	***Lower***	***Upper***	***Pr(<t)***
Ambient	Drought	196.84	55.17	8.79	3.57	71.57	322.11	0.006287
Elevated	Drought	442.88	67.97	8.99	6.52	289.11	596.66	0.000109
Ambient	Watered	433.41	55.48	8.98	7.81	307.87	558.95	2.71E-05
Elevated	Watered	665.92	67.95	8.99	9.80	512.15	819.68	4.28E-06

Comparison of gas exchange values and final yield:

Table 23 compares the final grain yield and average gas exchange values for the different varieties when exposed to different irrigation in the ambient tunnel. The reduction in stomatal activity under drought and the differences between the genotypes are visible. The correlation between G_S and yield seems more evident in the watered replicates, as the higher-yielding genotypes also have higher gas exchange. In the drought replicates, the results are more interesting. The DS-720-3-DH has the highest gas exchange and the lowest yield, which contradicts the expectations. Betong, however, is the highest yielding with both irrigation treatments and maintains G_S values under drought.

Table 24 shows a comparison of the final grain yield and average gas exchange values for the different varieties when exposed to different irrigation in the elevated tunnel. The yield values vary less than in the ambient tunnel, and the gas exchange values are noticeably higher. The DS-720-3-DH has interesting results in this tunnel as well, as it has significantly higher G_S in both treatments but comparatively similar yield values to the other varieties. Caress also stands out, as it has the best final yield values but the lowest and second lowest G_S in the dry and watered replicates.

Table 23: Comparison of final grain yield and average gas exchange values for the genotypes under drought and control conditions in the ambient tunnel. Calculated based on five time points; measured weekly from July 18 to August 18.

Genotype	***Watering***	***G_s***	***Yield***
Caress	Drought	196.49	365.3
DS-720-3-DH	Drought	228.14	307.5
Zombi	Drought	183.25	354
Betong	Drought	221.45	392.9
Runar	Drought	154.86	326
Caress	Watered	449.69	506.7
DS-720-3-DH	Watered	439.27	497.8
Zombi	Watered	433.47	518.1
Betong	Watered	472.71	555.8
Runar	Watered	371.91	495.9

Table 24: Comparison of final grain yield and average gas exchange values for the genotypes under drought and control conditions in the elevated tunnel. Calculated based on five time points; measured weekly from July 18 to August 18.

Genotype	***Watering***	***G_s***	***Yield***
Caress	Drought	406.79	469.5
DS-720-3-DH	Drought	603.7	449.4
Zombi	Drought	425.81	402.5
Betong	Drought	492	421
Runar	Drought	286.11	319.1
Caress	Watered	524.82	493.1
DS-720-3-DH	Watered	788.27	466
Zombi	Watered	664.48	465.6
Betong	Watered	704.27	454.7
Runar	Watered	647.73	359.8

5. Discussion

The plan for this trial was to create drought conditions leading to an approximate 50% reduction in yield. The temperature increases in the elevated tunnel aimed to simulate conditions that can be expected in the future due to climate change (an approximate 5° Celsius increase in temperature). By creating these conditions, the aim was to compare the 16 different genotypes and how they performed based on the evaluation of agronomic traits. By assessing their performance, the goal was to identify genotypes tolerant to drought and observe what traits contribute to the tolerance.

Assessing the performance of the infrastructure when conducting drought trials was another important goal of this experiment, as this was the first trial where the polytunnels were utilized. The polytunnels resemble static rainout shelters, but they are more versatile as their roof and walls can be adjusted to fit the objectives of the trials. This also makes it possible to manage them in a way that prevents unintended sheltering effects that often occur during trials conducted using rainout shelters. During this trial, the goal was to learn how to best manage the polytunnels to avoid such unintended effects, so they could be better utilized in later trials.

This thesis describes the pilot season of the drought trial in the polytunnels, focusing on gaining knowledge and documenting know-how on conducting such trials in the infrastructure.

5.1 Effect of the treatments

Analysis of variance showed a significant effect of the drought treatments on the different cultivars, and the genotypes responded differently to the treatments to which they were exposed. However, there were several issues during this trial that made reaching conclusions regarding the drought tolerance of the genotypes difficult. The interaction effects of drought treatments and genotype were not significant for any trait except plant height. This also reflects the issues experienced in the trial.

Many issues, especially in the tunnel with elevated temperature, affected the results for the different measurements, making it challenging to reach viable conclusions regarding the performance of the genotypes. In the ambient tunnel, the experiment was more successful. There were fewer issues regarding the manipulated environmental parameters, and the results were more reliable overall. However, the ambient tunnel was not immune to challenges, and this trial will need to be repeated and improved to get information useful in plant breeding. However, the results gathered from this trial can still be used to evaluate the experiment itself.

5.1.1 Grain yield:

The drought stress aimed to achieve a 50% decrease in grain yield. This did not occur, with the ambient tunnel reaching an average of 30% decrease and the elevated only a 6% decrease in final grain yield. The results for the yield in the ambient tunnel are satisfactory, showing a sizeable reduction in yield for the plots exposed to drought compared to the controls. The ANOVA analysis also shows a significant difference among the genotypes and that the yield is affected by drought, indicating the potential of identifying genotypes better adapted to drought by using the methodology. It is also an indication of the effectiveness of the tunnels. The result from the ambient tunnel highlights the potential for this trial and the use of polytunnels to study drought tolerance in wheat. In the elevated tunnel, the difference in yield was negligible, and in both tunnels, there was a delay in achieving drought stress severe enough to negatively affect the plants. Comparing the effects of temperature on yield in the two tunnels is therefore not possible, as well as observing the difference in the effects of drought in ambient and elevated temperatures.

Water deficit can cause a reduction in yield in many ways. The main reason is a reduction in photosynthetic activity due to the closure of stomata, which causes a lower supply of assimilates. Upholding carbon assimilation is necessary to support reproductive growth and allowing the grain to grow. Several yield components play into the final yield, each of which is impacted by drought. Such yield components include the number of heads per plant, the number of spikelets per head, thousand-grain weight and kernel size (Li et al., 2011). This was observed in this trial, as the grain analysis showed a reduction in TGW and kernel size for the drought-treated plots in the ambient tunnel. The size reduction was mainly caused by a decrease in kernel width, not length, and this reduction was large enough to affect the final grain size. This reduction coincided with the yield decrease observed in the ambient tunnel, and it is fair to assume that the decrease in TGW and kernel width caused the yield reduction in the replicates exposed to drought.

The correlation between the grain measurements and final grain yield for the ambient drought replicates shows a negative correlation between grain yield and TGW, kernel area, and kernel length. In the elevated tunnel, the correlation is strong and positive between kernel size and grain yield. These observations are interesting, but as these correlation coefficients are calculated based on only thirty-two observations per treatment, and all except 3 correlations are insignificant, any conclusions cannot be drawn from these results alone.

To what degree the yield is affected by drought depends on the severity of the deficit, its duration and at which developmental stage the stress occurs. During tillering, drought affects root and meristem development, leading to decreased leaf area and fewer heads and spikes per plant. (Hay and Porter, 2006). If occurred early in the season, the wheat head size is negatively affected, and the number of spikelets per spike could be reduced. Terminal (late season) drought impacts flowering and the grain-filling stage, which is more critical as it reduces photosynthesis and metabolic rates, and results in poor grain development (Zhang et al., 2018). Terminal drought can lead to large reductions in harvest index and lower yield due to lower starch deposition in the grain filling stage. (Hay and Porter. 2006). In this trial the water availability was not reduced significantly until the later stages of the season, and the drought achieved was terminal.

There were expectations of identifying responses to the combined stressors of both water deficit and elevated temperatures in the elevated tunnel. Heat stress during the growth season shortens the growth cycle length and reduces grain yield due to a reduction in grain size and number of grains per area (Ottman et al., 2012). Single heat events, along with elevated temperature during the entire season, results in a similar effect if occurring during critical growth stages (Langridge & Reynolds, 2021). However, these effects of increased temperature were not possible to observe in this trial as the temperature increase needed to be higher than what was achieved. Any possible combined effects of drought and increased temperature were also not observed, and it would be difficult to compare the effects of temperature on the different varieties as temperature treatment was only replicated once. The increased air humidity was also affecting the temperature in the tunnels, as humid air takes longer to heat up than dry air. This made assessing the effects of increased temperature on grain yield more difficult.

5.1.2 Grain protein content:

There was an increase in grain protein content in the watered replicates in both tunnels. This was unexpected, as it is established that drought generally leads to an increase in grain protein content (Li et al., 2011). The increase was larger in the elevated tunnel than in the ambient tunnel, with an increase of 1.56% and 0.47%, respectively.

Reduced water availability reduces grain size, resulting in higher grain protein content. The protein content is also closely linked with nitrogen acquisition, whereas nitrogen uptake requires sufficient water availability (Hay and porter. 2006). This could explain the increased protein in the watered zones. These plots had better water availability, which increased nitrogen uptake, which could have resulted in higher grain protein content compared to the dry replicates. The difference between watering treatments was greater in the elevated tunnel than in the ambient.

In the elevated tunnel, the increased temperature could also have affected the increased protein content. Heat stress inhibits the deposition of starch more than the deposition of protein in the grain. In stressful conditions, the starch deposition will slow down, leading to shrunken grains and lower grain weight. The accumulation of protein, however, will continue, leading to higher protein content relative to grain size and increased grain protein content. (Hay and Porter, 2006). This is widely established and is visible in the trial's correlation matrix, which shows the significant negative correlation between grain protein content and grain yield, especially for the ambient drought and elevated control replicate. Elevated temperatures during the growth season can shorten the growth cycle and result in reduced grain number and size, which could lead to increased protein content. The difference in yield between drought and control in the elevated was small, which could be the reason the protein content in the watered zones was so high.

Suppose the elevated temperature, high humidity, severe lodging, and aphid and powdery mildew infestation caused reduction in yield for both irrigation treatments. However, the watered zones still were able to acquire nitrogen through satisfactory water uptake. In that case, it could lead to these plots still being able to deposit protein, resulting in a higher GPC in the elevated watered treatments compared to the other replicates. These results have several possible explanations, but the exact reason is impossible to pinpoint after this trial alone, as there were several factors influencing the wheat in this trial that could have affected the protein content.

5.1.3 Plant height

In the ambient tunnel, the plots experiencing drought stress grew shorter than the plots receiving adequate irrigation, while there was minimal difference between the treatments in the elevated tunnel.

The cells that cause the elongation are developed early, and then the turgor pressure in the cells will cause them to extend and for the stem to elongate. Less water means less turgor pressure and less elongation; therefore, drought-stressed plants grow shorter. This explains the height difference observed in the ambient tunnel. The stem elongation happens simultaneously as the plant transitions from a vegetative to a reproductive state when the apex meristem differentiates from producing leaf primordia to producing spikelet primordia. Increased duration of the stem elongation increases the number of fertile florets due to longer spike growth and higher dry matter partitioning to the spike (González et al., 2003). On the other hand, a shortening of the stem elongation phase as a response to drought stress causes a reduction in height, in addition to loss of yield.

The introduction of reduced height genes, *Rht* genes, in new wheat varieties was a substantial part of the green revolution. As plant breeding led to increased grain yield, tall genotypes would be vulnerable to lodging, as they were too frail to support the growing weight of the heads (Hedden, 2003). Reducing the height prevented these problems and led to an increase in assimilate partitioning into the grain, leading to even greater yield increases. Shorter genotypes also benefited from more intensive nitrogen application, better allocation of assimilates to the spikes, and less lodging (Kronenberg et al., 2021).

Under drought conditions however, shorter genotypes with *Rht* genes have been shown to produce lower yields than taller varieties exposed to the same unfavorable conditions (Jatayev, 2020). The traits of dwarf varieties that are beneficial when well irrigated, like shorter and thicker stems, become unnecessary when grain shrinks in response to drought, making lodging less prominent (Liu, 2017). For the taller genotypes, however, the reduced risk of lodging is an improvement, in addition to them benefiting from a larger above-ground biomass and leaf area. Taller genotypes have therefore shown to be higher yielding under drought conditions compared to dwarf or semi-dwarf varieties (Jatayev, 2020).

In the elevated, there were minimal differences in plant height between the irrigation treatments. The probable cause being less drought stress due to the high air humidity. The drought-treated plots dried slowly, and drought stress did not occur until after the stem elongation phase, leading to the lack of height reduction. While in the ambient tunnel, there was sufficient drought stress during the stem elongation phase, and the height of the plants were negatively affected.

5.1.4 Heading date and maturity:

The difference in days to heading between the dry and control replicates was smaller than expected, due to a delay in achieving sufficient drought stress early in the trial. This was the case in both tunnels. On average, both heading and maturity occurred earlier in the drought replicates than in the control. This indicates that even moderate drought impacts the development of wheat.

The reproductive development of plants is vulnerable to water deficit (Saini & Lalonde, 1997) and heat. Elevated temperatures can lead to shortening of the life cycle, which also shortens critical developmental stages, causing decreased yield (Kronenberg et al., 2021). Despite being associated with undesirable yield penalties (Semahegn et al., 2020), early maturing genotypes are preferred in dry environments. They may be able to escape terminal drought stress due to early harvesting and can fit in multiple cropping cycles due to their relatively short growing cycles.

There were issues regarding the maturity data, as it was assessed incorrectly, which negatively impacted the accuracy of the data. Despite these issues, it is still possible to see that the dry replicates matured earlier than the well-watered ones on average. However, conclusions regarding the individual varieties cannot be made based on this trial.

5.1.5 Lodging:

There was severe lodging in both tunnels. Since the wheat was grown in a closed or semi-closed chamber, there was a lack of air movement that could increase straw stiffness. Later in the field season, there were periods of strong winds, which resulted in excessive lodging in both tunnels. Due to the management, the problem was greater in the elevated tunnel, where the tunnels had been closed entirely during the first part of the growth season, but later opened. Therefore, plants in the elevated tunnel were weaker than in the ambient, where the management allowed more air movement during the trial. Little to no acclimation to air movement led to the stems growing frail and susceptive to breaking and lodging.

Lodging is the permanent displacement of the stems from the vertical axis. It results either from the stem base's failure or the anchorage system (Foulkes et al., 2011). The risk of stem and root lodging is calculated in terms of the wind speeds required to cause the failure of the stem base and the anchorage system. Lodging is a common phenomenon that can decrease yield by 80%, and it is therefore necessary to try to prevent lodging in wheat (Foulkes et al., 2011).

There was more intensive lodging in the watered zones compared to the dry ones. Lodging is known to reduce yield, and it is fair to assume that the lodging in the elevated tunnel also contributed to the reduced yield values for the watered zones. Here every plot in the watered zones experienced partial or complete lodging. The dry zones were also largely affected by lodging but less severely than the watered zones. This may have affected the yield in some capacity and led to the smaller difference between the watered and dry plots.

In the ambient tunnel, the two southern replicates were less affected by lodging due to more acclimation during the growth period, as they grew close to the south end wall which was kept open the entire trial. The two northern replicates, however, experienced severe lodging, with the watered section being more affected than the dry replicates. In addition to the spatial effects in the tunnel and air movement that caused lodging, the watered zones grew taller and with heavier heads due to higher yield, therefore, they needed less air movement to reach the critical point where the stem would lodge. This could explain the increased amount of lodging in the watered replicates for both tunnels. This is also suggested by the correlation matrix, which shows a positive correlation between plant height and lodging for all replicates.

5.1.6 Gas exchange:

The measurements and data analysis showed differences in stomatal gas exchange between the varieties, and the different treatments affected the gas exchange rates. The result may be affected by issues that occurred during the trial, including lodging, humidity, and the time needed for the varieties to reach maturity.

The maturity of the genotypes affected the gas exchange data since the measurements with the KaRal were done on the same days, despite some varieties maturing earlier, and having started to wilt during the measurements. Desiccated leaves have no stomatal activity, and therefore, low gas exchange values. The drought affected the time it took for the varieties to reach maturity. It could have led the dry replicates to have lower gas exchange values as the stomatal activity were reduced towards the end of the trial. This would result in uneven values for the dry replicates compared to the control. It can also affect the result for the different varieties, as some matured earlier regardless of treatment, lowering the average values of those genotypes. The excessive lodging makes comparing the final yield to the gas exchange values challenging. The high humidity in the elevated tunnel, especially, is another challenge that can have affected the gas exchange data, as humidity lowers the crops' transpiration rates and water demand.

Transpiration is the process of water moving through a plant and evaporating into the atmosphere. The water is transpired through the stomata, which is the above-ground control point for the entry of carbon dioxide for photosynthesis and exit of water (Kulkarni et al., 2017). Opening the stomata allows water to evaporate from the leaves simultaneously as CO_2 enters the leaves to be used in photosynthesis. Transpiration is a critical process in controlling the plant's temperature, as it cools the leaves. Under elevated temperatures, the stomata will open, and transpiration will increase to cool the canopy, while under a water deficit, the stomata will close to prevent water loss. However, this also halts the influx of CO_2 to the leaf, which slows down photosynthetic capacity due to less carbon assimilation, reduced leaf water potential, and increased oxidative stress, all leading to a reduction in yield.

The expected changes in transpiration rate in response to drought was observed in this trial. The plots exposed to drought showed an average reduction in stomatal conductance compared to the control plots in both tunnels. The five genotypes also displayed different gas exchange rates across the different treatments, which indicates the presence of genetic differences in drought tolerance. The gas exchange data can be used to evaluate the performance of the varieties. If a variety can maintain stomatal activity under drought, it could be a sign of it being better adapted to tolerating drought. Reduction in gas exchange during drought means the stomata are closed to prevent water loss, which inhibits photosynthesis due to the lower availability of CO_2. If the gas exchange is not reduced, it indicates that the plant maintains stomatal activity and will not experience a lack of CO_2 and can maintain photosynthesis.

The gas exchange data show great potential of being used to learn about the role of transpiration, stomatal conductance, leaf temperature, and photosynthetically active radiation and its effect on the final grain yield. As the KaRal device also measured the light influx in the tunnels, it could be used as an indicator of the quality of light that reaches the canopy, and thereby give an indication about the polytunnels' usefulness. Apart from some outliers, most of the measurements with the KaRal are of good quality and could be used to get insight into the crops' physiology and how the different treatments affected stomatal conductance. However, due to the many issues experienced in the tunnels, it would be irresponsible to draw conclusions based on this trial. The excessive lodging is especially challenging. With improvements in future trials, however, the KaRal data could be used to evaluate the performance of genotypes included in the trial.

5.2 Evaluation of the varieties.

The trials were conducted with a split plot design, which gives very few degrees of freedom to identify interactions between irrigation treatments and agronomic traits. The design is primarily aimed at identifying variety differences. However, due to less-than-optimal results from this trial, comparing the performance of the varieties grown in the tunnels is challenging, and drawing conclusions regarding the drought tolerance of the genotypes would be ill-advised. The analysis shows significant differences between the varieties, however, and observations indicate that there are more tolerant lines among the genotypes included in the trial and that the trial has the potential of identifying said varieties if improved in the future.

The ANOVA analysis shows there are significant differences between the genotypes for all the agronomic traits measured, except for lodging in the ambient tunnel. The rankings for the different traits show the performances of the genotypes, presenting the difference in values between the drought and control replicate for each tunnel. Line 013-01 has the highest final yield in both tunnels, and a relatively small reduction when exposed to drought. When comparing the protein content of the varieties in the tunnels, line 876 is observed as the variety with the highest GPC in both tunnels for the control replicates. However, it shows a sizeable reduction in protein content when exposed to a water deficit. When observing the heading date and maturity ranking, DS-638-5-DH was the slowest for both traits in all treatments. In the ambient tunnel, 013-01 and Caress were also slow to reach maturity, while Runar used the least time. All varieties matured earlier when exposed to drought, compared to well-irrigated replicates. The same was seen for plant height in the ambient tunnel, with all growing shorter in the drought replicates, but there were still noticeable differences in height between the different genotypes. DS-720-3-DH had the least reduction in height under drought.

From the PCA one can see to what degree the drought treatments affected the traits of the different genotypes. Therefore, it can be used to identify lines that show better tolerance to drought. NW262 (DS-638-5-DH), NW158 (013-01), and NW265 (DS-655-7-DH) show less effect of the drought treatment of the final grain yield. In future trials, it would be beneficial to include these varieties to study their potential for drought tolerance more closely. However, lines that show a large effect of the treatment are NW63 (Caress), NW58 (Zombi) and NW154 (F-013-032), implying they are less tolerant to drought.

The gas exchange data can also be used as an indicator of the performance of the varieties. If a genotype can maintain stomatal activity and high yield simultaneously, it could mean that it has a mechanism to tolerate a water deficit. Betong manages to do this in the ambient tunnel, while Caress is the best performing variety in the elevated tunnel. They both maintain high gas exchange rates under drought, resulting in substantial final grain yield. The DS-720-3-DH has some interesting results, as it had elevated gas exchange values for all treatments but a relatively low grain yield.

The same 16 varieties were also grown in a field trial outdoors at Vollebekk in 2022 to study the effect of nitrogen fertilization on grain yield and protein content. By comparing the result from the outdoor trial with the results from the polytunnel, it is possible to compare the relevance of the polytunnel trials to field trials. Suppose the ranking is similar, with the same order of the varieties in the different trials. In that case, it indicates that they are similarly affected by the treatments, and that the differences are mainly caused by genotype and treatment. If the ranking changes a lot between the treatments, that could mean that genotype by environment interactions is affecting the results. When looking at the plot that compares the different trials, most of the varieties rank similarly, and there is not much variation among the genotypes. The Tunnel with the ambient temperature reproduced similar cultivar rankings as in the outdoor field, thus validating the tunnel as a reliable research platform to reproduce field conditions. There are some exceptions. DS-720-3-DH and DS-655-7-DH are two lines that went from being among the best performing varieties outdoors to being among the worst-performing under drought in the tunnels, indicating genotype by environment interactions. The comparison of the different trials shows that, despite the issues experienced during the trials, the experiment was still effective, and can be repeated in future trials.

5.3 Challenges that occurred during the field trial:

5.3.1 Temperature:

The temperature in the elevated tunnel was on average 1.2 degrees warmer than in the ambient tunnel and 1.9 degrees warmer than outside. The goal of increasing the temperature by 5 degrees still needs to be reached. In the ambient tunnel, the temperature increased by an average of 0.6 degrees compared to the outside, which is a satisfactory result. However, in future repetitions of this trial, it should be attempted to improve management to keep the temperature closer to the outside and increase the temperature in the elevated tunnel further.

For the elevated tunnel, the temperature increased rapidly in the morning when the sun hit the tunnels and declined rapidly after sunset. The nighttime temperature was only 0.4 degrees warmer than in the ambient. This does not coincide with what is expected with climate change, as climate models predict higher temperatures at nighttime as well as daytime (Prasad et al., 2008). Cool nighttime temperatures are vital during warmer periods, as it gives the plant time to recuperate from the stress they were exposed to during the day, for example, by regenerating photosystems that have been damaged. If the night temperatures increase, the plants cannot regenerate, thus causing losses in yield (Prasad et al., 2008). The attempt to elevate the temperature in the elevated tunnel was not entirely successful, and observing responses to the heat was not possible. In future experiments, the tunnels should be managed more efficiently in an attempt to create conditions expected in the future.

5.3.3 Humidity:
High humidity was prevalent in the elevated tunnel. The walls were closed during most of the season, which attributed to the delayed drought stress and possibly affected gas exchange values from the KaRal. High humidity negatively affects the evapotranspiration of the plots, leads to a lowered vapor pressure deficit, and reduces the water demand of the crops (Kundel et al., 2018), as the plants grown under such conditions have lower transpiration rates. At lower humidity levels, the transpiration rates are higher, leading to more water loss from the plants and greater water stress. The plants grown in the elevated tunnel possibly experienced a smaller negative impact on the water deficit due to reduced transpiration activity and reduced water demand.

High humidity, water droplets and dew negatively affected the accuracy of the KaRal measurements. This was observed in the first measurements, where some results showed impossibly high values. These were excluded in the final data set that was used for analysis. Later in the season, the roof was opened approximately 20 minutes before the measurement and stayed open during the measurement. This increased the airflow and allowed a substantial portion of the humidity to escape the tunnel and the accuracy of the results improved after this was implemented. This practice should be continued during future measurements with the KaRal system.

The humidity was also the cause of the extensive infestations that occurred in the polytunnels. Aphids were the main issue, and they riddled the field despite attempts to eliminate them with pesticides. Treatments with fungicides were more effective against powdery mildew that also occurred in the tunnels.

5.3.2 Delayed drought stress:
There was an issue with achieving the amount of drought stress which was aimed for in both polytunnels. After sowing, the field was irrigated generously to promote germination and good trial establishment. The soil retained moisture better than expected, however, and it took a longer time for the water potential in the soil to drop to the values necessary to achieve drought stress. This can be observed in both tunnels' soil moisture data. In the ambient tunnel, the water potential was eventually reduced to sufficient levels. Whereas in the elevated tunnel, the issue was greater. The elevated tunnel had less airflow and higher humidity levels which reduced the amount of water that evaporated from the soil and increased the time it took to induce drought stress. It also reduced the crops’ water demand, and therefore also reduced the amount of drought stress they experienced during the trial. The yield and other measured traits were less affected in the elevated tunnel than in the ambient tunnel because of this, which can be seen in the descriptive statistics for the different traits. For the ambient tunnel, the delay in achieving sufficient drought stress affected the data for days to heading, as the drought did not greatly affect the plants before they reached the heading stage.

5.3.4 Lodging:

There was excessive lodging in the elevated tunnels due to no acclimation to air movement at the beginning of the trial when the plants were in the stem elongation period. When the walls were lifted in July, and the wind could enter the tunnel, several plots lodged partially or entirely. In the ambient tunnel, the issue was not as severe, as the elevated walls allowed more air movement in the tunnel which caused the plants to acclimate. This was especially evident for the plots closer to the end wall, as these got more wind than the plots in the middle of the tunnel and suffered significantly less lodging. The middle plots got air movement from the gap on the middle walls, but strong winds at the end of the season also caused lodging. The lodging is also shown to be more severe in the watered sections. This can be explained by the wheat in the watered plots growing taller than the dry plots and therefore needing less air movement to reach the critical point where it would lodge.

5.4 Future improvements:

5.4.1 Better management of the tunnels

The priority when repeating this trial in the future is to improve the management of the tunnels to prevent the issues experienced during this season. This includes both the soil water potential, atmosphere, and temperature. Several of the issues during the trial were caused by unsatisfactory atmosphere regulation, mainly the lack of ventilation and inadequate temperature increase in the elevated tunnel. The lack of ventilation led to lodging, and high humidity caused infestations and reduced the quality of the results.

During this season, the atmosphere was regulated manually by adjusting the roof and walls of the polytunnels. A measure to improve the atmosphere and temperature regulation would be automation, making it possible to adjust the walls and roof remotely. If implemented, one could create a better schedule for ventilating the tunnels to prevent humidity, while simultaneously keeping the temperature increased in the elevated tunnel. A proposition for such a schedule could be opening the roof of the tunnels during the night, as the nighttime temperature did not increase significantly despite the closed roof and walls. Opening the roofs at night would improve ventilation in the tunnels, while still maintaining elevated daytime temperature. Automation of the atmosphere regulation would also make it easier to be more consistent with the adjustment of the walls, and it would be easier to adjust the opening of the roof according to weather forecasts.

Improved ventilation would also prevent lodging. The plants would be more evenly acclimated to movements by creating improved airflow. The inclusion of fans in the tunnels in future trials should also be considered. Other measures to stiffen the stems to prevent lodging could be manually rustling the plants. This could be done by moving through the field with a stick to move the plants or creating an installment that imitates such conditions.

The main issue with the soil was too high water potential to create satisfactory drought conditions. In future trials, it should be aimed to optimize the watering system to achieve the degree of drought the trial aims for. Heavy watering at the beginning of the trial should be avoided, while still irrigating enough to ensure satisfactory germination. In addition, the watering in the dry zones should be reduced, while it should be increased in the control sections to further differentiate between the two treatments.

By improving these issues, the results of the trial will be more reliable. The goal would be to create more control over the homogeneity and the stresses of interest in the trials (Blum, 2005). This reduces the number of other things that could affect the results. In this trial, there were many issues, including differences in soil water potential, soil variability, lodging, and bug and mildew infestations, all of which can muddle the results. By reducing these issues in future trials, it would be easier to identify varieties with better tolerance to drought.

5.4.2 Collection of more data:

Future trials would benefit from the collection of more data. In this trial, typical agronomic traits which give insight into the suitability of the genetic material were phenotyped. But extending the number of traits measured in the future could be beneficial. In addition to final grain yield, it is possible to measure different yield components. For example, count the number of florets per spike, the number of grains per floret and measure the length of the heads. This could give more detailed information on the effects of the drought and temperature on yield. Visual assessments can be used to study these traits, but new technologies like high-throughput phenotyping make it possible to study properties that cannot be seen.

It would therefore be beneficial to include high-throughput phenotyping in future trials. Red, green, and blue (RGB) and normalized difference vegetation index (NDVI) imaging is a possible method to collect data on spectral reflectance from plant canopies. Infrared imagery and remote sensing of canopy temperature are also options. NDVI and other spectral vegetation indices can detect differences in physiochemical and structural properties of the vegetation, such as pigment content, hydration status, photosynthetic area, and vegetative biomass. In addition, the estimation of canopy temperature using infrared thermography can be used to screen for water status and stomatal conductance (Langridge & Reynolds, 2021).

High throughput phenotyping is commonly done with the use of drones that are equipped with multispectral cameras. Due to insufficient space, it is impossible to fly drones inside the tunnels, and imaging would need to be collected in an alternative fashion. This could include the installation of rails in the roof to fasten the camera to or fastening the camera to the end of a rod and manually hovering it over the plots to get the images.

6. Conclusion

For this thesis, it was managed to conduct a satisfying pilot trial for this experiment. Despite the numerous issues, the trial was successful in creating drought conditions and observing the effects of the drought on the plants. With a 30% reduction in final grain yield caused by a reduction in grain width and thousand-grain weight, the results in the ambient tunnel were satisfactory. There were also observed significant effects of treatments on all agronomic traits, as well as a significant line by irrigation interaction for plant height in the ambient tunnel.

The analysis also highlighted genotypical differences between the varieties and their response to drought, and some varieties show potential of being more tolerant to water stress. These genotypes include DS-638-5-DH, 013-01, and DS-655-7-DH. There was also observed possible genotype by environment interaction for the genotypes DS-720-3-DH and DS-655-7-DH. The DS-720-3-DH breeding line was also interesting in regard to the KaRal gas exchange measurements, as it managed to maintain stomatal conductance under drought conditions without great losses to final grain yield.

As for the polytunnels, they were successful in creating drought stress in the ambient tunnel, while further improvements are needed in managing the elevated temperature to avoid creating excess humidity. Their utilization will need to be improved in future trials to get more accurate results. Possible improvements include better management of the atmosphere and irrigation system to improve the conditions in the tunnels, and implementation of high throughput phenotyping.

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Supplementary

Ranking of varieties

Grain yield pr m2

Table 25: comparison of grain yield for the varieties in the ambient tunnel under different irrigation treatments.

Grain yield ambient		
Variety	**Control**	**Drought**
013-01	647.56667	469.63333
ROBIJS	585.23333	375.06667
DS-17-16-DH	580.66667	402.6
Betong	555.83333	392.96667
876	555.56667	346.23333
F-013-032	552.46667	394.26667
013-074	541.23333	392.03333
DS-655-7-DH	539.46667	337.06667
Zombi	518.06667	354.03333
DS-638-5-DH	513	391.83333
Hiie	508.6	331.93333
Caress	506.73333	365.3
DS-720-3-DH	497.8	307.5
Voore	496.63333	396.7
Runar	495.9	326
990-2	489.86667	408.16667

Table 26: comparison of grain yield for the varieties in the Elevated tunnel under different irrigation treatments.

Grain yield elevated		
Variety	**control**	**Drought**
013-01	537.13	488.4
DS-655-7-DH	512.1	490.1
Caress	493.1	469.7
DS-17-16-DH	475.9	381.6
DS-638-5-DH	467.7	424.4
DS-720-3-DH	466	449.6
Zombi	465.6	402.5
Betong	454.6	421
990-2	453.4	430.5
F-013-032	443.2	418.2
013-074	437.2	412.3
Hiie	429.3	367.3
876	428.5	404.7
Voore	423.3	399.2
ROBIJS	369.5	377.1
Runar	359.8	319.1

Grain protein content

Table 27: Comparison of grain protein content for the varieties in the ambient tunnel under different irrigation treatments.

Grain protein content ambient		
Variety	**Control**	**Drought**
876	13.9	12.15
DS-655-7-DH	12.85	12.7
DS-720-3-DH	12.8	12.3
Zombi	12.75	11.5
Voore	12.45	12.2
Runar	12.4	11.6
Caress	12.05	11.65
DS-17-16-DH	11.95	10.8
013-074	11.7	11.2
990-2	11.7	11.45
Betong	11.6	11.3
F-013-032	11.45	11.5
Hiie	11.3	11.45
ROBIJS	10.8	10.95
013-01	10.65	10.6
DS-638-5-DH	10.1	9.55

Table 28: Comparison of grain protein content for the varieties in the elevated tunnel under different irrigation treatments.

Grain protein content elevated		
Genotype	**control**	**drought**
876	15	12.55
Runar	15	11.6
ROBIJS	14.7	13.2
Voore	13.95	12.55
DS-655-7-DH	13.9	12.4
DS-720-3-DH	13.85	13.45
013-074	13.8	12.4
990-2	13.6	12
Betong	13.6	11.45
Zombi	13.55	12
F-013-032	13.35	10.95
013-01	13.3	11
DS-17-16-DH	13.3	12.15
Caress	13.1	11.15
DS-638-5-DH	13.1	10.25
Hiie	13	10.85

Plant height

Table 29: Comparison of plant height for the varieties in the ambient tunnel under different irrigation treatments.

Plant height ambient		
Genotype	**Control**	**Drought**
DS-655-7-DH	105.8	101.2
Runar	103.5	95.8
876	102.6	95.6
990-2	102.5	96.6
DS-720-3-DH	102.3	100.8
ROBIJS	102.3	97.2
Hiie	100.3	96.6
F-013-032	99.6	92.1
013-074	98.3	93.5
Voore	97.3	92.6
DS-17-16-DH	97	92.3
013-01	96.1	92
Betong	94.1	87.6
DS-638-5-DH	94.1	87
Caress	88	83.6
Zombi	84.6	71.8

Table 30: Comparison of plant height for the varieties in the elevated tunnel under different irrigation treatments.

Plant height elevated		
Genotype	**Control**	**Drought**
DS-720-3-DH	105.6	101.3
990-2	102.8	102.1
ROBIJS	100.6	102.8
Runar	99.8	95.3
Hiie	97.8	98.8
DS-17-16-DH	97.3	96.3
876	97.1	100
DS-638-5-DH	97	96
013-01	96	96.8
F-013-032	95.5	96.3
013-074	95.3	96.3
Voore	94.6	96.3
DS-655-7-DH	91.8	99.6
Betong	87.6	86.6
Caress	84.1	91.3
Zombi	79	78.6

Days to heading

Table 31: Comparison of days to heading for the varieties in the ambient tunnel under different irrigation treatments.

Days to heading ambient		
Genotype	**Control**	**Drought**
DS-638-5-DH	56	55
013-01	53	52
DS-720-3-DH	52.5	51
ROBIJS	51.5	50
990-2	51	48
DS-17-16-DH	50.5	49
Caress	49	47
013-074	48.5	46.5
DS-655-7-DH	48	47
Zombi	48	46
876	47.5	44.5
F-013-032	47	46.5
Betong	47	46.5
Voore	46.5	46
Runar	46	44.5
Hiie	45	45

Table 32: Comparison of days to heading for the varieties in the elevated tunnel under different irrigation treatments.

Days to heading elevated		
Genotypes	**Control**	**Drought**
DS-638-5-DH	55.5	54
013-01	52.5	52
DS-720-3-DH	52	52
ROBIJS	51	49
DS-17-16-DH	48.5	48.5
013-074	48	47.5
990-2	48	48.5
Caress	47.5	47
Zombi	47.5	46.5
Betong	47.5	46.5
DS-655-7-DH	47	46
Voore	47	47
876	46	46.5
F-013-032	46	46
Runar	45.5	45
Hiie	44.5	45

Days to maturity

Table 33: Comparison of days to maturity for the varieties in the ambient tunnel under different irrigation treatments.

Days to maturity ambient		
Genotype	**Control**	**Drought**
013-01	98	92
Caress	96	84
ROBIJS	96	86
DS-720-3-DH	95	90
990-2	94	84
F-013-032	94	83
DS-17-16-DH	93	85
DS-655-7-DH	93	87
013-074	92	80.5
GN11644	90	80.5
Voore	90	79.5
876	88	79
DS-638-5-DH	103	94
GN13618	87	83
Hiie	87	82
Runar	87	79

Table 34: Comparison of days to maturity for the varieties in the elevated tunnel under different irrigation treatments.

Days to maturity elevated		
Genotype	**Control**	**Drought**
DS-638-5-DH	98	92
013-01	97	90
DS-655-7-DH	95	88
F-013-032	95	87
Caress	94	84
DS-17-16-DH	94	88
DS-720-3-DH	93	90
013-074	92	84
ROBIJS	92	91
GN13618	91	84
Voore	89	85
990-2	88	86
GN11644	88	85
Hiie	88	83
Runar	87	82
876	86	80

Correlation scatterplots for grain analysis

Ambient drought

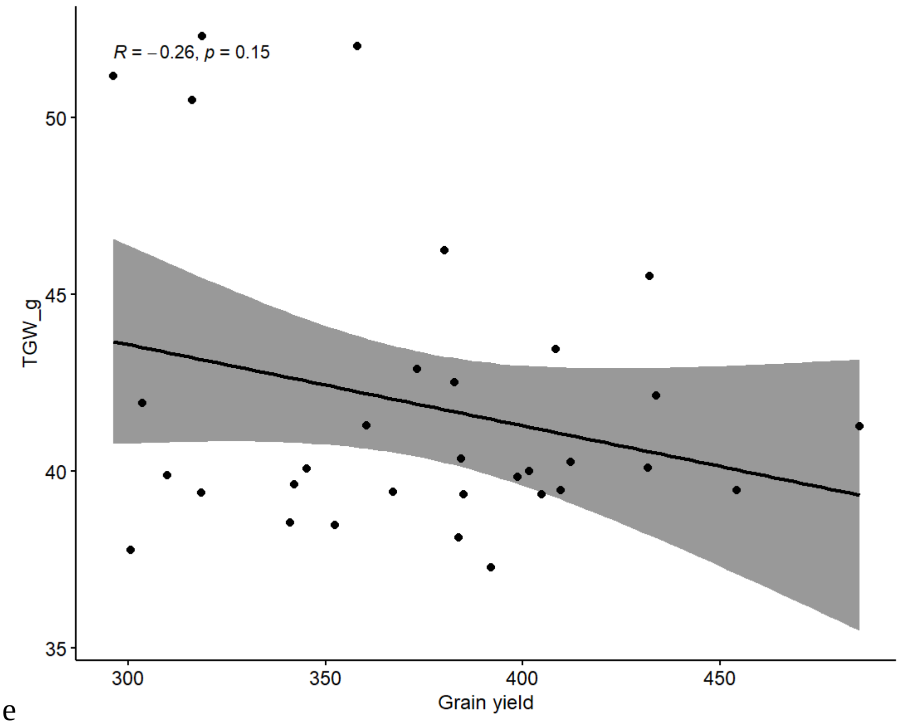

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Figure 37: Scatterplot for correlation between TGW and GY in the ambient drought replicates.

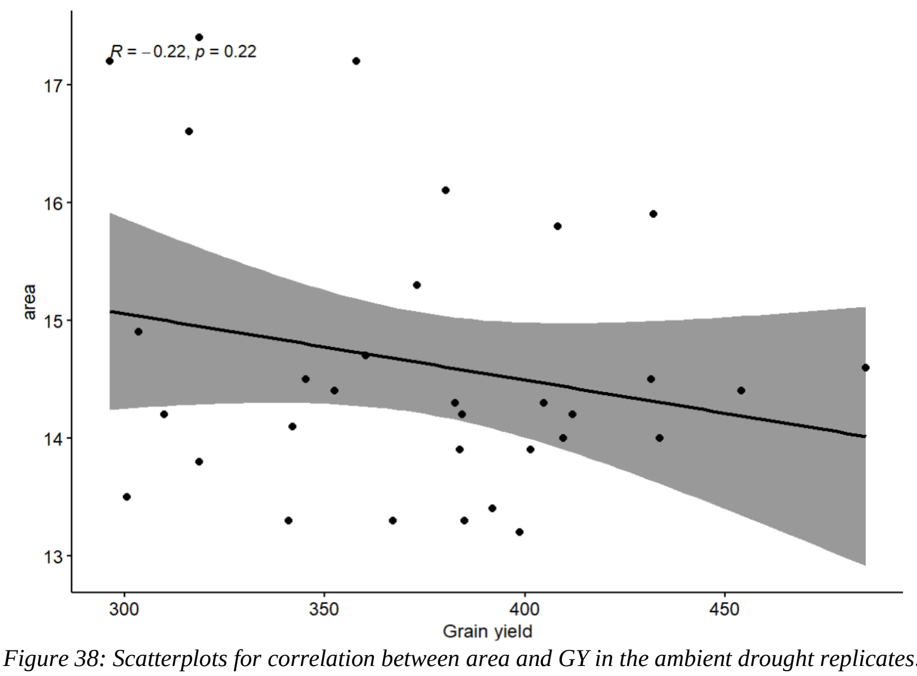

Figure 38: Scatterplots for correlation between area and GY in the ambient drought replicates.

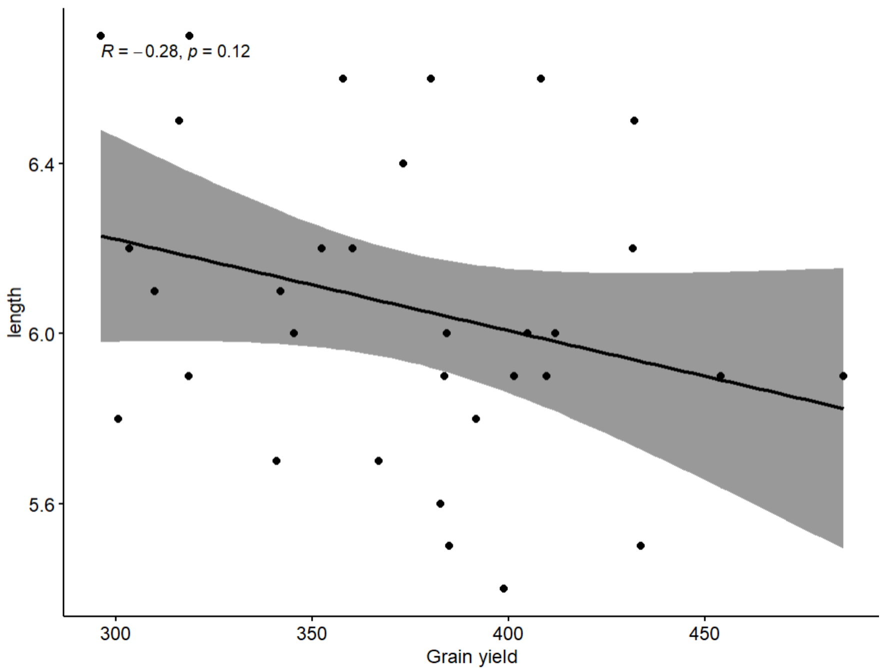

Figure 39: Scatterplots for correlation between length and GY in the ambient drought replicates.

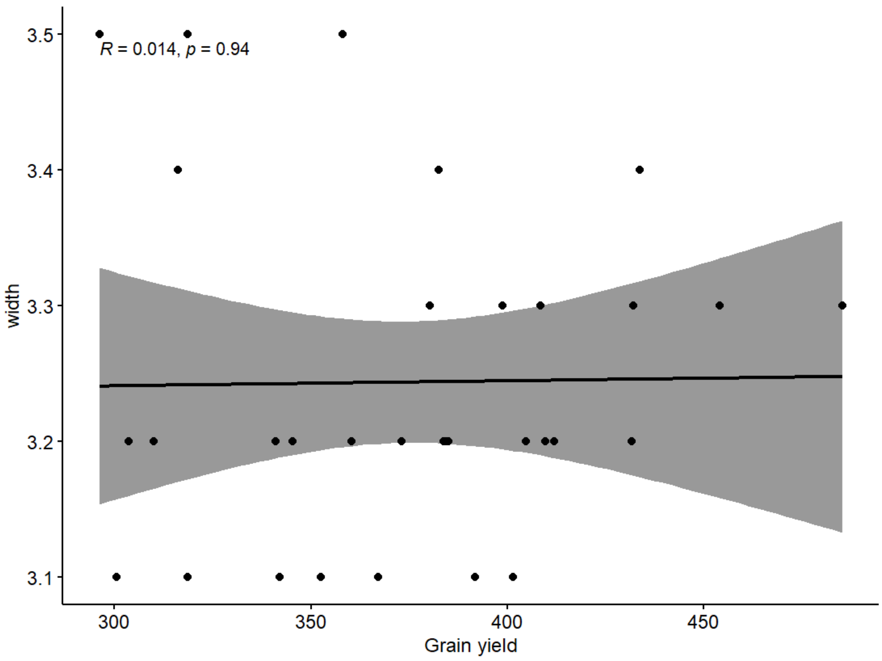

Figure 40: Scatterplots for correlation between width and GY for the ambient drought replicates.

Ambient control

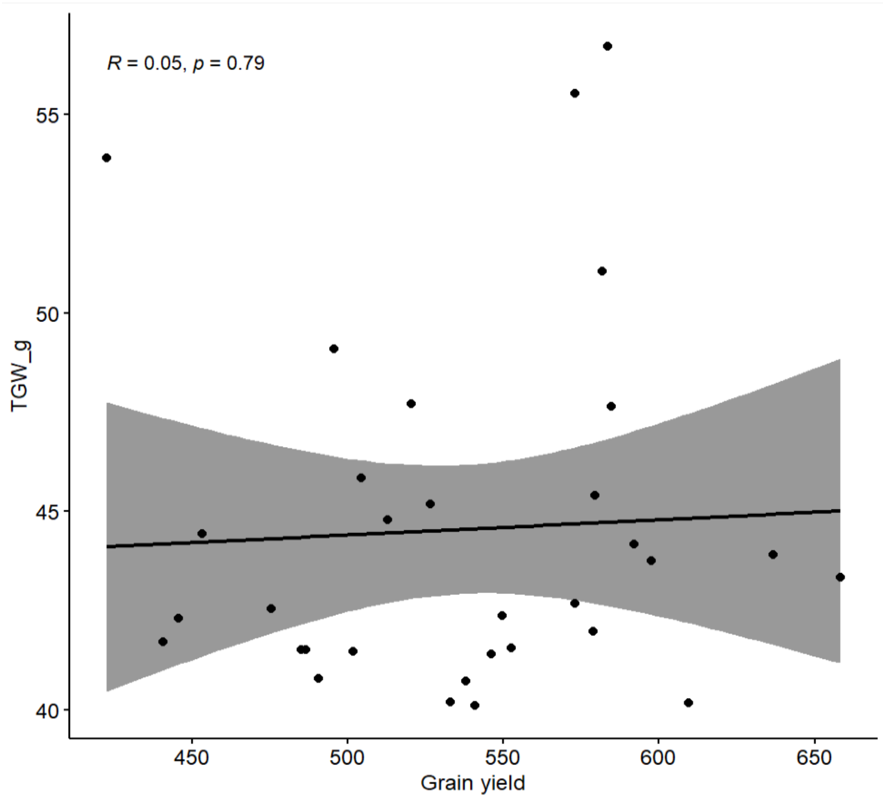

Figure 41: Scatterplots for correlation between TGW and GY for the ambient control replicates.

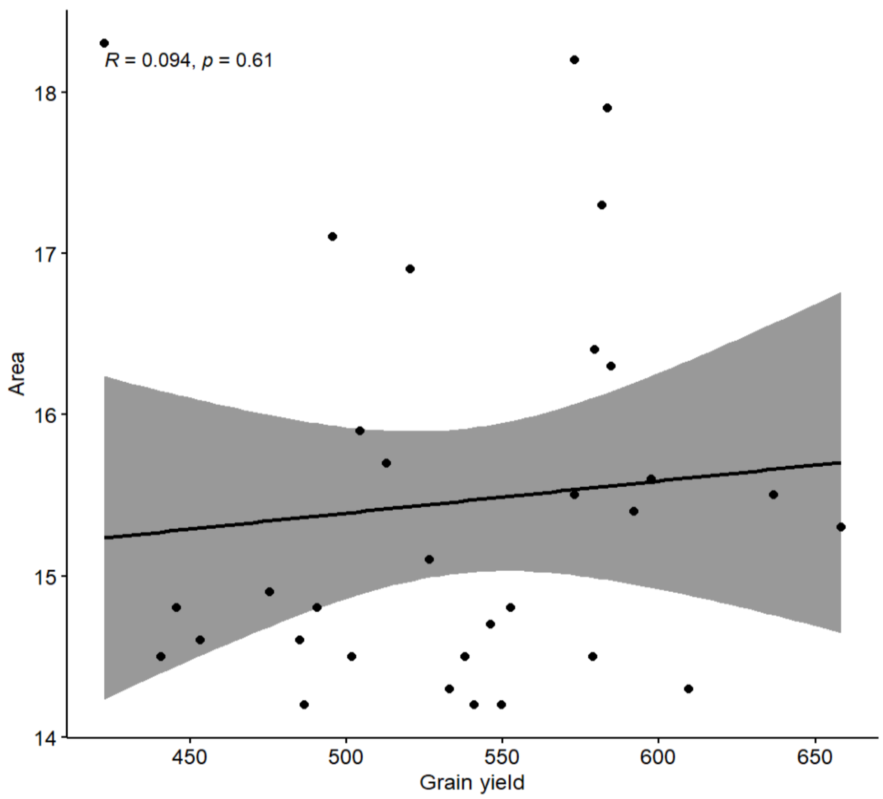

Figure 42: Scatterplots for correlation between area and GY for the ambient control replicates.

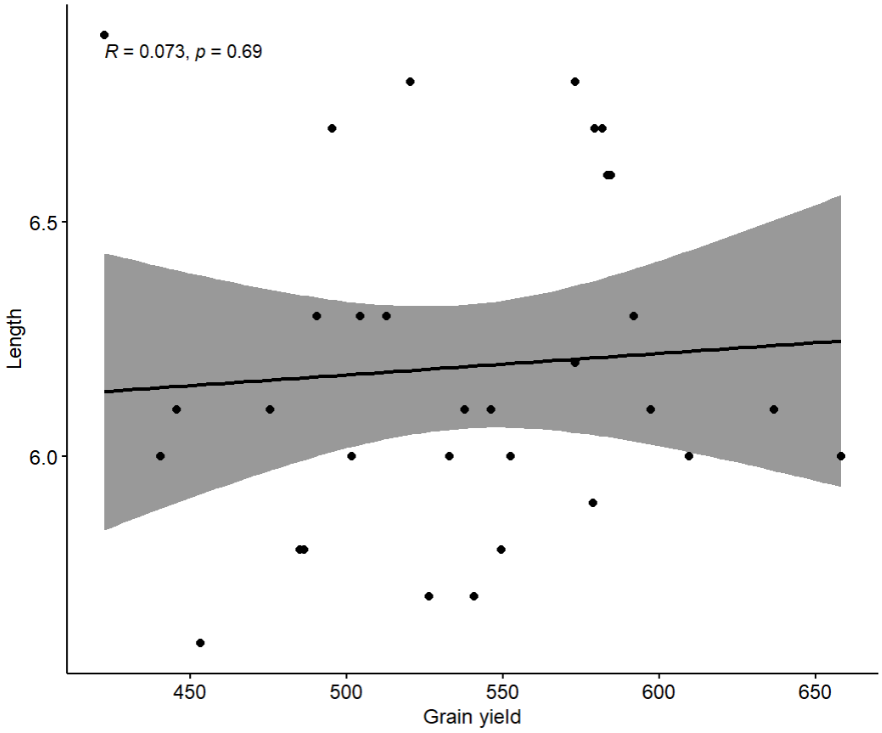

Figure 43: Scatterplots for correlation between length and GY for the ambient control replicates.

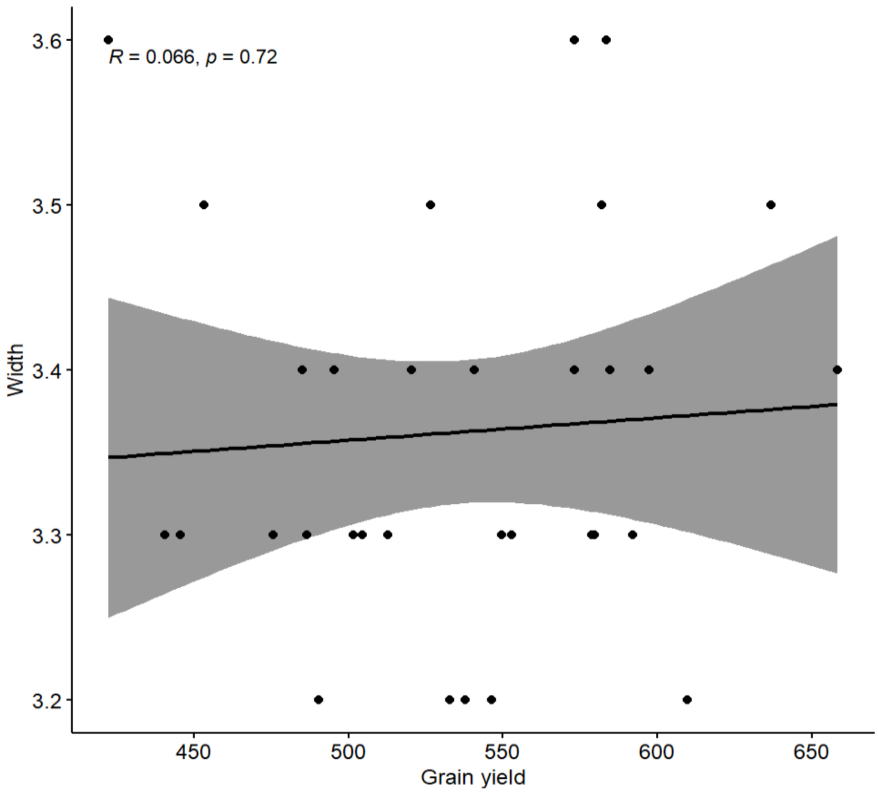

Figure 44: Scatterplots for correlation between width and GY for the ambient control replicates.

Elevated drought

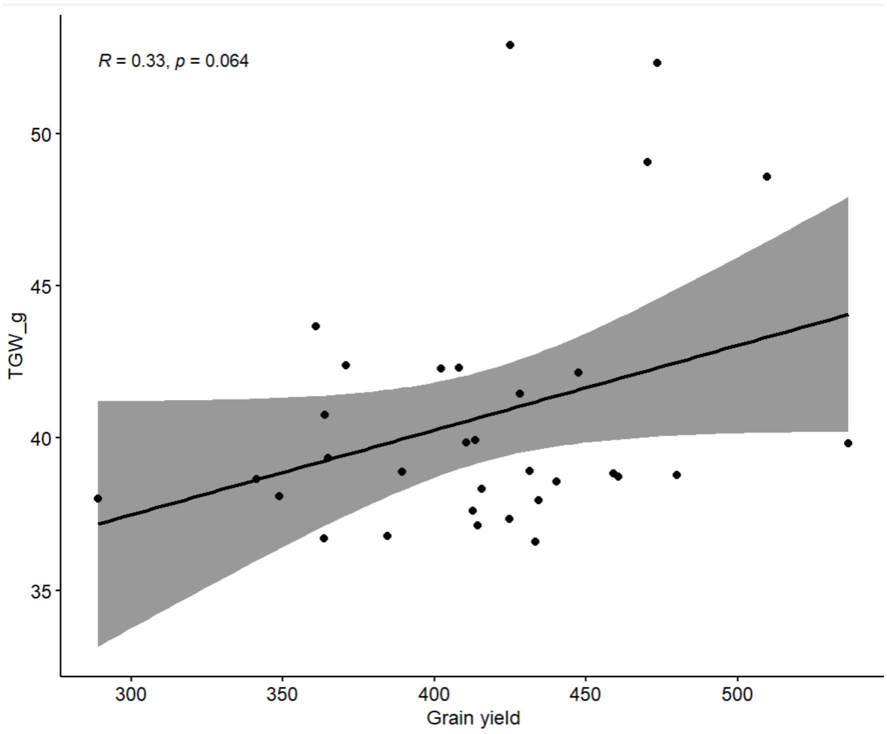

Figure 45: Scatterplots for correlation between TGW and GY in the elevated drought replicates.

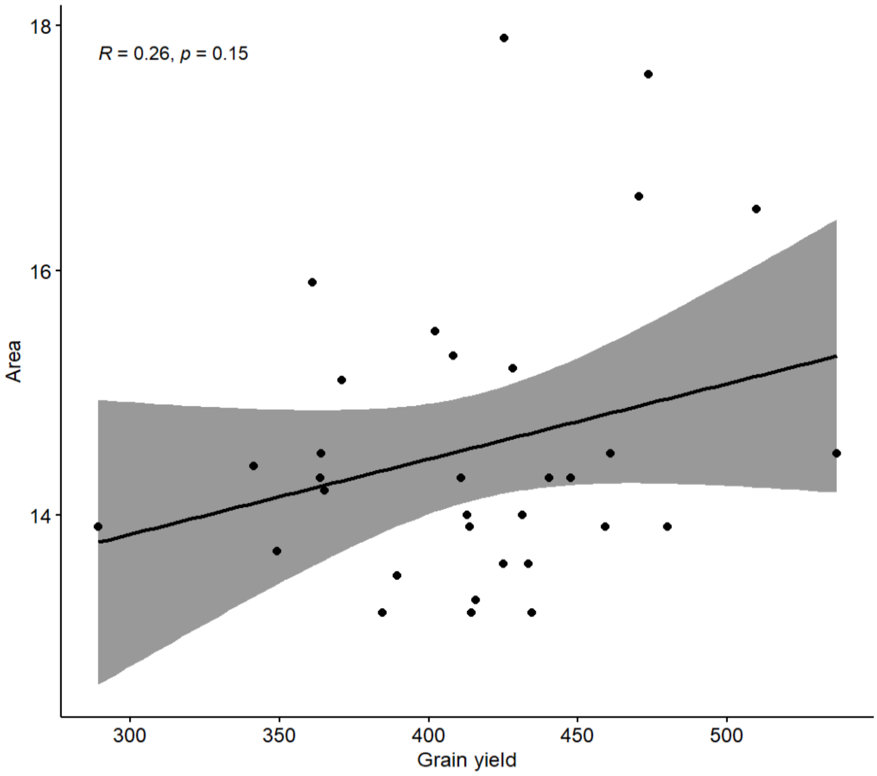

Figure 46: Scatterplots for correlation between area and GY for the elevated drought replicates.

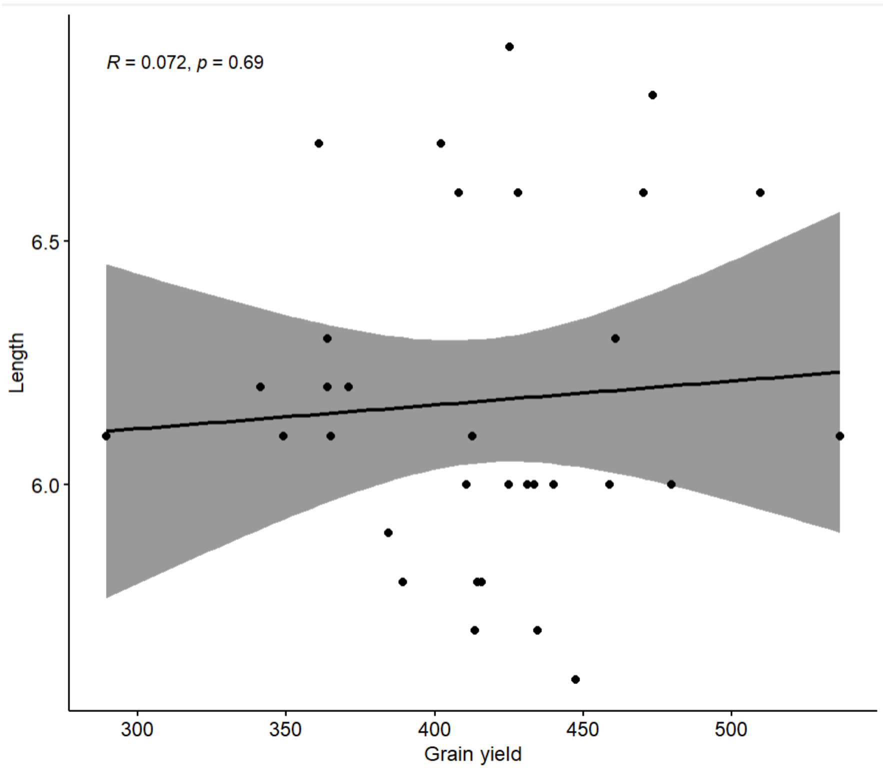

Figure 47: Scatterplots for correlation between length and GY for the elevated drought replicates.

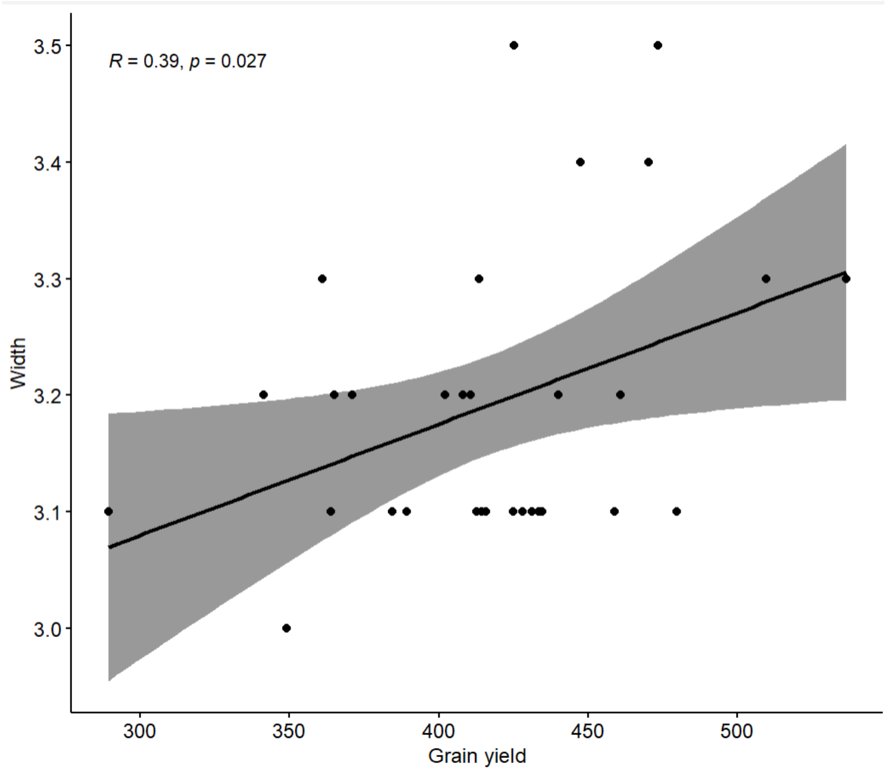

Figure 48: Scatterplots for correlation between width and GY for the elevated drought replicates.

Elevated control

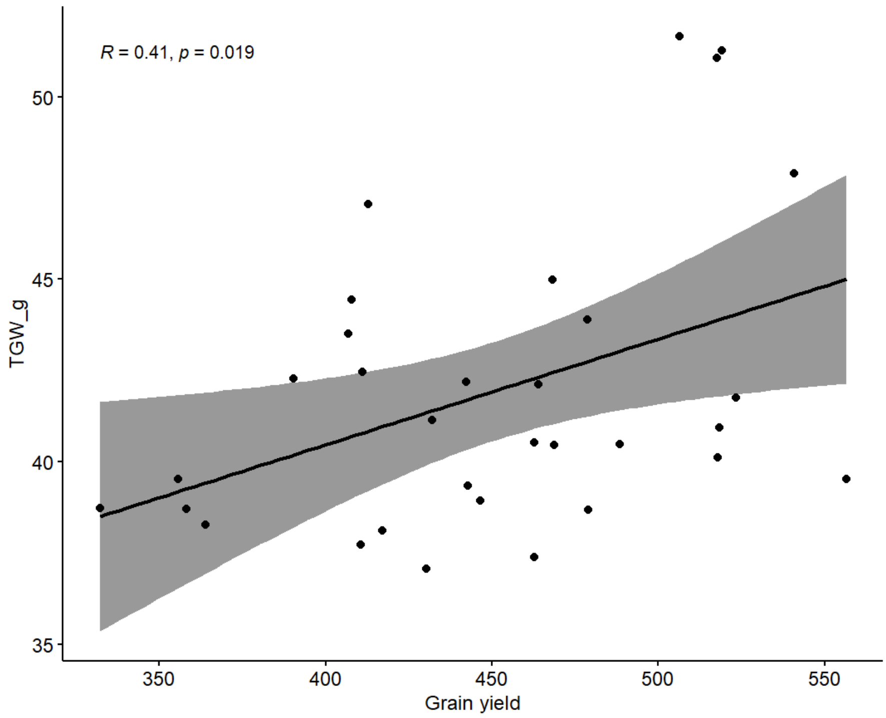

Figure 49: Scatterplots for correlation between TGW and GY for the elevated control replicates.

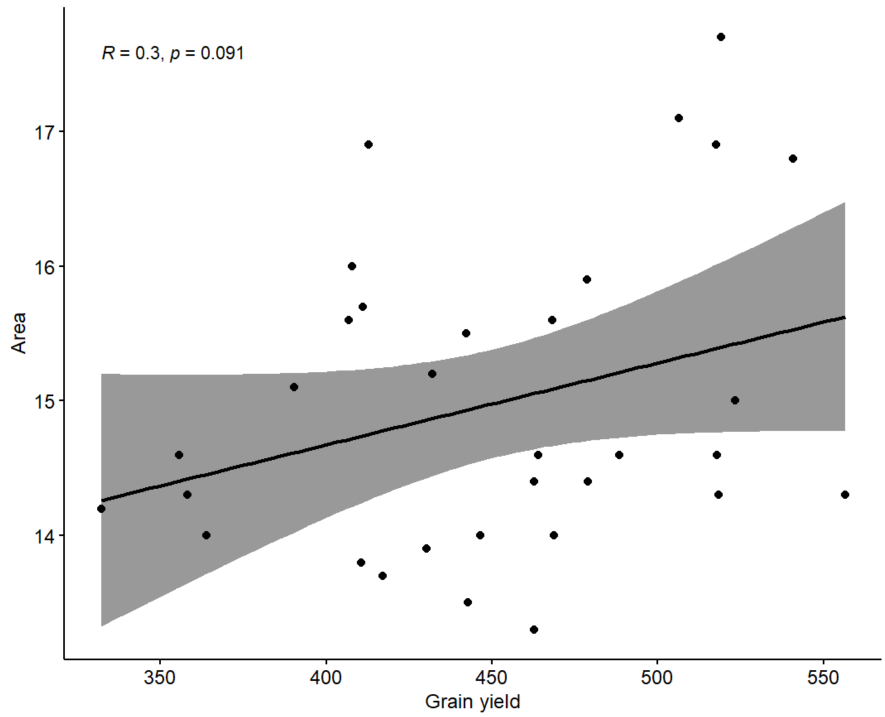

Figure 50: Scatterplots for correlation between area and GY for the elevated control replicates.

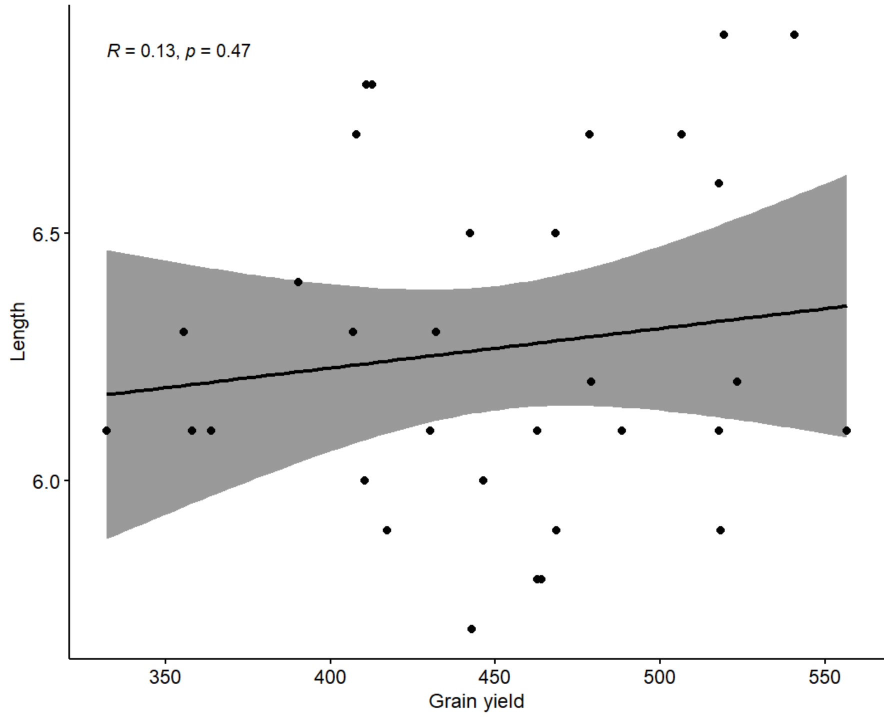

Figure 51: Scatterplots for correlation between length and GY for the elevated control replicates.

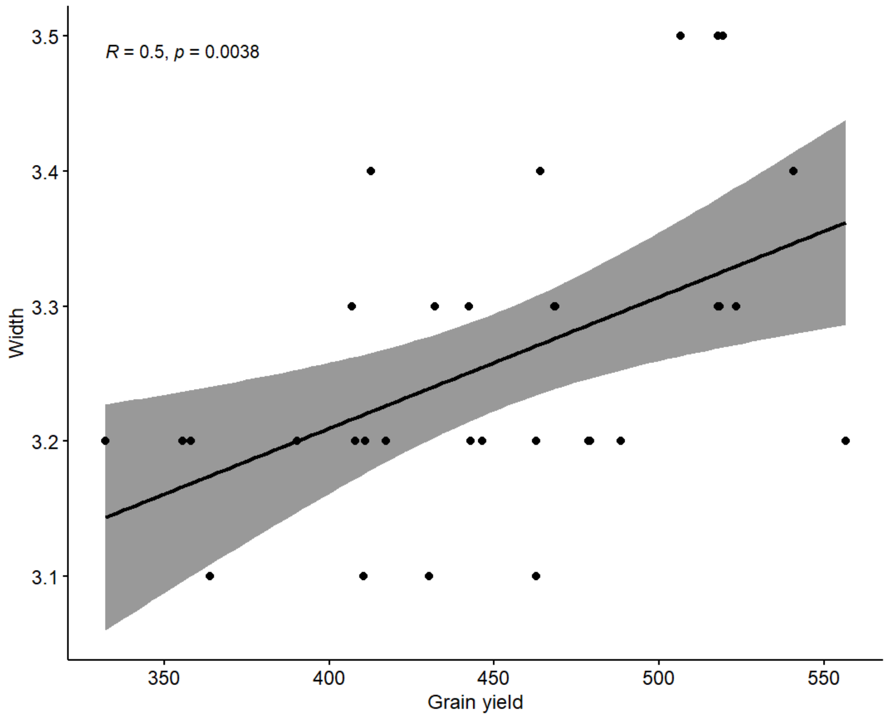

Figure 52: Scatterplots for correlation between width and GY for the elevated control replicates.

Least-square means for KaRal:

Table 35: The average gas exchange values under the different treatments. Calculated based on five timepoints, measured from July 18 to August 18.

genotype	temp	watering	G_s	Std. Error	df	t value	lower	upper	Pr(>|t|)
	Ambient		315.12	53.82	7.96	5.85	190.90	439.35	0.000388326
	Elevated		554.40	66.02	8.01	8.40	402.18	706.61	3.06E-05
		Drought	319.86	43.77	8.91	7.31	220.69	419.03	4.77E-05
		Watered	549.66	43.86	8.98	12.53	450.41	648.91	5.41E-07

Table 36: The average gas exchange values for the varieties under different temperature treatments. Calculated based on five timepoints, measured from July 18 to August 18.

genotype	temp	watering	G_s	Std. Error	df	t value	lower	upper	Pr(>|t|)
Caress	Ambient		323.09	59.80	12.09	5.40	192.91	453.27	0.000155118
DS-720-3-DH	Ambient		333.70	60.09	12.32	5.55	203.17	464.24	0.000113411
Zombi	Ambient		308.36	59.13	11.57	5.22	179.00	437.73	0.000244005
Betong	Ambient		347.08	59.80	12.09	5.80	216.90	477.26	8.17E-05
Runar	Ambient		263.38	59.13	11.57	4.45	134.02	392.75	0.000859682
Caress	Elevated		465.81	73.01	11.94	6.38	306.65	624.96	3.58E-05
DS-720-3-DH	Elevated		695.99	73.01	11.94	9.53	536.84	855.14	6.22E-07
Zombi	Elevated		545.15	73.01	11.94	7.47	386.00	704.30	7.77E-06
Betong	Elevated		598.14	73.01	11.94	8.19	438.98	757.29	3.04E-06
Runar	Elevated		466.92	75.09	13.34	6.22	305.11	628.73	2.79E-05

Table 37: The average gas exchange values for the Varieties under different irrigation treatments. Calculated based on five timepoints, measured from July 18 to August 18.

genotype	temp	watering	G_s	Std. Error	df	t value	lower	upper	Pr(>|t|)
Caress		Drought	301.64	52.42	18.08	5.75	191.55	411.74	1.84E-05
DS-720-3-DH		Drought	415.92	51.95	17.47	8.01	306.55	525.30	2.99E-07
Zombi		Drought	304.53	51.60	17.02	5.90	195.68	413.38	1.73E-05
Betong		Drought	356.72	52.42	18.08	6.81	246.63	466.82	2.21E-06
Runar		Drought	220.49	53.48	19.52	4.12	108.76	332.21	0.000551046
Caress		Watered	487.25	52.36	18.01	9.31	377.25	597.26	2.66E-08
DS-720-3-DH		Watered	613.77	53.17	19.10	11.54	502.52	725.02	4.69E-10
Zombi		Watered	548.98	52.42	18.08	10.47	438.88	659.07	4.16E-09
Betong		Watered	588.49	52.36	18.01	11.24	478.49	698.49	1.43E-09
Runar		Watered	509.82	53.48	19.52	9.53	398.09	621.54	8.81E-09

Table 38: The average gas exchange values for the varieties under both different temperature and irrigation treatments. Calculated based on five timepoints, measured from July 18 to August 18.

genotype	***temp***	***watering***	***G_s***	***Std. Error***	***df***	***t value***	***lower***	***upper***	***Pr(>|t|)***
Caress	Ambient	Drought	196.49	65.26	17.02	3.01	58.81	334.18	0.007864768
DS-720-3-DH	Ambient	Drought	228.14	66.37	18.15	3.44	88.79	367.49	0.002908434
Zombi	Ambient	Drought	183.25	65.26	17.02	2.81	45.57	320.94	0.012093492
Betong	Ambient	Drought	221.45	65.26	17.02	3.39	83.77	359.13	0.003454582
Runar	Ambient	Drought	154.86	65.26	17.02	2.37	17.18	292.54	0.029699568
Caress	Elevated	Drought	406.79	82.05	18.81	4.96	234.95	578.64	8.98E-05
DS-720-3-DH	Elevated	Drought	603.70	79.93	17.02	7.55	435.07	772.33	7.86E-07
Zombi	Elevated	Drought	425.81	79.93	17.02	5.33	257.18	594.44	5.54E-05
Betong	Elevated	Drought	492.00	82.05	18.81	6.00	320.15	663.84	9.44E-06
Runar	Elevated	Drought	286.11	84.73	21.26	3.38	110.03	462.19	0.002814274
Caress	Ambient	Watered	449.69	67.66	19.53	6.65	308.34	591.04	2.03E-06
DS-720-3-DH	Ambient	Watered	439.27	67.66	19.53	6.49	297.92	580.62	2.80E-06
Zombi	Ambient	Watered	433.47	65.26	17.02	6.64	295.79	571.15	4.14E-06
Betong	Ambient	Watered	472.71	67.66	19.53	6.99	331.36	614.05	1.01E-06
Runar	Ambient	Watered	371.91	65.26	17.02	5.70	234.22	509.59	2.60E-05
Caress	Elevated	Watered	524.82	79.93	17.02	6.57	356.19	693.45	4.78E-06
DS-720-3-DH	Elevated	Watered	788.27	82.04	18.81	9.61	616.43	960.11	1.10E-08
Zombi	Elevated	Watered	664.48	82.05	18.81	8.10	492.64	836.33	1.50E-07
Betong	Elevated	Watered	704.27	79.93	17.02	8.81	535.64	872.90	9.49E-08
Runar	Elevated	Watered	647.73	84.73	21.26	7.64	471.65	823.81	1.56E-07

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