Drought tolerance prediction of potato by automatic phenotyping of morphological and physiological traits

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Abbreviations

ATW	Average tuber weight
A2D	Leaf area 2D
A3D	Leaf area 3D
cc	Optimal water supply
cs	Late stress
CT	Canopy temperature
CTD	Canopy temperature depression
DB	Digital biomass
DFP	Days from planting
DRYM	Deviation of relative starch yield from the experimental median
DRYMp	Deviation of relative starch yield from the parental median
FAO	Food and Agriculture Organization of the United Nations
FGH	Polytunnel Screenhouse
LA	Leaf angle
LAI	Leaf area index
LI	Leaf inclination
LPD	Light penetration depth
PH	Plant height
Pop	Population
RH	Relative humidity
sc	Early stress
ss	Long-term stress
SY	Starch yield
TN	Tuber number
TY	Tuber yield
VPD	Vapor pressure deficit

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Abstract

Potato is the 4th most important food crop in the world. Especially in tropical and sub-tropical potato production, drought is a yield limiting factor. Potato is sensitive to water stress. Potato yield loss under water stress could be reduced by using tolerant varieties and adjusted agronomic practices. Direct selection for yield under water-stressed conditions requires long selection cycles. Thus, identification of markers for marker-assisted selection may speed up breeding. The objective of this thesis is to identify morphological markers for drought tolerance by continuously monitoring plant growth and canopy temperature with an automatic phenotyping system.

The phenotyping was performed in drought-stress experiments that were conducted in population A with 64 genotypes and population B with 21 genotypes in the screenhouse in 2015 and 2016 (population A) and in 2017 and 2018 (population B). Drought tolerance was quantified as deviation of the relative tuber starch yield from the experimental median (DRYM) and parent median (DRYMp). Relative tuber starch yield is starch yield under drought stress relative to the average starch yield of the respective cultivar under control conditions in the same experiment. The specific DRYM value was calculated based on the yield data of the same experiment or the global DRYM that was calculated from yield data derived from data combined over yeas of respective population or across multiple experiments including VALDIS and TROST experiments (2011-2016).

Analysis of variance found a significant effect of genotype on DRYM indicating that the tolerance variation required for marker identification was given in both populations.

Canopy growth was monitored continuously six times a day over five to ten weeks by a laser scanner system and yielded information on leaf area, plant height and leaf angle for population A and additionally on leaf inclination and light penetration depth for population B. Canopy temperature was measured 48 times a day over six to seven weeks by infrared thermometry in population B. From the continuous IRT surface temperature data set, the canopy temperature for each plant was selected by matching the time stamp of the IRT data with laser scanner data.
Mean, maximum, range and growth rate values were calculated from continuous laser scanner measurements of respective canopy parameters. Among the canopy parameters, the maximum and mean values in long-term stress conditions showed better correlation with DRYM values calculated in the same experiment than growth rate and diurnal range values. Therefore, drought tolerance index prediction was done from maximum and mean values of canopy parameters.

The tolerance index in specific experiment condition was linearly predicted by simple regression model from different single canopy parameters under long-term stress condition in population A (2016) and population B (2017 and 2018). Among the canopy parameters maximum light penetration depth (2017), mean leaf angle (2017, 2018, and 2016), mean leaf inclination or mean canopy temperature depression (2017 and 2018), maximum plant height (2017) were selected as tolerance predictors. However, no single parameters were sufficient to predict DRYM. Therefore, several independent parameters were integrated in a multiple regression model.

In multiple regression model, specific experiment DRYM values in population A was predicted from mean leaf angle (2016). In population B, specific tolerance could be predicted from maximum light penetration depth and mean leaf inclination (2017) and mean leaf inclination (2018) or mean canopy temperature depression and mean leaf angle (2018).

In data combined over season of population A, the multiple linear regression model selected maximum plant height and mean leaf angle as tolerance predictor. In Population B, mean leaf inclination was selected as tolerance predictor. However, in population A, the variation explained by the final model was too low.

Furthermore, the average tolerances respective to parent median (2011-2018) across FGH plants or all plants (FGH and field) were predicted from maximum plant height (population A) and maximum plant height and mean leaf inclination (population B). Altogether, canopy parameters could be used as markers for drought tolerance. Therefore, water stress breeding in potato could be speed up through using leaf inclination, light penetration depth, plant height and canopy temperature depression as markers for drought tolerance, especially in long-term stress conditions.

Vorhersage von Trockentoleranz in Kartoffel durch automatische Phänotypisierung morphologischer und physiologischer Eigenschaften

Zusammenfassung

Die Kartoffel ist die viertwichtigste Nahrungspflanze der Welt. Besonders in den Tropen und Subtropen ist Trockenheit ein ertragsbegrenzender Faktor für die Kartoffelproduktion. Kartoffeln sind empfindlich gegen Trockenstress. Der Ertragsverlust von Kartoffeln unter Wasserstress könnte durch die Verwendung von toleranten Sorten und angepasste Anbaupraxis verringert werden. Die direkte Selektion für Ertrag unter Trockenstressbedingungen erfordert lange Selektionszyklen. Daher kann die Identifizierung von Markern für marker-assisted Selektion die Züchtung beschleunigen. Das Ziel dieser Arbeit ist es, morphologische Marker für Trockentoleranz mit Hilfe von kontinuierlichen Messungen von Pflanzenwachstum und Bestandstemperatur mittels automatischer Phänotypisierung zu identifizieren.

Die Phänotypisierung wurde in Trockenstressexperimenten durchgeführt, welche mit 64 Genotypen aus Population A und 21 Genotypen aus Population B in einem Foliengewächshaus in 2015 und 2016 (Population A) bzw. 2017 und 2018 (Population B) stattgefunden haben. Die Trockentoleranz wurde als Abweichung des relativen Stärkeertrags der Knollen vom experimentellen Median (DRYM) und dem Elternmedian (DRYMp) quantifiziert. Der relative Stärkeertrag ist der Stärkeertrag unter Trockenstress relativ zum mittleren Stärkeertrag der Sorte unter optimaler Bewässerung im gleichen Experiment. Der spezifische DRYM wurde auf der Basis der Ertragsdaten des gleichen Experiments berechnet oder der globale DRYM wurde auf der Basis der Ertragsdaten kombinierter Experimente aus mehreren Jahren für die gleiche Population oder für mehrere Experimente auch aus VALDIS und TROST (2011-2016) berechnet.

Die Varianzanalyse zeigte einen signifikanten Effekt des Genotyps auf DRYM, so dass die für die Identifizierung von Markern erforderliche Toleranzvariation in beiden Populationen gegeben war.

Die Bestandsentwicklung wurde mit einem Laserscanner-System kontinuierlich sechsmal täglich über fünf bis zehn Wochen gemessen und lieferte Informationen zu Blattfläche, Pflanzenhöhe und Blattwinkel für Population A sowie zusätzlich Blattneigung und Lichteinfalltiefe für Population B. Die Oberflächentemperatur wurde 48mal täglich für sechs bis sieben Wochen mittels Infrarot-

Thermometrie in Population B gemessen. Aus dem kontinuierlichen IRT-Oberflächentemperatur-Datensatz wurde die Oberflächentemperatur jeder Pflanze bestimmt, indem die Zeitstempel der IRT-Daten mit denen der Laserscannerdaten abgeglichen wurden. Mittelwert, Maximum, Streubereich (*range*) und Wachstumsrate wurden für die Bestandsparameter der Laserscannermessungen bestimmt. Unter den Bestandsparametern zeigten die Maxima und Mittelwerte unter Langzeitstress die bessere Korrelation mit dem Toleranzindex DRYM, der aus dem gleichen Experiment berechnet wurde, als die Wachstumsrate und der Streubereich. Die Trockentoleranzprognose wurde daher aus den Maxima und Mittelwerte der Bestandsparameter gemacht.

Der Toleranzindex spezifischer Versuche wurde linear mit einem einfachen Regressionsmodell aus verschiedenen einzelnen Bestandparameters unter Langzeitstressbedingungen in Population A (2016) und Population (B) (2017 und 2018) vorhergesagt. Toleranz-Prognoseparameter wurden unter den Bestandparametern maximale Lichteinfalltiefe (2017), mittlerer Blattwinkel (2017, 2018 und 2016), mittlere Blattneigung und mittlere Oberflächentemperatur-Abweichung (2017 und 2018), maximale Pflanzenhöhe (2017) ausgewählt. Kein einzelner Parameter war jedoch ausreichend um DRYM vorherzusagen. Daher wurden mehrere unabhängige Parameter in einem multiplen Regressionsmodell integriert.

Im multiplen Regressionsmodel wurde der spezifische Experiment-DRYM in Population A aus dem mittleren Blattwinkel (2016) vorhergesagt. In Population B konnte die spezifische Toleranz aus der maximalen Lichteinfalltiefe, der maximalen Blattneigung (2017) und der mittleren Blattneigung (2018) oder der mittleren Oberflächentemperatur-Abweichung und dem mittleren Blattwinkel (2018) vorhergesagt werden.

In Daten aus mehreren Anbauperioden von Population A wählte das multiple lineare Regressionsmodel maximale Pflanzenhöhe und mittleren Blattwinkel als Prognoseparameter für Toleranz aus. In Population B wurde mittlere Blattneigung als Prognoseparameter für Toleranz ausgewählt. In Population A war jedoch die Variation, die durch das Endmodell erklärt wurde, zu niedrig.

Die mittlere Toleranz hinsichtlich des Medians der Eltern (2011 – 2018) über alle FGH Pflanzen oder alle Pflanzen (FGH und Feld) wurde ferner aus der maximalen Pflanzenhöhe (Population A) und der maximalen Pflanzenhöhe und mittleren Blattneigung (Population) vorhergesagt.

Insgesamt konnten Bestandsparameter als Marker für Trockentoleranz genutzt werden. Dementsprechend könnte Trockenstresszucht in Kartoffeln beschleunigt werden, indem Blattneigung, Lichteinfalltiefe, Pflanzenhöhe und Oberflächentemperatur-Abweichung als Marker für Trockentoleranz, insbesondere unter Langzeitstressbedingungen, genutzt werden.

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1. General introduction

1.1 Yield stability under water stress conditions

1.1.1 Drought

Drought stress is the most prevalent environmental factor limiting crop productivity and ultimately the food security (Farooq *et al.* 2009; Basu *et al.* 2016). Global food security is being pressurized by the rapid population growth and drastic changes in the climate (Mancosu *et al.* 2015; Lesk *et al.* 2016). There is increasing evidence that human-induced climate change is changing the precipitation and the hydrological cycles, especially floods and droughts (Trenberth 2008). Warming increases the potential incidence and severity of drought through accelerated land-surface drying by evaporation (Dai *et al.* 2004).

Drought stress occurs when the available soil moisture is insufficient to meet the transpiration needs of the crop (Lobell *et al.* 2011; Tuberosa 2012; Rauf *et al.* 2016). Water moves from the soil into the plant, through the plant and into the atmosphere in response to a series of water potential differences. Water potential is the difference in potential energy between a given water sample and pure water (at atmospheric pressure and ambient temperature). The system that involves the soil, the plant's roots, the xylem, the leaf and the atmosphere is called the soil-plant-atmosphere continuum (SPAC), which is a pathway for the movement of water from the soil into the atmosphere (Blum 2011). The value of the water potential is highest in the soil and decreases along the transpiration pathway (Bittelli 2010). This strong water potential gradient allows water movement through plants and ultimately for transpiration to take place. The SPAC responds primarily to the seasonal, daily and hourly change of net radiation. Other environmental factors are also affect the water potential gradient, such as wind, passing clouds, and the vapor pressure deficit of the air (Blum 2011). The vapor pressure deficit (VPD) of the air is the difference between the actual water potential of the air and the water potential at full saturation (Ficklin and Novick 2017) and it is a combination of temperature and relative humidity in a single value (Eaton and Kells 2009).

Drought stress can be aggravated by soil salinity, physical properties of the soil, and high air temperature (Rauf *et al.* 2016). Severe droughts substantially reduce crop yields through negative impacts on plant growth, physiology, and reproduction (Fahad *et al.* 2017). Drought stress not only limits crop productivity but also reduces the available area for crop cultivation. Out of the

potentially arable area, only 16% are under cultivation (Alexandratos and Bruinsma 2012). Drought primarily affects crops cultivated under rain-fed conditions, which represent 80% of the total cultivated area worldwide (Rockstrom 2003). Globally, the percentage of the cultivated area permanently affected by drought is estimated to be about 28% in sorghum, 20% in wheat, 19% in barley and 19% in maize (Li *et al.* 2009).

1.1.2 Specific effects of drought on Ethiopia as a case study for subtropical Africa

Drought is frequent in East African countries, especially in Ethiopia (Simane *et al.* 2016). Since 85% of the population depends on predominantly rain-fed agriculture (Mersha and Boken 2005; Babikir *et al.* 2015), food production in Ethiopia is highly vulnerable to the effect of climate change.

Ethiopian food production is insufficient already under the current climate condition, mainly because of recurrent droughts. During the last decade, frequency and intensity of drought increased in southern Ethiopia (Mera 2018). A major drought occurred following the 2015 *El Nino* event, resulting in severe acute food insecurity for more than 15 million people (FEWSNET 2015). Ethiopian annual top-40-cm soil moisture was reduced in the recent decades as compared with the average soil moisture between 1981-2014 (Funk *et al.* 2015).

For the future, several models predict that tropical dry areas will become drier (Hantson *et al.* 2017). Figure 1 shows the spatial and temporal pattern of rainfall and temperature in Ethiopia estimated for 2010-2039. Changes in precipitation are predicted to affect the short rainy season between March to June. During the local rainy season, rainfall will decline in a range from 50-150 mm in south-central, eastern, western and southern parts of Ethiopia (Funk *et al.* 2012). Furthermore, the predictions suggest an increase in air temperature by more than 1°C thus increasing the risk of drought-driven yield loss (Zhao and Li 2015; Leng and Hall 2019).

In conclusion, the change in soil moisture due to decreased rain fall and the temperature increase mainly affect the short rainy season, in which more than 62% of the Ethiopian potato is produces (Kolech *et al.* 2015; Gebru *et al.* 2017). Drought tolerance breeding is thus important to adapt Ethiopian agriculture to global change. This is especially the case for potato (see 1.2.5).

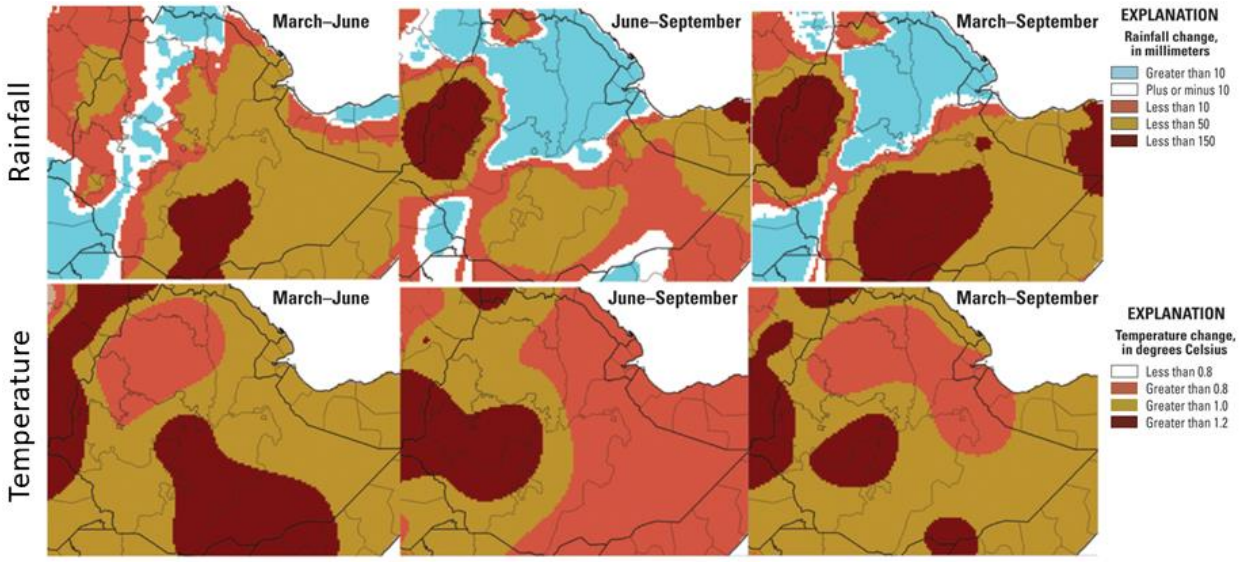


Figure 1. Projected rainfall (top) and temperature (bottom) changes in March–June, June–September and March–September in 2010-2039. The model predicted future values based on the changes observed between 1960 and 2009, assuming persistence of the observed trends. Adapted from (Funk *et al.* 2012) by include rainfall and temperature labels on the left side of the original figure.

1.1.3 Plant strategies to cope with water deficits

Plants respond to drought by inducing several morphological, physiological and molecular mechanisms that enable them to cope with the stress. Drought resistance mechanisms can be grouped into three categories: drought escape, drought avoidance, drought tolerance (Blum 2011; Aslam *et al.* 2015; Fang and Xiong 2015).

Drought escape is defined as the ability of a plant to complete its life cycle before the drought (Manavalan *et al.* 2009). Plants can escape drought stress with rapid development, e.g. early flowering and early maturity. Developmental plasticity and remobilization of assimilates from reserve organs (like stems) to economically important parts (e.g. grain) contribute to the escape mechanism (Turner 1979; Farooq *et al.* 2014). Developmental plasticity permits increased growth during the wet season to produce grains or tubers in spite of limited growth during the stress season (Basu *et al.* 2016). This mechanism of drought resistance minimizes yield loss in terminal stress scenarios. However, short crop duration and early maturity reduce yield (Turner *et al.* 2001). In potato, early maturing (Verkort 1994; Rana *et al.* 2011) and increasing dry matter partitioning to the tubers (Verkort 1994) are means to escape drought that gradually increases towards the end of the growing season.

Drought avoidance is the ability of plants to maintain (relatively) high tissue water content despite low soil water contents (Luo 2010). Various adaptive traits minimize water loss (water savers) and optimize water uptake (water spenders) (Fang and Xiong 2015; Basu *et al.* 2016). Under drought stress, water spenders achieve higher tissue water status by maintaining the water uptake through a well-developed root system (especially increased rooting depth, rooting density or root/shoot ratio). In contrast, water savers use water efficiently by reducing transpiration rate, leaf area and radiation absorption. Some of the mechanisms, through which plants manage drought effects, are increasing investment in the root, reallocation of nutrients from older leaves and higher rates of photosynthesis (Chaves *et al.* 2002).

Drought tolerance is the ability of plants to sustain a certain level of physiological activity at reduced tissue water contents by reducing or repairing stress damage (Morgan 1984; Fang and Xiong 2015). Drought may reduce the cellular water potential, resulting in higher solute concentration, osmosis and turgor loss. Tolerance to low tissue water potential may involve osmotic adjustment, more rigid cell walls or smaller cells, which will help to maintain cell turgor (Obidiegwu *et al.* 2015). Osmotic adjustment is achieved through accumulation of compatible solutes or osmoprotectants called osmolytes. So called compatible solutes can accumulate to high levels without disrupting protein function (Bray 1997). Osmolytes accumulated in response to water stress include mannitol, proline, glycine, betaine, trehalose, fructan, inositol, and inorganic ions (Bray 1997; Fang and Xiong 2015). Osmotic adjustment decreases the osmotic potential and thus helps to maintain turgor (Tessema 2017; Turner 2018).

Other water stress induced compounds like dehydrins, which belong to highly hydrophilic proteins known as late embryogenesis abundant (LEA) proteins (Borovskii *et al.* 2002), also have an important role in preserving the structural integrity of cells subjected to dehydration (Allagulova *et al.* 2003). Besides osmotic adjustment, reactive oxygen species (ROS) scavenging is reported to have an important role in protecting a plant from osmotic stress (Miller *et al.* 2010). ROS are toxic molecules that may cause oxidative damage to proteins, DNA, and lipids (Apel and Hirt 2004). When ROS are produced during water stress, accumulated ROS scavenging enzymes such as superoxide dismutase, ascorbate peroxidase, catalase and peroxiredoxin prevent damage (Miller *et al.* 2010).

1.1.4 Tolerance index

Yield under drought stress is a complex integration of constitutive plant traits and stress-responsive processes which depend on stress intensity, duration and timing with respect to growth stage (Tardieu 1996; Blum 2011). A drought tolerant plant maintains production during mild stress (Tardieu 1996; Tardieu *et al.* 2018). Drought tolerance is most widely expressed as the rate of yield or biomass reduction by stress in comparison to non-stress conditions. Several yield-based drought tolerance indices, based on mathematical relationships between yield under irrigated and drought conditions, have also been proposed to characterize the behavior of genotypes in stress and non-stress environments, and to screen drought tolerant genotypes (Mitra 2001). Some of the tolerance indices used in different studies are Stress Susceptibility Index (SSI) (Fischer and Maurer 1978), the Geometric Mean Productivity (GMP) and the Stress Tolerance Index (STI) (Fernandez 1992), Tolerance index (TOL) and Mean Productivity (MP) (Rosielle and Hamblin 1981), Yield Index (YI) (Gavuzzi *et al.* 1997), Yield Stability Index (YSI) (Bouslama and Schapaugh 1984), Modified Stress Tolerance Index (MSTI) (Farshadfar and Sutka 2002) and Deviation of the Relative starch Yield from the experimental Median (DRYM) (Sprenger *et al.* 2015). However, the different indices have different levels of precision. According to (Fernandez 1992; Zangi 2005), a good selection index allows distinguishing genotypes that express uniform superiority in both stress and non-stress environments.

According to (Sprenger *et al.* 2015), DRYM is more powerful to distinguish between tolerant and sensitive genotypes independent of the yield potential than the more frequently used SSI , GMP and STI indices. In addition to its differentiation power, the interpretation of DRYM value is straightforward. A DRYM value of zero is median tolerance, with tolerant genotypes showing a positive value and sensitive genotypes a negative value. Therefore, the subsequent evaluation of our drought stress experiments was based on the DRYM index.

1.1.5 Breeding strategies for drought tolerant crops

Conventional breeding for drought tolerance

The challenges resulting from global climate change require the breeding of tolerant cultivars adapted to the new conditions (Jarvis *et al.* 2015). However, identification of traits related to tolerance/resistance to abiotic stresses is difficult because they are governed by many genes

(Tardieu 1996), affected by genotype × environment interactions, stress timing and stress intensity (Tardieu 2012).

There are two main approaches to improve economical yield: the empirical approach and the analytical approach (Rauf *et al.* 2016). In the empirical approach, the plant breeder directly selects the breeding material for yield or for yield components. For example, grain yield of wheat was doubled since 1940 throughout Australia through conventional breeding and management (Richards *et al.* 2014). In the analytical approach, yield is improved indirectly through selection for morphological, physiological or biochemical traits associated with yield. The trait(s) should be, as far as possible, easily measurable using non-destructive techniques, and highly heritable (Rauf *et al.* 2016). According to (Lanceras *et al.* 2004; Rauf *et al.* 2016), genetic variances of yield contributing traits generally decrease with the intensity of water stress. When the heritability for yield is low and the heritability for secondary traits is high, and the genetic correlation between secondary trait and yield is high, breeders select tolerant genotypes based on secondary traits (Lafitte *et al.* 2003). As an example: in maize, anthesis-silking interval is more useful for selection of drought tolerance than yield (Rauf *et al.* 2016).

Marker assisted selection for drought tolerance

Most traits contributing to drought tolerance are quantitative and strongly influenced by the environment. The progress of molecular genetics has made it possible to identify regions that are associated with a quantitative trait. The term quantitative trait locus (QTL) applies to genome regions that control these traits. After the development of mapping populations and identification of polymorphic markers, the linkage between the molecular markers and QTLs is established (Rauf *et al.* 2016). Molecular markers have been classified in protein-based markers (such as isozymes) and DNA-based markers, like restriction fragment length polymorphisms (RFLP), amplified fragment length polymorphisms (AFLP), simple sequence repeats (SSR) and single nucleotide polymorphisms (SNP).

These molecular markers can then be used for marker-assisted selection (MAS). In various crop species such as wheat, rice, cotton, oil seeds and forage species, MAS has been shown to be a useful additional breeding tool to enhance yield in dry environments (Venuprasad *et al.* 2009).

Microarray techniques have been widely exploited to understand the differential pattern of gene expression. Drought stress increases the expression of different genes in various species like *Arabidopsis thaliana* (Seki *et al.* 2002; Huang *et al.* 2008), potato (Sprenger *et al.* 2018), wheat

(Aprile *et al.* 2009) and maize (Gullì *et al.* 2015). In drought-responsive genes like *GmSYP2*, expression increases under drought stress (Chen *et al.* 2019). Drought-inducible genes like RAB18 are exclusively express under drought stress (Pieczynski *et al.* 2018).

Transgenic breeding for drought tolerance

Transgenic breeding is genetic improvement of plants through biotechnology (Low *et al.* 2018). Transgenic plants are plants that have had their genomes modified through genetic engineering techniques either by the addition of a foreign gene or removal of a certain detrimental gene (Jhansi Rani and Usha 2013) or by gene editing (Mohanta *et al.* 2017). Genetically modified organisms (GMO) have been successfully created in various crop species. Transgenic approaches have mainly concentrated on plant survival rather than plant productivity under drought stress (Ahanger *et al.* 2017; Low *et al.* 2018).

Drought tolerance of rice and wheat can be improved by overexpressing the transcription factor *OsNAC14* (Shim *et al.* 2018) and a synthetic bacterial cold shock protein gene (*SeCspA)* (Yu *et al.* 2017). Transformation of tomato with gibberellic acid methyl transferase (*ATGAMT1*) (Nir *et al.* 2014) and alfalfa with *GsWRKY20* (Tang *et al.* 2014) improves drought tolerance in the respective crops. Until recently, GMO with increased drought tolerance rarely produced higher yields compared to their parent genotypes in agronomic settings. However, the transgenic maize variety MON 87460, which overexpresses the cold shock protein B, has been released for cultivation in water-deficit prone areas of the US (Chang *et al.* 2014). The other successful story is delivering drought-resistant GMO canola plants to farmers in Canada and Australia (Blum 2014).

1.1.6 Phenotyping

Phenotyping is the quantitative description of the plant's anatomical, developmental, physiological and biochemical properties (Walter *et al.* 2015). Linking genomics with high-throughput phenotyping may improve the efficiency of selection during molecular breeding (Montes *et al.* 2007; Vadez *et al.* 2015; Marko *et al.* 2018; Peirone *et al.* 2018). Thus, phenotyping is an important aspect of crop breeding. Phenomics is the study of phenotypes by high-throughput technologies including biochemical and imaging methods (Lanktree *et al.* 2010). High-throughput phenotyping techniques allow to quantify complex traits - such as growth, development, tolerance, resistance to stresses, yield - in one run, repeatedly through the plant's life-span, efficiently and with high accuracy in large populations in breeding and genetics studies (Chen *et al.* 2014; Humplik *et al.*

2015; Marko *et al.* 2018). However, phenotyping is still lagging behind genotyping (Furbank and Tester 2011; White *et al.* 2012; Fahlgren *et al.* 2015a; Singh *et al.* 2018).

Different high-throughput phenotyping platforms (HTPP) have been set up in several countries (Pratap *et al.* 2015). These HTPPs are mostly run by large seed companies and advanced crop research institutes around the world. Some of the popular HTPPs around the world include the Biotron Canada, the Julich Plant Phenotyping Centre, LEPSE-Montpellier Plant Phenotyping Platform, PhenoFab Wageningen, Phenopsis Arabidopsis Platform, INRA, and the Australian Plant Phenomics Facility (Pratap *et al.* 2015). Most of the HTPPs were built in Europe (https://www.plant-phenotyping.org/IPPN-Participating-Organisations). The major plant phenotyping centers are represented by the international plant phenotyping network (IPPN). IPPN has different regional partners (https://www.plant-phenotyping.org/IPPN-home): European Phenotyping Plant Network (EPPN), The European Infrastructure for Multi-Scale Plant Phenomics and Simulation (EMPHASIS), The North American Plant Phenotyping Network (NAPPN) and Nordic Plant Phenotyping Network (NPPN).

Depending on the research objective, HTPP facilities use various camera systems to generate plant images or information by capturing signal from the visible (VIS) and infrared (IR) spectrum of light (Fahlgren *et al.* 2015b). VIS cameras are used to measure the morphological, geometric, and color properties of plants. Infrared, near-infrared (NIR), thermal infrared (TIR) and hyperspectral cameras are used to detect leaf water content, leaf temperature or indices related to stress response in plants.

Laser scanner

Digital growth analysis is one of the least complicated and most useful methods for quantitatively determining stress tolerance (Furbank and Tester 2011). Digital growth analysis can be done based on VIS imaging or based on surface scans by lasers. The laser scanner system PlantEye (PlantEye, Phenospex B.V., Herleen, The Netherlands) uses an NIR laser beam to acquire 3D point clouds of plant surfaces. The system projects a narrow, oblong NIR laser beam on the plant and captures the light scattered by the plant surface with an integrated camera (Kjaer and Ottosen 2015). A carrier moves the laser scanner with constant speed over the scanning field. The scanning field is divided into a number of subfields. From each subfield image, depth profiles of the x-z plane are compute. These depth profiles are arranged as histograms or displayed as a raw 3D point cloud of the subfield canopy. Automatic segmentation of the 3D point cloud estimates different morphological

parameters like leaf area, plant height and digital biomass. Correlation between digital estimation of leaf area and that obtained by destructive harvesting on cowpea, peanut and pear millet achieves r^2 values of 0.80, 0.82, and 0.96, respectively (Vadez *et al.* 2015). This method efficiently estimates plant parameters in a diurnal cycle (Fahlgren *et al.* 2015b) without interacting with the photosystem or the plant's clock (Kjaer and Ottosen 2015). Laser imaging has been used in maize (Reis 2013), sugar beet and wheat ears (Paulus *et al.* 2014a), triticale (Busemeyer *et al.* 2013), barley (Paulus *et al.* 2014b), rice and soybean (Fang *et al.* 2009), and canola (Kjaer and Ottosen 2015) to determine canopy parameters and physiological parameters.

Infrared thermometry

Infrared thermometry measures an object's surface temperature without touching it by sensing the long-wave infrared radiation emitted from the object. The measurement is based on the relationship between emitted radiation and surface temperature (Blum 2011). The total energy radiated per unit time per unit surface area of a blackbody is proportional to the fourth power of the temperature of the body (Stephen Boltzmann law). Canopy temperature measurements by IR thermometry was used for the first time by Blum (Blum *et al.* 1982) as a screening technique for dehydration avoidance in a wheat breeding program.

Canopy temperature (CT) is a parameter that can be monitored nondestructively on a whole-plant level to monitor the plant's response to environmental stresses including drought (Blum *et al.* 1982). The relationships between CT, air temperature and transpiration is not simple, involving atmospheric conditions (VPD, air temperature and wind velocity), soil parameters (mainly available soil moisture) and plant features (canopy size, canopy architecture, and leaf adjustments to water deficit) (Mahan *et al.* 1995; Blum 2009). Moreover, CT is best assessed at full canopy stage with high VPD conditions associated with low relative humidity and warm air temperature (Blum 2009; Tuberosa 2012). Relatively low CT in drought-stressed plants indicates a better capacity for taking up soil moisture (Lopes and Reynolds 2010) and thus maintain a better plant water status by various plant adaptive traits (Burke *et al.* 1988; Balota *et al.* 2008). CT measurements have been widely used to study the drought response of various crops (Stark *et al.* 1991; Mutava *et al.* 2011; Gerhards *et al.* 2016; Hirut *et al.* 2017). Canopy temperature depression (CTD) is expressed as the difference between air and foliage temperature (Jackson *et al.* 1981; Balota *et al.* 2007; Mahmud *et al.* 2016). CTD correlates more closely with stomatal aperture than CT. The underlying concept is that a decrease in plant water status leads to a reduction in leaf

transpiration as a result of active regulation of stomatal aperture, consequently increasing the leaf temperature due to a reduced evaporative cooling (Inoue *et al.* 1990). In contrast, a well-watered plant will have a lower temperature relative to the ambient air temperature. Under high VPD and low RH condition, the difference between canopy temperature and air temperature may reach 12^{o}C (Duffková 2006). CTD is a highly integrative trait resulting from the effects of several biochemical and morphophysiological features acting at the level of root, stomata, leaf, and canopy (Tuberosa 2012). Under drought, canopy temperature depression is the single most drought-adaptive trait contributing to a higher performance. CTD is a highly heritable secondary trait in drought tolerance breeding (Olivares-Villegas *et al.* 2007). High stomatal conductance cools leave and thus leads to CT less than air temperature. This results in a negative correlation of CTD with stomatal conductance, photosynthesis, the maximal quantum yield of primary photochemistry and chlorophyll content (Roohi *et al.* 2015). CTD is widely used to study the drought response of various crops (Singh *et al.* 2014; Mahmud *et al.* 2016; Kumar *et al.* 2017; Thapa *et al.* 2018). However, CTD is influenced by several factors like the capacity of the crop plant to extract water, transpiration differences, phenological stages of crop growth (Kumar *et al.* 2017), individual environments and the species (Thapa *et al.* 2018).

1.2 Potato as a system for drought tolerance breeding

1.2.1 Potato origin and distribution

Potato (*Solanum tuberosum* L.) belongs to the Solanaceae family and to the large and diversified genus Solanum. The genus Solanum contains approximately 2000 species, including over 100 tuber-bearing species, which form a polyploidy series ranging from diploids to hexaploids (Magoon *et al.* 1962). Cultivated potato varieties are diploid or tetraploid (4n=48) and wild species are diploid to hexaploid (Hawkes 1994).

Potato is native to the Andes Mountains in Chile, Peru and Bolivia in South America, where it has been cultivated for about 2400 years (Acquaah 2012). It was later introduced into Europe in 1570 and then taken from Europe and cultivated in all corners of the globe (Hawkes and Francisco-Ortega 1993). Potato was introduced to Africa in the mid-19th century (Haverkort 1990) and to Ethiopia in 1858 by the German botanist Schimper (Pankhurst 1964). Over the following decades, farmers in Ethiopia's highlands began cultivating the new tuber - known as ‘Denech’ in Amharic. At present, potato is the most widely grown root and tuber crop in Ethiopia (CSA, 2018).

1.2.2 Importance of potato

Potato is the fourth most important food crop in the world after maize, wheat, and rice in terms of volume of production (FAOSTAT, 2017). In 2017, worldwide potato production was more than 388 million tones. Out of these, Asia alone produced 50% (FAOSTAT, 2017). Potatoes produce more quantity food per unit of land and water than any other major crop (Villamayor 1984). Around 1.3 billion people worldwide eat potato on a regular basis (Devaux *et al.* 2020). Potato also constitutes part of the diet of half a billion consumers in the developing countries (Mondal 2003). In addition to its importance as human food, potato is an important commodity in the starch industry (Sawicka and Gupta 2018). The tubers contain starch, minerals, protein, antioxidants and vitamins (Hussain 2016). The high nutrient content, ability to adapt to marginal environments, relative ease of cultivation, and low cost and high productivity make potatoes one of the principal and most important sources of food and income for developing countries (Gildemacher *et al.* 2009). Consequently, the share of developing countries to the world potato production has increased over the last decades and exceeded the production of the developed word in 2005 (FAO, 2008).

1.2.3 Potato production in subtropical Africa: case study in Ethiopia

In 2017, Ethiopia was the 9th top potato producing country in Africa (POTATOPRO 2019). Ethiopia has possibly the highest potential for potato production of any country in Africa as 70% of the country's arable land is potentially suitable for potato cultivation (FAO, 2008). However, presently only 2% of the potential area in Ethiopia is used for potato production (Tufa *et al.* 2015). Potato is a high-potential food security crop in Ethiopia, because it is grown by many small-holders in the country. For example, in the 2017/2018 season, it was grown by more than one million households on 7000 ha (CSA, 2018). Potato is an important crop to fill the gap in food supply during the 'hungry' months July and August before grain crops are harvested (Helen 2016). Besides its use as a food security crop, potato is a source of cash income in the densely populated highlands of Ethiopia (Gildemacher *et al.* 2009). In Ethiopia, potato is grown in four major areas: the Central, the Eastern, the North-Western and the Southern regions (Helen 2016) (see Figure 2). The climatic zones of potato growing areas are *Woina Dega* and *Dega.* The *Woina Dega* climate zone occurs in regions between 1500 and 2300 m a.s.l. and has an average annual temperature of 17.5 – 20.5°C with an average annual rainfall of 800 – 1200 mm. The *Dega* climate occurs in regions above 2300 m a.s.l. and has an average annual temperature of 11.5 – 17.5°C with an

average annual rainfall of 900 – 1200 mm (Varshney *et al.* 2005). More than 62 % of potato is grown during the short rainy (*Belg*) season in order to fill the food gap in July and August. Furthermore, avoiding the main rain season *Kiremit* (Figure 3) reduces the late blight risk (Kolech *et al.* 2015; Gebru *et al.* 2017).

Potato productivity in Ethiopia is much lower (13.9 tones/ha) than the current world average (20 tones/ha) (FAOSTAT, 2017). The low productivity results from the lack of good quality seed tubers and improved cultivars (Tufa *et al.* 2015), insufficient agricultural inputs (fertilizer, pesticides), poor agronomic practices (Dersseh *et al.* 2016), abiotic stress (Kolech *et al.* 2015; Hirut *et al.* 2017) and biotic stress (Guchi 2015; Abewoy 2018). Drought is one of the main abiotic stresses in the subtropics especially in Ethiopia because of erratic rainfall and the lack of irrigation facilities. Breeding drought-resilient germplasm for Ethiopia is crucial especially in drought-sensitive crops like potato.

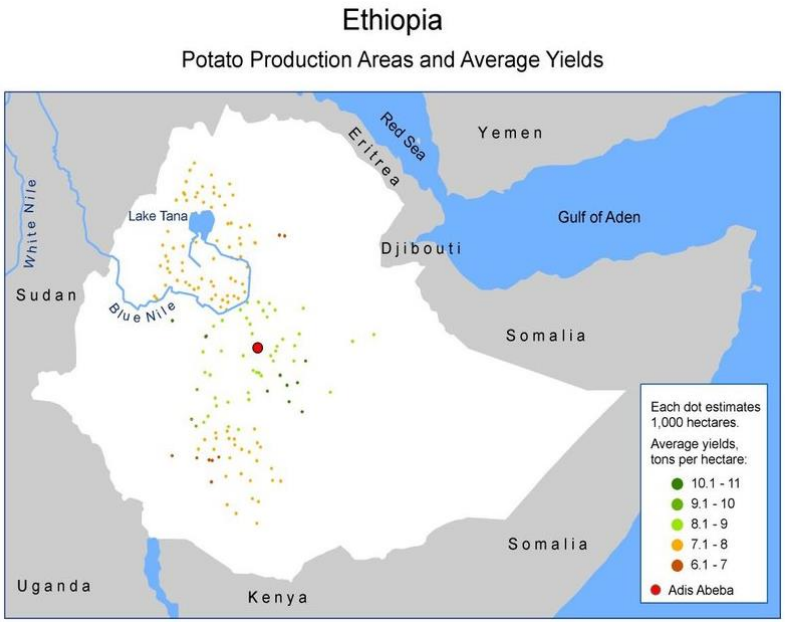


Figure 2. Potato production areas and average yields in Ethiopia (Hirpa *et al.* 2010).

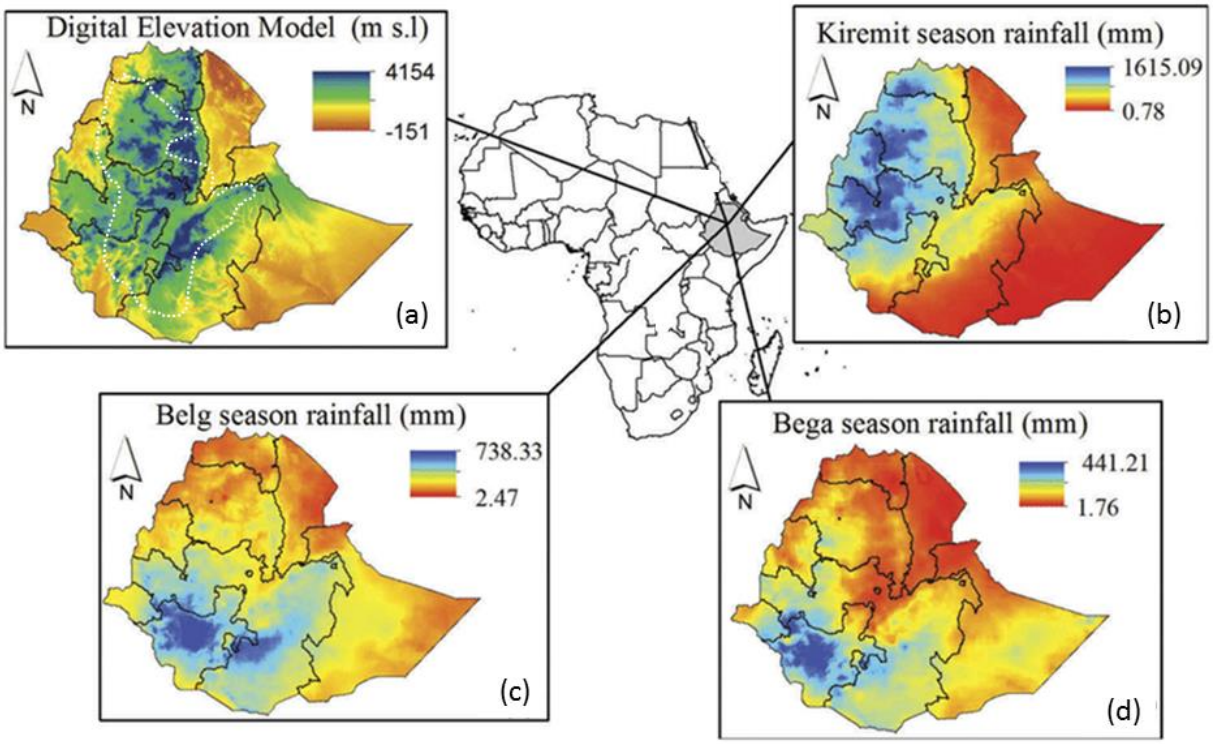


Figure 3. Ethiopian topography and potato growing areas (inside broken line) (a) and seasonal rainfall distribution of *Kiremit (Jun-Sep)* (b), *Belg (Feb-May)* (c) and *Bega* (*Oct-Jan*) (d). Note: seasonal rainfall is calculated using long-term CHIRPS rainfall data from 1983 to 2015. Modified from (Bayissa *et al.* 2019) by demarcating the potato growing areas in digital elevation model.

1.2.4 Drought effects on potato

Potato is best suited to cool and humid climate conditions. Potato grows best at 14 - 22^{o}C (Struik 2007b) . However, optimum temperature range varies depending on the developmental stage of the potato plant (Struik 2007b) and the photoperiod (Wheeler 2006). Potato is drought-sensitive (van Loon 1981; Schafleitner 2009; Obidiegwu *et al.* 2015; Romero *et al.* 2017) because of its shallow and low-density root system (Gregory and Simmonds 1992). About 85 % of the roots are concentrated in the upper 0.3 m of the soil and the maximum root pentation depth is about 1 m (Gregory and Simmonds 1992). Thus, potato plants extract less of the available water from the soil than other crops (Weisz *et al.* 1994).

The magnitude of drought effects on potato depends on the phenological timing, duration and severity of stress (van Loon 1981; Obidiegwu *et al.* 2015). Drought reduces plant growth (Deblonde and Ledent 2001), shortens the phenological development (Obidiegwu *et al.* 2015) and decreases the number and size of tubers (Schafleitner *et al.* 2007b). Water-limited conditions reduce leaf growth (Walworth and Carling 2002; Lahlou *et al.* 2003), leaf size (Jefferies and Mackerron 1987), leaf area index (LAI) (Lahlou *et al.* 2003; Schafleitner *et al.* 2007b) and ground

coverage (Ojala *et al.* 1990). Water deficiency increases the rate of leaf senescence (Fleisher *et al.* 2008). Drought events reduce nitrate reductase activity, which consequently affects nitrogen use efficiency (Schafleitner *et al.* 2007a). Water stress also makes the plants more susceptible to pest and diseases, such as early blight caused by *Alternaria solani*, common scab caused by *Streptomyces scabies* and powdery mildew (Nolte *et al.* 2003).

Under drought, a reduction in radiation interception as a result of reduced canopy expansion (Jefferies and Mackerron 1987) reduces photosynthetic rate and eventually tuber yield. A prolonged soil moisture deficit results in small tubers, while intermittent water stress produces tubers with secondary growth (Nolte *et al.* 2003). Water shortage also increases the content of reducing sugar in the stem, and promotes quality problems like tuber cracking and malformation, surface abrasions, hollow heart, brown center, internal brown spot, vascular discoloration or bruising, degradation of starch in the tuber stem end and accumulation of total glycoalkaloids (Papathanasiou *et al.* 1999). Under drought stress, potato accumulates the compatible solutes proline, inositol, raffinose, galactinol, and trehalose (Evers *et al.* 2010; Obidiegwu *et al.* 2015; Sprenger *et al.* 2016; Rudack *et al.* 2017).

The yield loss is highest when water deficiency occurs during tuber formation (Deblonde and Ledent 2001; Aliche *et al.* 2018) or tuber bulking (van Loon 1981) as many physiological traits are most sensitive during these stages (Rudack *et al.* 2017). Water shortage at plant establishment also affects final yield and recovery potential of the plant (Deblonde and Ledent 2001). Drought-resistant genotypes have higher root mass, high leaf/stem ratio (Deguchi *et al.* 2010), high harvest index (Deblonde and Ledent 2000; Deguchi *et al.* 2010), high water use efficiency (WUE), increased root elongation (Anithakumari *et al.* 2012) and rapid recovery on re-watering (Bansal and Nagarajan 1987; Anithakumari *et al.* 2012). Since potato is a drought-sensitive crop, adaptation by breeding is important to sustain future potato production.

1.2.5 Potato breeding for drought tolerance

Drought tolerant varieties can improve yield stability in drought-prone areas (Hijmans 2003; Chapman *et al.* 2012; Jarvis *et al.* 2015). Furthermore, the use of drought tolerant varieties may reduce the competition for limited freshwater resources (Anithakumari 2011). However, breeding for drought tolerance can be complicated by simultaneous occurrence of other abiotic and biotic stresses (Atkinson and Urwin 2012), low heritability of drought tolerance, genotype × environment interaction, and the lack of suitable selection tools for the traits of interest (Langridge and Reynolds

2015; Çalişkan 2016). The genotype × environment interaction (Obidiegwu *et al.* 2015) makes drought tolerance breeding complex, as genotypes have to be bred for specific target environments.

Potato could be improved through conventional or genetic approaches (Bradshaw 2007; Anithakumari 2011). Conventional breeding in potato involves evaluation and selection based on several traits (yield and yield components and other secondary traits) within the clonally propagated progeny of a cross between two tetraploid clones. These clones can be existing cultivars or clones with introgressions from wild species. Many wild species, which can be crossed directly with cultivated potato, can serve as a great source of genetic variation for drought tolerance (Plaisted *et al.* 1989). The progress of gene transfer is impeded by linkage between desirable and undesirable genes from the wild species (Bethke *et al.* 2017). In addition, genetic improvement of cultivated potato is hampered by its high level of heterozygosity, tetrasomic inheritance and incompatibility barriers (Muthoni *et al.* 2015). Conventional breeding is thus time consuming and the outcome is hard to predict (Pacilly *et al.* 2016).

The availability of the potato genome sequence provides a great resource to develop molecular markers and identify QTLs linked to these traits (Xu *et al.* 2011). Modern drought tolerance breeding in potato exploits natural genetic variation to map tolerance QTL and establish marker-assisted selection (Bradshaw 2007; Anithakumari 2011) or even introduce new genes from species that cannot be crossed with potato (Bradshaw 2007; Si *et al.* 2018).

So far, QTLs linked to drought tolerance have been identified in diploid potato mapping populations (Anithakumari *et al.* 2011; Anithakumari *et al.* 2012; Khan *et al.* 2015; Tessema 2017). However, selection markers have not yet been validated (Çalişkan 2016). In European potato cultivars, (Sprenger *et al.* 2018) predicted potato yield stability by using a combination of transcript and metabolite markers.

Pest resistance and quality traits have been improved through novel gene transfer from another organism (Bradshaw 2007; Si *et al.* 2018). However, similar approaches to increase drought tolerance seem to be more challenging. There are reports indicating that transformation of the transcription factor CaPF1 gene from *Capsicum annuum (Youm et al. 2008)*, the ScCBF1 gene from *Solanum commersonii* (Pino *et al.* 2013), the ibMyb1 gene from *Ipomoea batatas* (Cheng *et al.* 2013) and the StDREB1a gene from potato (Bouaziz *et al.* 2013) into potato improve drought tolerance under greenhouse conditions.

Objectives of this thesis

In order to improve potato yield, we need to identify best production practices and develop new potato cultivars that best fit in the predicted climate change. Yet the lack of data on the precise mechanisms of plant resistance to abiotic stress and the subsequent ability to predict future outcomes constitute a major knowledge gap. Therefore, we aim to find a breeding approach that works independently of extensive genomic information of the crop by identifying phenotypic markers.

The general aim of this thesis is to identify morphological and physiological markers for drought tolerance in potato that could be easily measured under field conditions and thus speed up variety development under stress conditions.

Specific objectives

- To determine drought tolerance in potato germplasm as the dependent parameter of the prediction model.
- To identify suitable morphological and physiological parameters for drought tolerance prediction by automatic phenotyping with laser scanner and IR thermometry
- To determine the appropriate conditions for the measurement of the predictive parameters with respect to the diurnal cycle, the developmental stage and the environmental conditions.

To achieve this aim, the following questions were addressed:

- Which morphological and physiological traits differ between sensitive and tolerant genotypes during stress?
- How can these morphological and physiological markers be easily measured in early developmental stages and under field conditions?
- Are those morphological and physiological markers stable over different environments and developmental stages?
- Are those morphological and physiological markers specific to a population or do they work in different populations?

2. Materials and methods

2.1 Plant materials and experimental conditions

Plant materials

Drought tolerance (population A and B) and recovery potential (population B) were studied in two populations of potato (*Solanum tuberosum* ssp. *tuberosum* L.) genotypes (supplementary Table S1). Populations included progenies from two crosses between tolerant and sensitive cultivars and commercial varieties. Commercial varieties and the seeds from crosses between the cultivars Euroresa x Albatros and Albatros x Ramses were obtained from breeding companies. In the project VALDIS, 200 seedlings of both crosses were evaluated for drought tolerance, genotyped by Prof. Horn ((University of Rostock), manuscript in preparation) and maintained in tissue culture facilities of MPI-MP. The parent Albatros (At) identified as tolerant lines and Euroresa (Es) and Ramses (Rs) as sensitive parent lines. From this population, 60 progeny lines with different tolerance levels (supplementary Table S1) were selected based on their relative tuber starch yield (24 tolerant lines (PPt)) in three stress experiments or on the tolerance predicted from metabolite and transcript measurements (23 tolerant lines (MPt) and 22 sensitive lines (MPs)) (Haas et al. 2020). In PPt sub-population, the share of AxR and ExA progenies were about 58 % and 42 %. In MPt sub-population, 83 % of lines were from AxR progeny whereas in MPs sub-population, 95% of lines were from ExA progeny. There were three shared lines between PPt and MPs sub-populations and six shared lines between MPt and PPt sub-populations (Haas et al. 2020). The population A contained 64 different genotypes, namely the three parent cultivars, 60 progeny lines, and the reference genotype Desiree.

The population B included 21 different genotypes, namely ten progeny lines, three parent cultivars and reference genotype Desiree from population A plus seven commercial cultivars. Fourteen genotypes were represented in both populations. This allowed to link the two populations and generated more data in different environmental conditions (season). Plus, to this, more predictive parameters were generated by advanced software version used in Pop B under different treatment conditions.

Pot/screenhouse experiments

Pot experiments were performed on the populations A and B in the polytunnel screenhouse (FGH) at the MPI-MP in Potsdam-Golm, Germany in 2015 to 2018 (Table 1). The experimental design was a split-plot randomized complete block design. Water supply levels were the main plot treatments and genotypes were the subplot factors.

In population A, plants were established from tissue culture plantlets in 2015 and 2016. After two weeks of acclimatization, plantlets were transferred to big-bags (30 l) filled with a fertilized potting substrate (2/3 white peat, 1/3 Quarz sand, 1kg/m³ Novatec Classic, pH Wert 5,5-6,6) (Fritz Kausek GmbH & Co KG, Germany).

Twelve plants per genotype were randomized within four blocks (three plants per block). Two blocks received optimal water supply, two blocks drought stress regime. After 12 (2015) or 27 (2016) days of optimal water supply, irrigation was reduced to 50 % of the optimal water supply in the drought stress blocks (see Table 1).

Plants were irrigated through manually controlled drip irrigation system. The optimally irrigated (cc) block received water as required by the plant and determined from previous trials for normal growth at the site. Soil water content, environmental and seasonal conditions were considered in deciding on timing and amount of irrigation. Stress treatments (ss) received 50 % of the control irrigation. Altogether, plants from the stress block got 39 l per plant (2015) or 40 l (2016) water in the screenhouse experiment, whereas the control plants got 81 l (2015) or 74 l (2016) (Figure 4).

Table 1. Experimental setup: trial type, location and number of genotypes, start of treatment and end date of trial in 2015-2018, Golm, Germany (52°23’55’’N13°03’56’’E).

Trial type	Trial-Id	Culture	Location	T	Rep	Pl	Number of lines	Start date	Stress initiation date	Treatment switching date	End date
Big-bag	P2015	72247	FGH	2	6	1	64	9.4.15	21. 4.15		21.7.15
Big-bag	P2016	76240	FGH	2	6	1	64	13.4.16	9. 5.16		18.7.16
Big-bag	P2017	81251	FGH	4	7	1	21	11.4.17	5.5.17	25. 5.17	21. 7.17
Big-bag	P2018	85178	FGH	4	7	1	20	17.4.18	8. 5.18	24. 5.18	9. 7.18
Field	F2015	72275	Field	2	3	5	64	22.4.15	8. 6.15		17.8.15
Field	F2016	76219	Field	2	3	5	64	21.4. 16	12. 6.16		9.8.16
Field	F2017	81256	Field	4	2	5	21	25. 4.17	28. 5.17	17. 6.17	14. 8.17
Field	F2018	85442	Field	4	2	5	21	2. 5.18	25. 5.18	09. 6.18	2. 8.18

Culture - experiment reference Id in the MPI database limsdb2 (http://dx.doi.org/10.1186/1746-4811-4-11). T- number of treatment levels:2 (cc and ss) and 4 (cc, ss, cs and sc). cc - optimal water though out the plant growth, ss - water stress during the entire treatment period, cs - optimal water until flower initiation then switch to stress and sc - stress until flower initiation then switch to water stress. Rep - number of replicates. Treatment switching date - date of switching optimal water to reduced irrigation water in cs block and stress to optimum water level in sc block. Pl - number of plants per replicate. Number of lines - number of genotypes used in the experiment including parents, progeny and commercial cultivars. Start date - date of planting into final pot size or field. End date - date of shoot destruction.

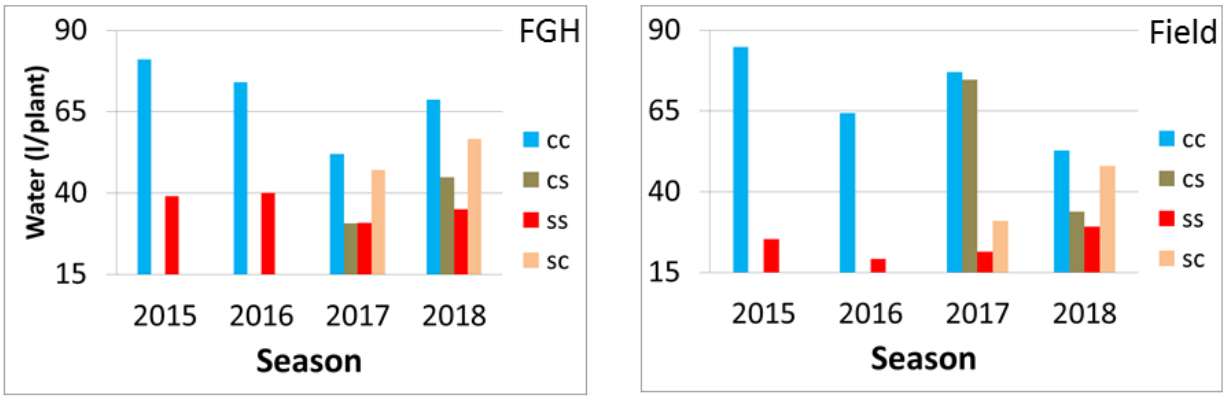


Figure 4. Total volume of water (irrigation and precipitation) received by the plant in the screenhouse and field experiment, 2015-2018. In the 2017 field experiment, cc and cs treatment received 67 l of rainfall (85% of the total volume of water) (see supplementary Figure S1).

Population B was tested for drought tolerance and recovery in two independent experiments in 2017 and 2018 in the screenhouse. Plants were established from seed tubers in the same potting substrate and pot size as described before. Plants were subjected to four treatments: cc, cs, ss, and sc (see Table 1). In the treatment cc, plants received optimal water supply for the entire cultivation period, in the treatment ss, plants were water-stressed during the entire treatment period. However, at the time of switching the treatments (cs and sc blocks), the ss block was irrigated to optimal soil water content for three to four days. In the cs treatment, plant received optimal water supply until flower initiation, after which they were switched to stress conditions. Treatment sc was switched from stress to control conditions at flower initiation. After 25 (2017) and 22 (2018) days of optimal water supply, irrigation was reduced in the ss and sc blocks. The treatment switch from optimum to stress in cs block and from stress to optimum in sc block was done 45 (2017) or 38 (2018) days from planting (DFP). The total amount of water received by the plants in the different treatments is depicted in Figure 4.

In both populations, soil moisture and temperature in pots were monitored by plantCare soil moisture sensors (PlantCare Ltd. Switzerland).

The soil moisture sensor was used to measure temperature and moisture content of soil in pots (pot experiment) and at a depth of 15 to 20 cm in the ridges of field experiments. The daily soil moisture content is presented in Figure 5, the soil temperature in supplementary Figure S2. As expected, the soil moisture in stress blocks was lower than in the optimal water blocks. As a result, the soil temperature of the stress blocks was higher than under optimal water conditions.

Plants were scored for developmental stage and plant height from plant establishment until full canopy stage according to the stage table of the Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie (BBCH) scale (Hack *et al.* 1997). Plant growth time was expressed as thermal time with a base and maximum limit temperature of 6ºC and 30ºC respectively (Data from M. Haas, Max Planck Institute of Molecular Plant Physiology). Shoots were removed when the target thermal time had been reached. Tubers were harvested and their numbers, sizes, and fresh weight were determined. Tuber starch content was measured gravimetrically. Starch yield was calculated as the product of tuber mass and tuber starch content. Finally, tubers were disinfected with MENNO solution (2%) and stored at 5ºC in cold store to be used as planting material in the field experiments.

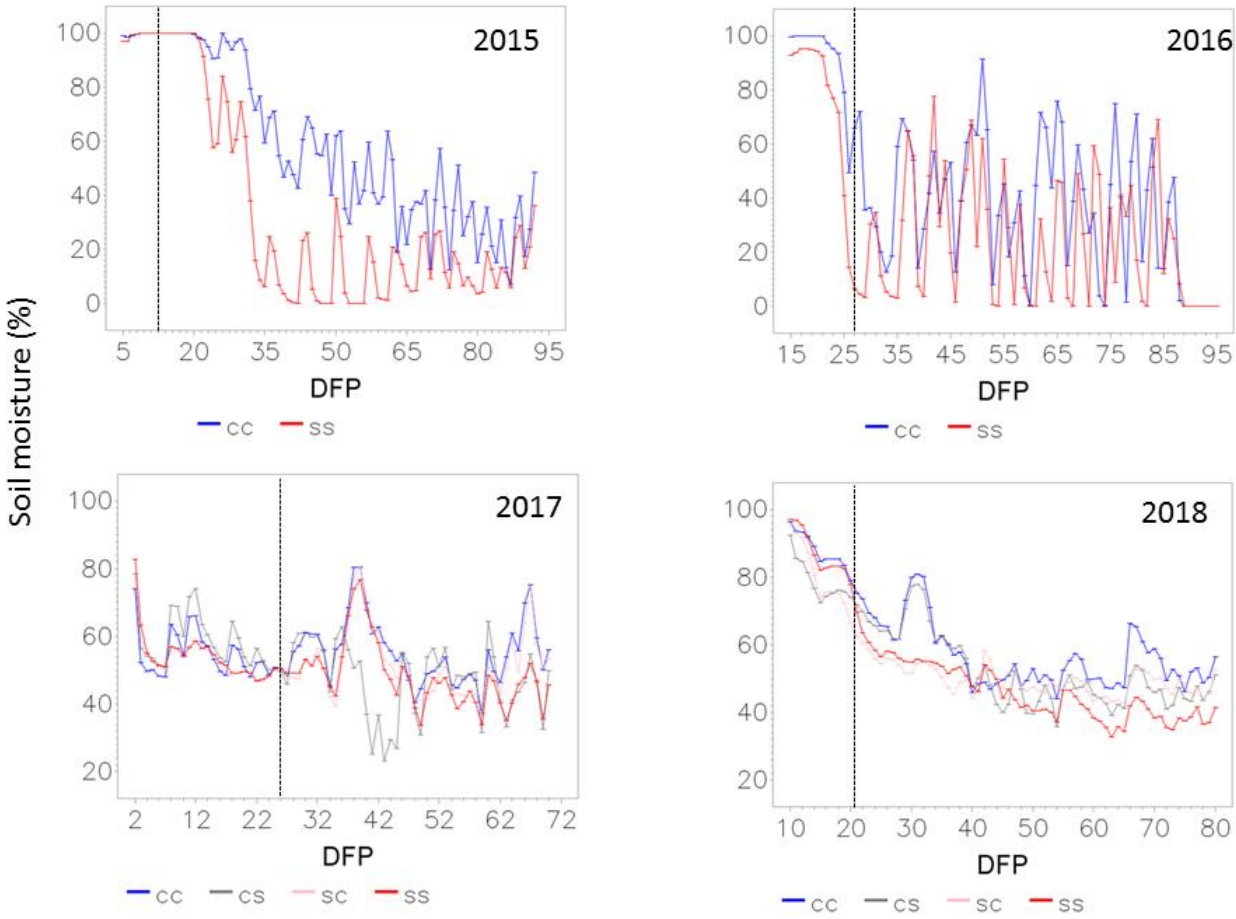


Figure 5. Daily mean soil moisture in treatments performed in the screenhouse in 2015 to 2018 depicted against plant age in days from planting. Vertical broken line indicates stress initiation date.

Field experiments

Four field experiments were carried out at the site of Potsdam-Golm, Germany as split-plot experiments with five plants per plot in 2015 to 2018 (see Table 1). Treatments and genotypes were the same as with the screenhouse experiment.

In the field experiments, plots were arranged in four blocks, half of which were planted under a rain-out shelter. Within blocks, plots were separated by one plant of a red-skinned cultivar. The two marginal ridges and the beds at both ends were planted with buffer plants. Based on soil test result, 201, 93, 109 and 109 g/m^2 NovaTec classic (12-18-16+3) fertilizer were added to the soil in 2015, 2016, 2017, and 2018, respectively. Seed potatoes were planted manually into the ridges at a depth of ~10-15 cm, with a spacing of 30 cm within and 75 cm between rows. Plants were drip-irrigated from the top of the ridges with about 11 mm per m^2 water during the night when plants in control plots showed signs of decreased turgor at noon. Drought stressed plants were irrigated when they showed signs of water stress a few hours after sunrise. The total amount of water applied per growth season (Figure 4) and daily soil moisture are presented in Figure 6. The daily rainfall per meter square (supplementary Figure S2) and soil temperatures are presented as supplementary Figures (supplementary Figure S3 and supplementary Figure S4). The figures, indicates that soil moisture of the stress blocks was lower than in the optimal water blocks and vice versa for soil temperature.

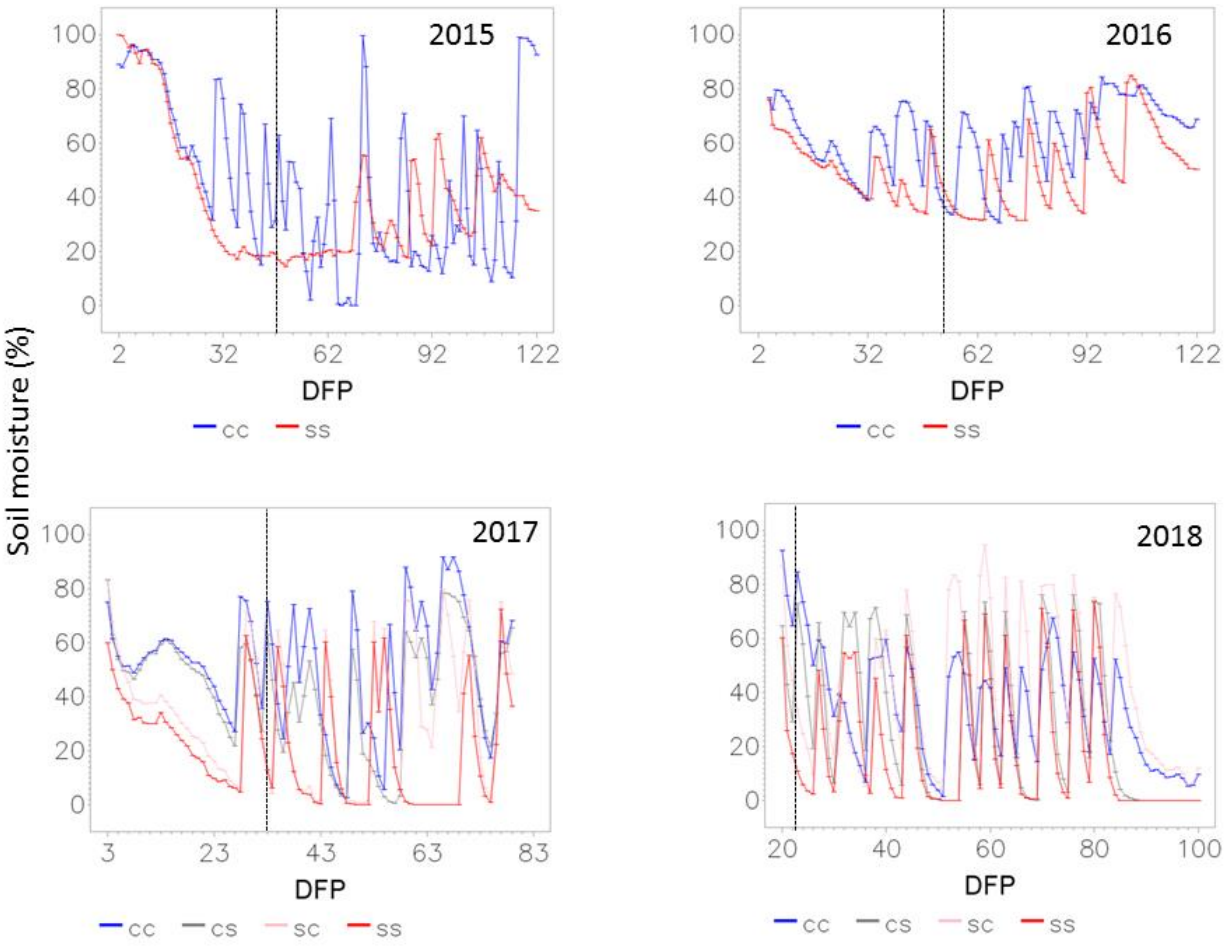


Figure 6. Daily mean soil moisture of treatments under field condition in 2015 to 2018. Vertical broken line indicates stress initiation date.

For population A, three replicate plots (five plants per plot) per genotypes were planted in 2015 and 2016. After 47 (2015) and 50 (2016) days of optimal water supply, irrigation was reduced in the drought stress blocks. In population B, two replicate plots with five plants per plot were grown for each genotype in 2017 and 2018. The reference and parent cultivars were replicated in four plots in 2018 experiment. After 33 (2017) and 22 (2018) days of optimal water supply, irrigation was reduced to 50 % of the control level in the ss and sc blocks. Water supply was switched from optimum to stress in the cs block and from stress to optimum in the sc block 53 DFP in 2017 and 38 DFP in 2018.

The ss treatment was planted under the rainout shelter in all field experiments. In population B, sc was planted under the shelter in 2017, cs in 2018 (see Figure 7).

Pests and diseases were controlled as required by pesticide application. Shoots were killed by herbicide (Reglone 200 g/l Diquat) application at the onset of haulm (above ground plant part) senescence. Phenotyping of harvested tubers was done as described for the pot experiments.

Figure 7. Phenotyping platform and experiments. (a) Laser scanner and infrared thermosensor (IRT) (b) Weather station with sensor for temperature and relative humidity (c) Field experiment with rainout shelter and (d) without rainout shelter 22 Jun, 2017.

Meteorological measurements

In all experiments (FGH and field), meteorological data were recorded automatically by GP2 data logger (Figure 7). In the FGH experiment, temperature and relative humidity were recorded every 15 min at 2 m above ground in the polytunnel compartment where the experiment was conducted. In field experiment, temperature and relative humidity, light radiation, wind speed and rainfall

were recorded every min with sensors situated 2 m above ground level and about 300 m from the test site where the experiments were conducted. The daily midday (10:00 -14:00) VPD (data from M. Haas) are plotted against the plant age in Figure 8, the mean midday VPD in Figure 9. As expected, the VPD under FGH was higher than the field conditions.

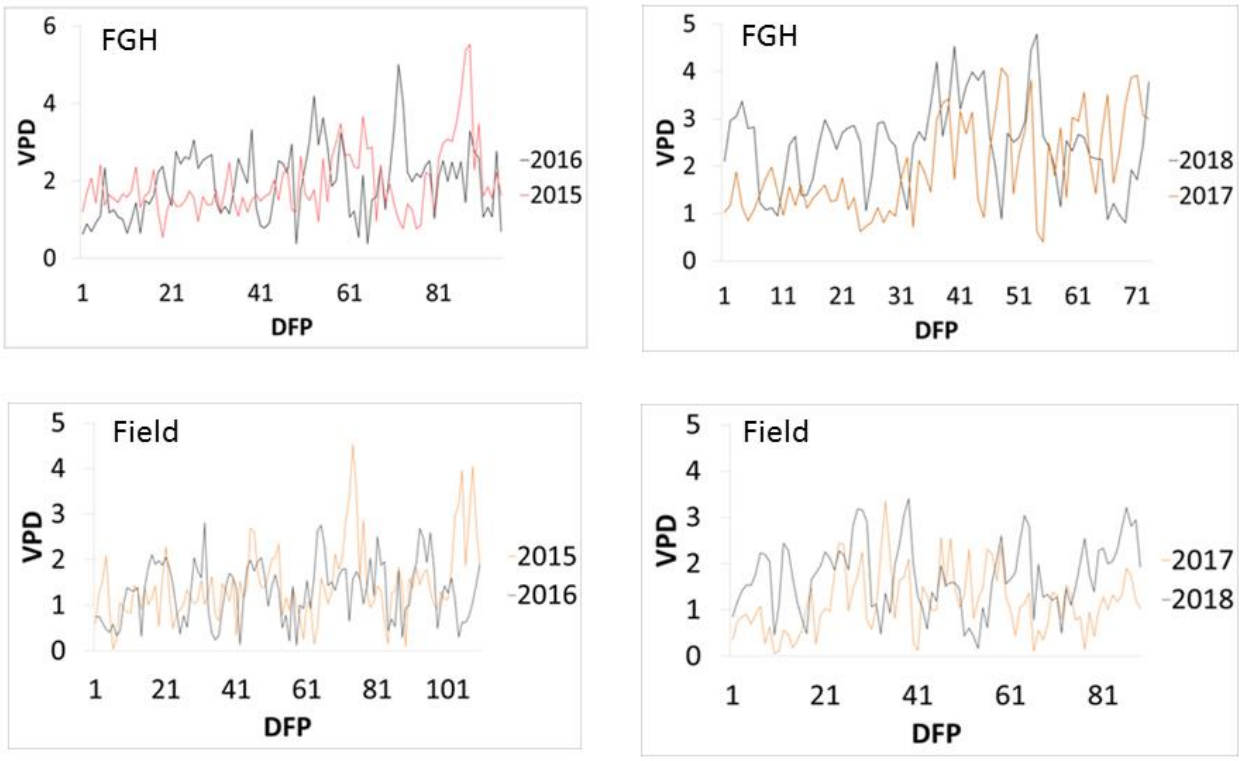


Figure 8. Daily midday (10:00 – 14:00) VPD in FGH and field experiments in 2015-2018.

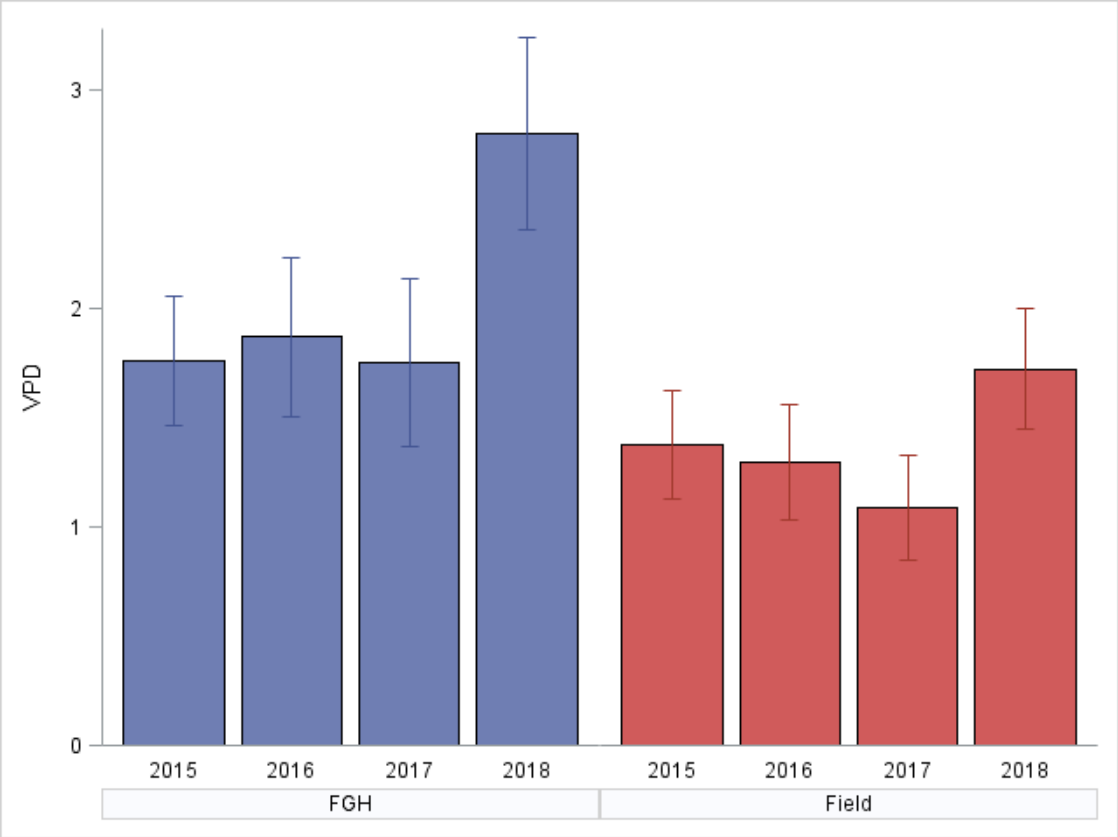


Figure 9. Average midday VPD during the cultivation time in FGH (left) and field (right) experiments. Bars represent mean ± SD of daily values of phenotyping period.

2.2 Automated Phenotyping

2.2.1 Laser scanner

In the FGH experiments, plant growth of both populations was monitored by laser scanner (PlantEye system, Phenospex, Heerlen, Netherlands) mounted on a Fieldscan (Phenospex) carrier system that moves the scanner automatically over the plants (Figure 7 A), generating 50 height profiles per second. In this study, the Fieldscan carrier was set to a scanning speed of 35 mm/s and a scanning interval of 4 h between repeated measurements of the same plant. The control and the stress blocks were scanned by two independent PlantEye scanners, which were positioned on right and left side of the Fieldscan carrier. The data from the laser scanner are automatically merged into a 3D point cloud (Figure 10 C) with a resolution of around 0.8 × 0.8 × 0.2 mm into the xyz-direction, respectively (Kjaer and Ottosen 2015). The PlantEye software Hortcontrol computes a diverse set of plant parameters on the flight by meshing neighbouring points with a nearest neighbour search (Fanourakis *et al.* 2014). From this triangle mesh, a subsequent surface triangulation algorithm computes different morphological parameters (Figure 10) within a second

(Kjaer and Ottosen 2015; Vadez *et al.* 2015). Hortcontrol does not dissect the continuous mesh into individual plants automatically. The separation is performed based on the location information uploaded to the software, which identified plant position relative to a reference barcode.

In this study, plant parameters in population A and population B were generated by using two different software versions. The plant parameters (see below), plant height, total leaf area, projected leaf area and leaf angle were generated by both versions. The more advanced software version generated the additional parameters digital biomass, leaf inclination, light penetration depth and leaf area index for the experiments performed on population B.

Plant parameters estimated from the laser scanner data of this study are listed below (Phenospex ; Vadez *et al.* 2015):

- **Plant height** (PH) (mm) is the height of the plant in the Z-axis above the pot surface.
- **Total leaf area** (A3D) (mm^2) is the total leaf area measured by scanner. Starting from the 3D point could, all points that belong to the same sector are triangulated. The total 3D leaf area of the plant is then calculated by taking the sum of the elementary triangle's area.
- **Projected leaf area** (A2D) (mm^2) measures the area of the projection of the plant onto the X-Y-plane.
- **Leaf inclination** (LI) (mm^2/mm^2) is the ratio of leaf area 3D to leaf area 2D. It expresses how erect the leaves of a plant are on average.
- **Leaf area index (LAI)** (mm^2/mm^2) is the ratio of leaf surface area to unit ground area.
- **Light penetration depth** (LPD) (mm) denotes how deep light can penetrate into the canopy.
- **Digital biomass** (DB) (mm^3) is calculated as the product of height and 3D leaf area.
- **Leaf angle** (LA) (^{o}) is the mean angle of a leaf's surface to the y-axis

The following descriptive traits per plant were estimated from the above plant parameters:

- Maximum (plant height, leaf area, leaf area index, digital biomass) values per plant
- Average (leaf angle, leaf inclination, and canopy temperature depression) values per plant
- Range of leaf movement was calculated from each canopy parameters per day per plant.

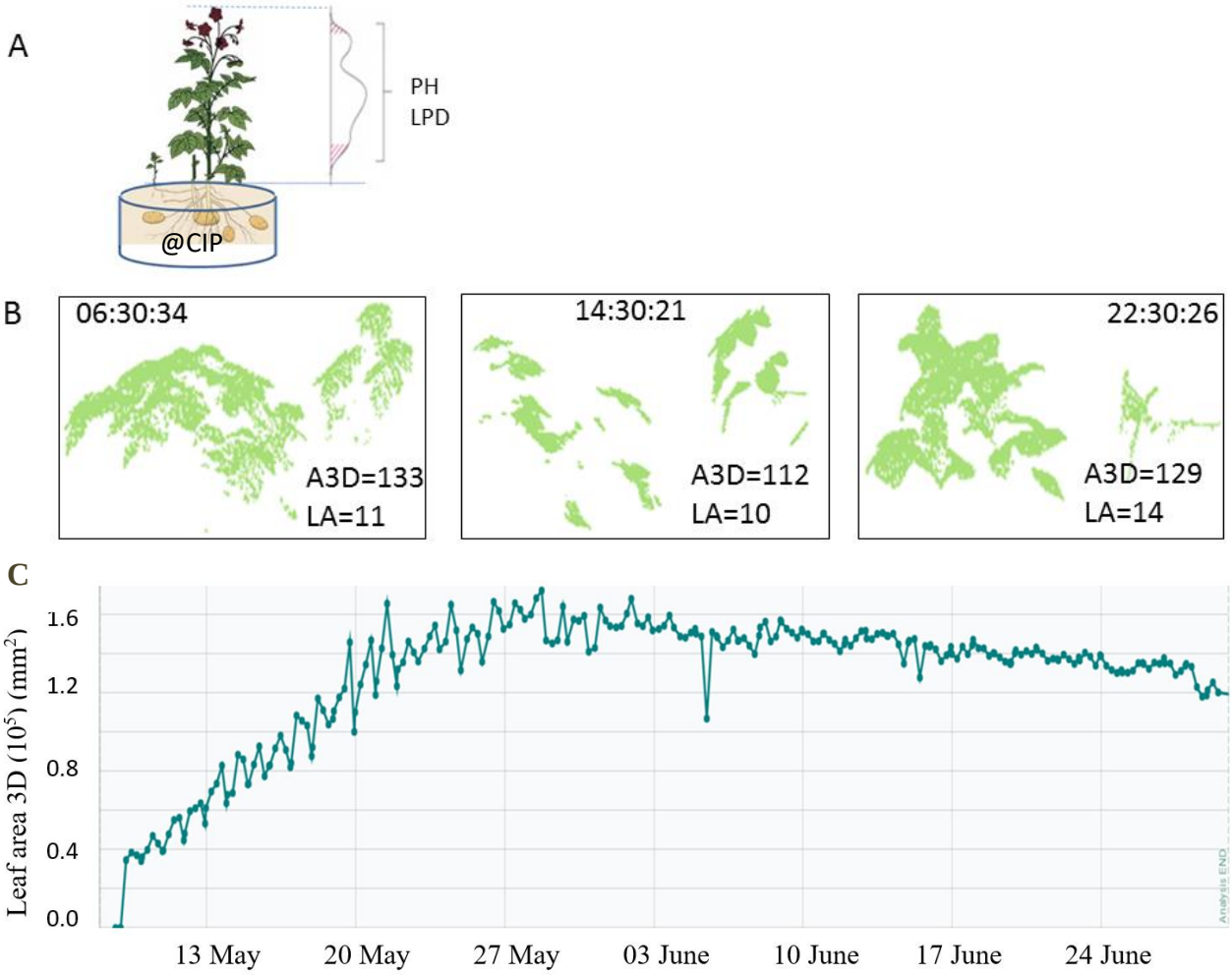


Figure 10. Laser scanner, 3D point cloud and sample plant parameters. (a) Plant height (PH) and light penetration depth (LPD). (b) 3D point cloud of plant id-858641 (position 8:10:1) at different time of 10 Jun, 2018. A = leaf area (3D) (10^3) mm^2 and LA =leaf angle (^{o}). (c) Leaf area (3D) (mm^2) of genotype 858641 at plant position of 8:10:1 depicted against the measurement date (treatment ss, 2018). The separation of specific plant position is performed based on the location information uploaded to the software, which identified plant position relative to a reference barcode**.**

2.2.2 Infrared thermosensor (IRT)

Canopies emit long-wave infrared radiation as a function their temperature. The IRT senses this radiation and converts it to an electrical signal, which is displayed as temperature (Blum 2011). In this study, canopy temperature of population B plants was measured by 16 IRT (IR100, Campbell Scientific Ltd.), which were mounted on the Fieldscan carrier (Figure 7 A) and stored in CR1000

data logger (CR1000, Campbell Scientific Ltd.). The distance between sensors and plant canopy were approximately 80 cm and scanning diameter was around 30 cm. The distance between the sensors and the plants was maintained by manually adjusting the height of the thermosensors relative to the plant height. The IRT measurements were programed with a three second integration interval. On average, the sensor passed the canopy of a single plant within nine seconds, yielding three data points per plant. For each plant, canopy temperature was monitored two times per hour between 27 to 66 DFP in 2017 and 24 to 74 DFP in 2018. From the continuous IRT surface temperature data set, the canopy temperature for each plant was selected by matching the time stamp of the IRT data based on the rapid temperature changes caused by the reflecting barcodes or the bare soil at the parking position to the timestamps of laser scanner dataset taking the time lag (47 sec) caused by the mounting distance (150 cm) between laser scanner and IRT into account.

2.3 Data evaluation and statistical analysis

Data evaluation was performed in SAS 9.4 (SAS, Cary, NC, USA). Starch yield was calculated as the product of tuber mass and tuber starch content. The average tuber weight (ATW) was determined by dividing total tuber weight by total tuber number.

Drought tolerance of starch yield

Drought tolerance level was calculated for each experiment as deviation of relative starch yield from either experimental (DRYM) (Sprenger et al; 2015) and parental median (DRYMp) (Eqn 1, (Hass et al; 2020)). DRYMp value was calculated relative to parent median by using equation below (Eqn 1), with parent median in place of experimental median.

$$\mathrm{DRYM}_{\mathrm{Gx,Ei}} = \mathrm{RelSY}_{\mathrm{Gx,Ei}} - \mathrm{median}(\mathrm{relSY}_{\mathrm{Ei}}) \quad (1)$$

Where

$\mathrm{DRYM}_{\mathrm{Gx,Ei}}$ - tolerance of genotype x in experiment i, Gx - genotype x, Ei - experiment

$$\mathrm{RelSY}_{\mathrm{Gx,Ei}} = \frac{\mathrm{starchyield}_{\mathrm{Gxs,Ei}}}{\mathrm{starchyield}_{\mathrm{Gxc,Ei}}} \quad (2)$$

Where

$\mathrm{RelSY}_{\mathrm{Gx,Ei}}$ - relative starch yield of genotype x in experiment i, Gxs - genotype x under drought conditions, Gxc - Genotype x under control conditions

The DRYMp was used to analysis a joint analysis across season or experimental conditions (FGH and Field) of respective population.

Descriptive morphological and physiological parameters

Plant traits used in this study were obtained from laser scanner and IRT. Data sets were checked for outliers by using *proc sql* (SAS) procedures. A normally distributed data set was obtained after the outliers were trimmed at plus or minus two standard deviations. *Proc mean* procedure was used for the normally distributed data set to calculate different parameters like maximum (max), mean, range from different morphological (see laser scanner) and physiological (CTD) traits. Maximum values of plant height, leaf area, digital biomass, leaf area index and light penetration depth per plant were calculated from continuous values of the measuring periods of respective canopy traits. Among the canopy traits considered in this study, LA, LI and CTD values oscillated around mean values through the plant age. Therefore, mean values were calculated instead of maximum values. Daily range was calculated as the difference between the daily maximum and minimum of a particular parameter value. Range per season was calculated as the mean of the daily range.

Canopy temperature and canopy temperature depression

Canopy temperature depression (CTD) was calculated as the difference between canopy temperature and air temperature. The data from plant temperature logger and metrological logger were matched by considering the data logging frequency of the loggers. The metrological data were logged less frequently (four times per hour) than plant temperature so that data of the two loggers were matched based on logging interval of the metrological data.

CT is best assessed at full canopy stage with high vapor pressure deficit (VPD) conditions associated with low relative humidity and warm air temperature (Blum 2009; Tuberosa 2012). Therefore, in this study the daily midday VPD was calculated as the median VPD from hourly temperature (T(t)) and relative humidity Rh(t) readings between 10 am and 2 pm (Eqn 3, (Sprenger *et al.* 2015)) (VPD data from M. Haas):

$$\mathrm{VPD(t)} = 0.61365\mathrm{e}^{\frac{17.502\mathrm{T(t)}}{240.97+\mathrm{T(t)}}} \cdot \left(1 - \mathrm{Rh(t)}\right) \qquad (3)$$

2.4 Statistical evaluation

The effects of experiment, genotype, treatment and the treatment × genotype interaction, genotype × year, genotype × treatment × year were tested by analysis of variance (ANOVA) (Eqn 4 and Eqn 5) with *proc glm* (see supplementary syntax). Means separation was done based on *regwq* grouping and *lsd*. Data normality was tested with *proc sql* and *proc univariate* (see supplementary syntax). In case of *proc sql*, plus or minus two standard deviations (s.d) was used as cut point for outlier. Based on the normally distributed data set, analysis of variance was run for yield parameters (TN, TY, SY, ATW), DRYM, canopy parameters (max or mean values) for particular experiment (Equ 4) as well as combined experiments over years for the respective experimental conditions (Eqn 5 (FGH or Field).

Model equation for particular experiment

$$R_{GT} = \mu + \alpha G + \beta T + \gamma GT + \varepsilon \quad (4)$$

Where R_{GT} is the data value observed for the sample on levels Genotype (G) and treatment (T), μ is the common effect for the whole experiment, αG is the model parameter for factor α on level G, βT is the model parameter for factor β on level T, γGT is the interaction term, and ε is the error. Genotype and genotype by treatment interaction were random factors.

Model equation for combined experiments over season

$$R_{GTY} = \mu + \alpha G + \beta T + \gamma Y + \delta_1 GT + \delta_2 GY + \delta_3 GTY + \varepsilon \quad (5)$$

Where R_{GTY} is the data value observed for the sample on levels genotype (G), treatment (T) and year (Y), μ is the common effect for the whole experiment, αG is the model parameter for factor α on level G, βT is the model parameter for factor β on level T, γY is the model parameter for factor γ on level Y, $\delta_1 GT$, $\delta_2 GY$, $\delta_3 GTY$ were interaction terms and ε is the error. Genotype, treatment and year were random factors. This general equation was used to analysis the data across experiments of the same population and experimental condition.

Growth rate

Growth rate of genotypes was determined by fitting linear (see Eqn 6) and second-degree polynomial (Eqn 7) mathematical models to the daily mean values of respective traits with *proc reg* (see supplementary syntax). Morphological parameters were treated as dependent and plant age DFP as independent parameters (see Eqn 6 and Eqn 7). The data used for the linear model was censored to the range of the approximately linear increase of the respective parameter, whereas for

the polynomial model the entire data sets were used in the model. The approximately linear increase of the respective parameter was identified from scatter plot graphs of morphological parameters against days from planting. Growth rate / rate of change of canopy parameters varied with time so that the linear model was fitted to the part of the data that showed linear incase up to some point in the plant growth stage (plant establishment until it reaches its biological maximum).

$$y = \mu + \alpha(\mathrm{DFP}) \quad (6)$$

$$y = \mu + \alpha(\mathrm{DFP}) + \beta(\mathrm{DFP})^2 \quad (7)$$

Where μ - constant, α -linear coefficient, β - quadratic coefficients, DFP- days from planting

Correlation analysis

The Pearson correlation coefficients were calculated between tolerance index either assessed within one experiment (specific tolerance index) or as mean from all screenhouse experiments or mean from all experiments (FGH and field) of each treatment with mean tuber parameters (starch yield and tuber fresh yield) or canopy parameters (maximum, mean, range of leaf movement, and growth rate / rate of change over time) of respective treatment. Parameters measured in optimal condition were correlated with DRYM values calculated in long-term stress conditions.

Data visualization and grouping

Different data visualizations and groupings were done by using *proc factor* (PCA), *proc hpsplit* (Decision tree) and *proc cluster* (homogeneity of merged clusters determined by Centroid distance) in SAS (see supplementary syntax). Centroid distance is the Euclidian distance between the centroid of the two clusters that are to be joined.

Multiple linear regressions

To identify parameters that could predict drought tolerance, multiple regression analysis and LASSO regression analysis were performed. Multiple regression analysis was used to determine linear combinations of morphological and physiological parameters that predict drought tolerance (see Eqn 8). The analysis was performed with *proc glmselect* (see supplementary syntax), selection=stepwise, 10-fold cross validation. Morphological and physiological parameters (see descriptive morphological and physiological parameters**)** were used as independent variable and DRYM of pot experiment as dependent variable. A joint evaluation across season or across trial conditions (FGH and Field) was calculated based on the mean DRYM of parental median.

$$Y = \beta 0 + \beta 1X1 + \beta 2X2 + \cdots + \beta pXp + \varepsilon \quad (8)$$

Where

Y - DRYM, X - morphological or physiological parameter, β - regression coefficients, ε - pooled error

LASSO regression is a type of linear regression that uses regularization. Regularization is a way to avoid overfitting by penalizing high-valued regression coefficients. In simple terms, it reduces parameters and shrinks (simplifies) the model (James *et al.* 2017). In this study, LASSO regression was performed on standardized data with *proc glmselect* (SAS) (Eqn 9). All predictor variables were standardized to have a mean of zero and a standard deviation of one in *proc glmselect*. Standardized data were randomly split into a training set that included 70% of the observations and a test set that included 30% of the observations. This criterion was used for the selection of the values for the penalty parameters, based on which the final model was trained.

$$\hat{\beta}_{lasso} = \sum_{i=1}^{n} (yi - \dot{x}\imath * \hat{\beta})^{2} + h1 \sum_{j=1}^{m} |\hat{\beta} j| \quad (9)$$

where y denotes the response, $\dot{x}$ denotes the matrix of covariates, $\hat{\beta}$ is the solution to the constrained least squares problem, h1 is the penalty parameter

3. Results

3.1 Drought effects on tuber parameters

The drought tolerance assessment of potato genotypes was based on the determination of tuber parameters (TY, SY, TN and ATW) under control and different water stress conditions. These parameters were measured for two populations in four screenhouse and four field experiments between 2015 and 2018. Data for the 2015 and 2016 trials were obtained from M. Haas. Analysis of variance was done for the effects of genotype and treatment and their interaction on TY and SY, ATW and TN. The summary tables of the ANOVA results are presented below for each trial in the screenhouse (Table 2) and the field (Table 3). Additionally, the analysis was done on pooled data of both experiments performed on each population (Table 2 and Table 3). All tuber parameters considered in this study were significantly affected by water stress. The responses of genotypes to water stress varied from genotype to genotype as indicated by the significant genotype × treatment interactions. However, no significant interaction was observed in population B under field condition. This was mainly because of the unusually high rainfall during the 2017 experiment and the small difference between cc and sc treatments in the 2018 experiment. The significant genotype x treatment x year interaction indicated that the response of genotypes to water stress were varied between years under field conditions. This suggests that several trials in different years and under different environmental conditions are required to obtain a general assessment of a genotype's drought tolerance.

Table 2. ANOVA summary: The effect of genotype, treatment and their interaction on tuber yield, starch yield, average tuber weight and tuber number in screenhouse experiment in 2015 -2018. F values are shown. With the exception of one interaction value (indicated with ns), all main effects and their interactions were significant at a p-value of 0.01. ns - non-significant at p-value of 0.05.

Pop	Year	Para	Model	G	T	G×T	Y	G×Y	G×T×Y
A	2015	DF	131	63	1	63			
		TY	2710.2	1373.2	239988	424.7			
		SY	2931.4	870.8	306693	373.4			
		ATW	10.9	14.7	336.08	2.35			
		TN	10.7	11.5	429.38	3.62			
	2016	DF	131	63	1	63			
		TY	4539.4	2621.9	389461	369.3			
		SY	3664.6	1516.5	369945	273.6			
		ATW	12.7	18.6	317.14	2.3			
		TN	11.6	11.6	637.08	2.2			
B	2017	DF	90	20	3	60			
		TY	61.8	130.7	853.05	3.6			
		SY	46.4	81.9	744.70	2.5			
		ATW	10.8	33.8	58.41	1.2^{ns}			
		TN	17.2	49.3	144.90	2.2			
	2018	DF	86	19	3	57			
		TY	22.2	35.7	360.33	2.0			
		SY	25.3	36.8	443.51	2.5			
		ATW	12.3	31.5	105.29	1.9			
		TN	14.2	27.3	186.97	3.3			
A	com	DF	258	63	1	63	1	63	64
		TY	8515.5	5163.5	1035502	901.7	399577	993.5	1123
		SY	7759.9	3185.1	1171206	840.9	254235	744.2	1353
		ATW	13.7	28.2	629.45	3.3	557.2	3.55	1.85
		TN	11.4	19.4	1016.52	3.	94.2	3.18	2.31
B	com	DF	170	20	3	60	1	19	60
		TY	47.3	129.9	1176.92	3.4	1007.3	22.1	2.51
		SY	49.2	91.0	1138.82	2.9	2011.4	22.2	3.43
		ATW	12.9	49.1	143.26	1.7	145.4	12.1	1.58
		TN	16.8	61.1	317.96	2.9	142.4	8.8	3.59

Where G - genotype, T - treatment, Y - year, Pop - population, com - pooled data from two years, para - parameters, and DF - degree of freedom

Table 3. ANOVA summary: The effect of genotype, treatment and their interaction on tuber yield (TY), starch yield (SY), average tuber weight (ATW) and tuber number (TN) under field conditions in 2015 - 2018. F-values and significance levels are shown. ** and * significant at p-value of 0.01 and 0.05, respectively, and ns - none significant at p-value of 0.05.

Pop	Year	Para	Model	G	T	G×T	Y	G×Y	G×T×Y
A	2015	DF	131	63	1	63			
		TY	28.82**	10.21**	2778.08**	4.23**			
		SY	30.02**	10**	2891.92**	4.27**			
		ATW	13.65**	12.62**	833.80**	2.51**			
		TN	15.00**	10.10**	1030.03**	3.52**			
	2016	DF	131	63	1	63			
		TY	24.61**	7.14**	2588.23**	1.70**			
		SY	28.77**	6.94**	3085.53**	2.31**			
		ATW	17.46**	16.33**	1054.62**	2.36**			
		TN	7.13**	10.17**	156.83**	1.68**			
B	2017	DF	84	20	3	60			
		TY	9.73**	11.76**	173.36**	0.97ns			
		SY	9.24**	13.79**	145.10**	1.24**			
		ATW	5.66**	15.59**	24.91**	1.45ns			
		TN	5.52**	11.57**	47.47**	1.20ns			
	2018	DF	84	20	3	60			
		TY	16.42**	4.99**	354.49**	1.08ns			
		SY	18.52**	13.25**	362.45**	1.33ns			
		ATW	8.29**	8.62**	139.73**	1.19ns			
		TN	3.70**	10.84**	6.90**	1.26ns			
A	com	DF	257	63	1	63	1	63	64
		TY	27.77**	15.40**	5289.23**	4.76**	15.85**	2.70**	3.65**
		SY	30.21**	15.23**	5957.59**	5.31**	11.40**	2.33**	2.00**
		ATW	15.96**	26.98**	1859.36**	3.17**	153.33**	1.52**	1.80**
		TN	10.99**	16.36**	910.03**	2.91**	161.26**	3.78**	3.88**
B	com	DF	168	20	3	60	1	20	63
		TY	14.91**	11.87**	334.88**	1.00ns	369.86**	8.02**	9.00**
		SY	14.46**	22.82**	302.68**	1.33ns	270.04**	7.15**	8.48**
		ATW	7.12**	20.25**	115.07**	1.30**	9.16**	3.30**	3.81**
		TN	5.94**	18.17**	45.24**	1.43*	156.19**	8.53**	2.10**

Where G - genotype, T - treatment and Y - year, pop - population, com - pooled data from two years, and para - parameters, and DF - degree of freedom

The trials on both populations contained optimal water supply (cc) and long-term stress (ss) (Figure 11 to Figure 14). Additionally, trials on population B contained early stress (sc) and late stress (cs) treatments (Figure 11 to Figure 14). Comparisons between all pairs of means were done by *regwq* method. The means comparison between treatments in each experiment are presented in Figure 11 to Figure 14. The means of pooled experiments of the respective populations and trial

conditions (FGH and Field) are presented in supplementary Figure S7. Among the tuber parameters mentioned above, the mean SY values of genotypes are presented for each experiment in the FGH (Figure 15. left panel) and the field (Figure 15. right panel). Genotypes in cc condition indicated that long-tail distribution below the first quartile. The *regwq* means comparison of starch yield of the genotypes for each experiment is presented in the supplementary Table S2, supplementary Table S3 and supplementary Table S4.

In both populations and trial conditions (FGH, field), long-term stress resulted in significantly reduced mean starch and tuber yield (Figure 11 and Figure 12). The experiments on population B (Figure 11 and Figure 12) indicated that late stress was more devastating to tuber and starch yield than early stress. This trend was clearly observed in both experiments under FGH conditions. Almost all yield parameters were significantly affected by time and duration of water stress. In terms of tuber and starch yield reduction, late stress was more important than early stage stress.

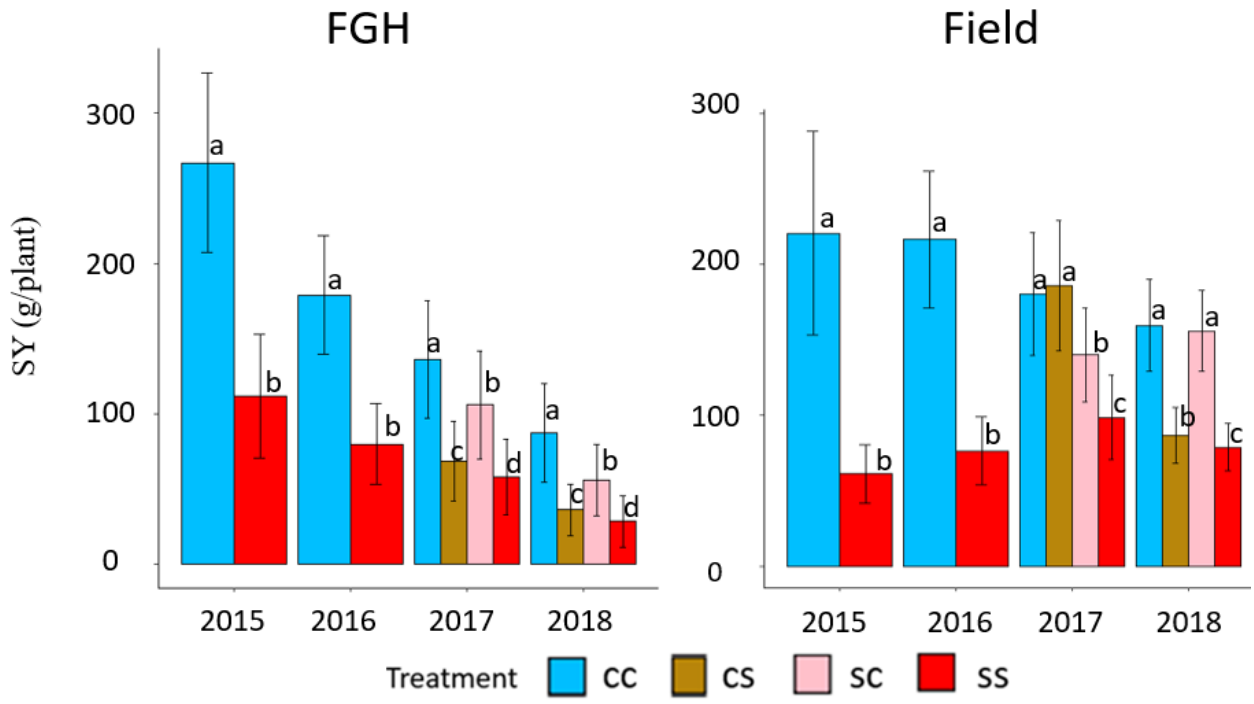


Figure 11. Mean starch yield (SY) per plant of population A and B in FGH (left) and field (right) experiments. Means comparison was done by *regwq test.* Treatments assigned the same letter are not significantly different at a p-value of 0.01. Bars represent mean ± SD of replicates. In the 2017 field experiment, the cs treatment was affected by rainfall (see supplementary Figure S2).

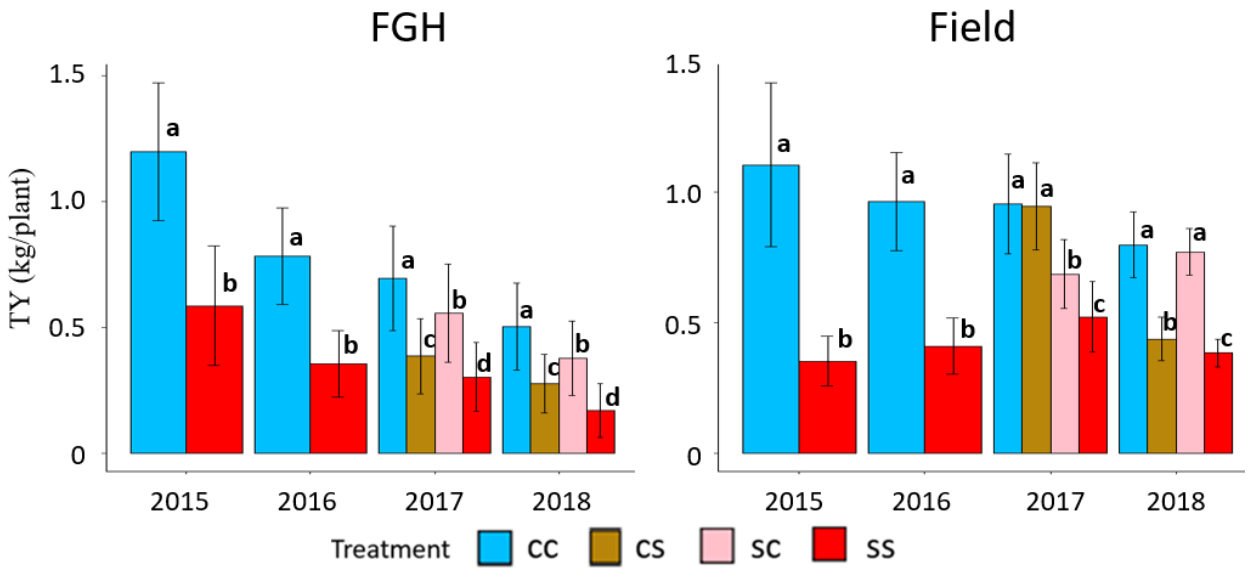


Figure 12. Mean tuber yield (TY) per plant of population A and B in FGH (left) and field (right) experiments. Means comparison was done by *regwq*. Treatments assigned the same letter are not significantly different at a p-value of 0.01. Bars represent mean ± SD of replicates. In the 2017 field experiment, the cs treatment was affected by rainfall (see supplementary Figure S2).

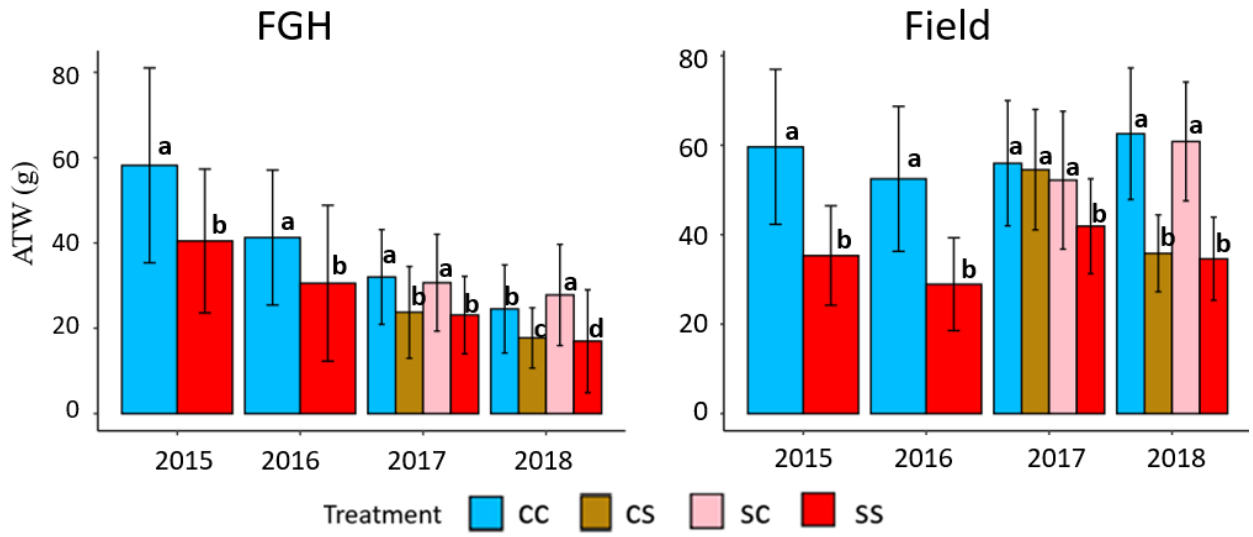


Figure 13 . Mean average tuber weight (ATW) (g) per plant of population A and B in FGH (left) and field (right) experiments. Means comparison was done by *regwq.* Treatments assigned the same letter are not significantly different at a p-values of 0.01. Bars represent mean ± SD of replicates.

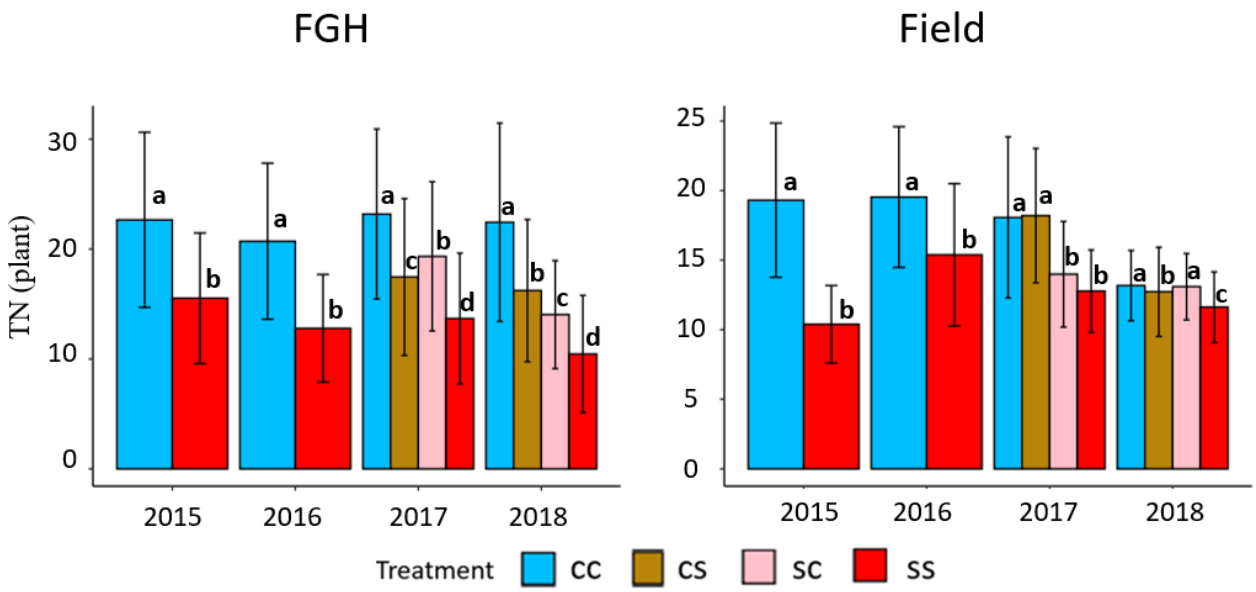
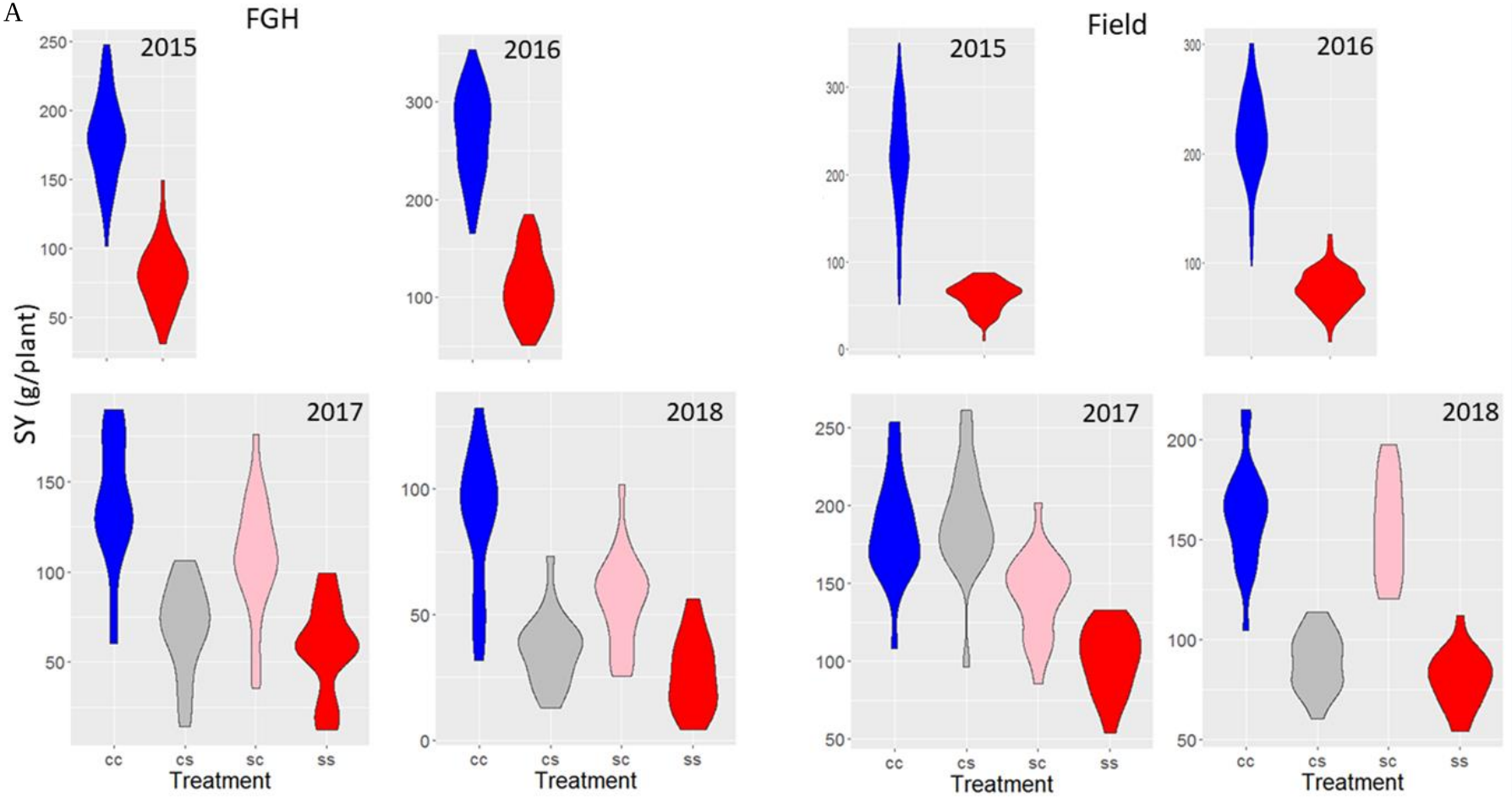


Figure 14 . Mean tuber number (TN) per plant of population A and B in FGH (left) and field (right) experiments. Means comparison was done by *regwq*. Treatments assigned the same letter are not significantly different at a p-value of 0.01. In the 2017 field experiment, the cs treatment was affected by rainfall (see supplementary Figure S2). Bars represent mean ± SD of replicates.

A



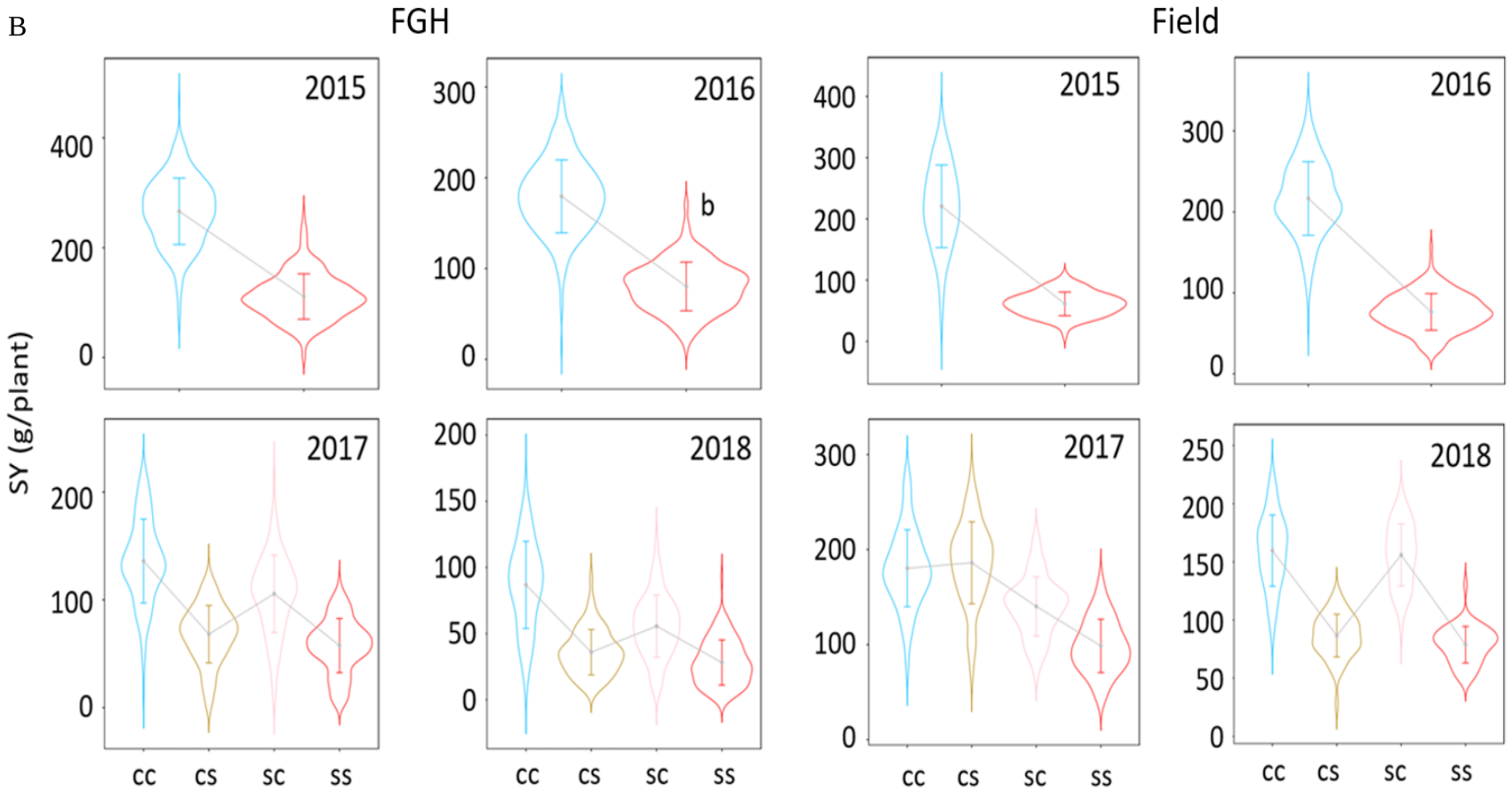


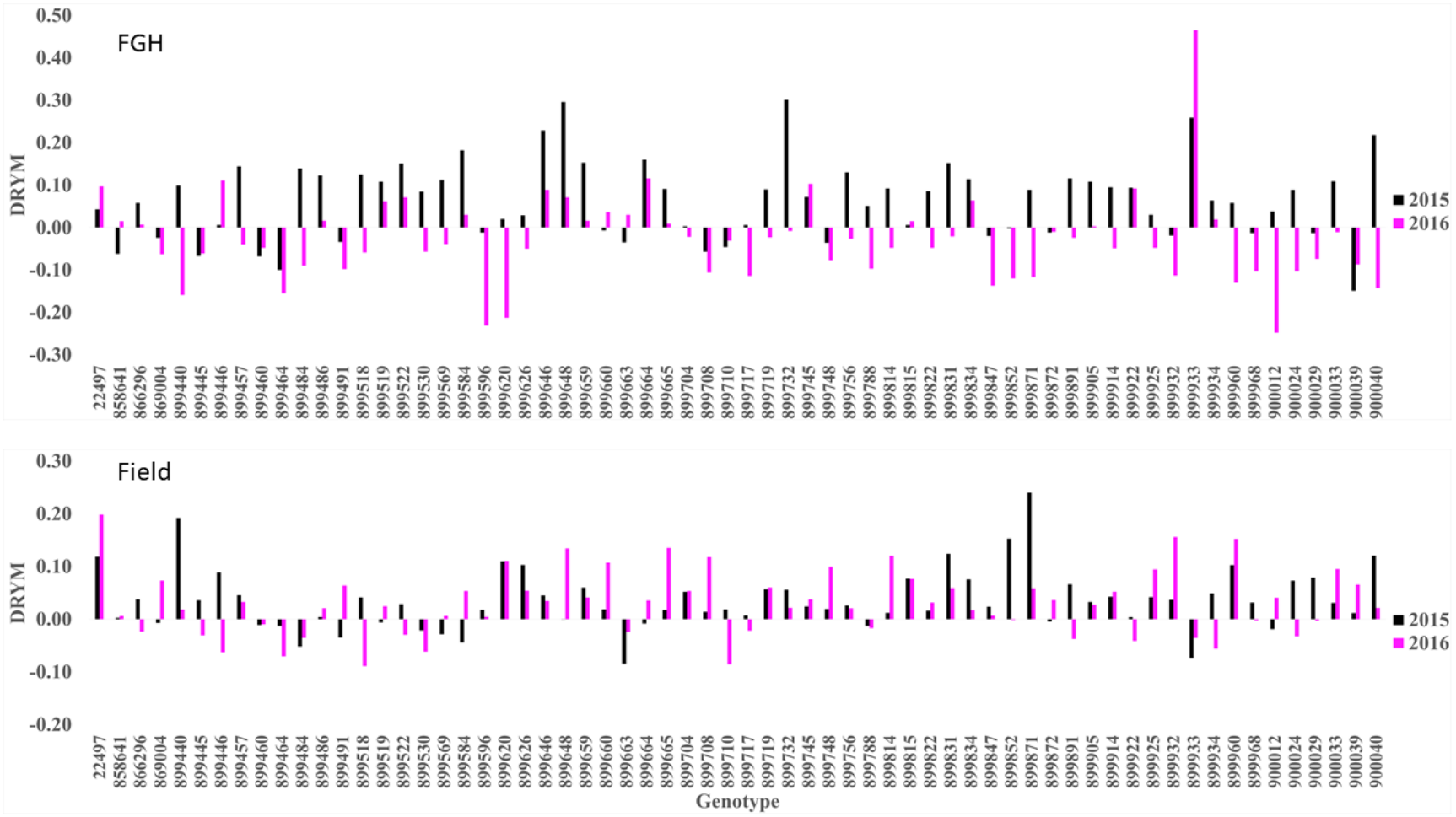
Figure 15. Distribution of genotype means of tuber starch yield in FGH (left) and field (right) in Pop A (top) and Pop B (bottom), 2015-2018. The old figure in (A) and new figure (B). Bars represent mean ± SD of replicates. In the 2017 field experiment, the cs treatment was affected by rainfall (see supplementary Figure 2).

3.2 Drought tolerance in FGH and field trials

The drought tolerance index (DRYM) was calculated for each genotype, treatment (except control) and experiment (FGH and Field). A DRYM value of zero indicates median tolerance, negative values indicate sensitivity and positive values indicate enhanced tolerance compared to the median of the parent cultivars (DRYMp) or experimental median (DRYM). In population A, DRYMp ranged from 0.47 (FGH-2016) and 0.24 (Field-2015) in the most tolerant genotype to -0.25 (FGH-2016) and -0.09 (Field-2016) in the most sensitive genotype (Figure 16). In population B, the most tolerant genotypes had DRYMp values of 0.20 (sc treatment in 2017 and ss treatment in 2018) and the lowest DRYMp was -0.27 under cs treatment (Figure 17 and Figure 18) in 2017 FGH experiment. In the field experiments, the lowest (-0.21) and the highest (0.28) DRYMp values were found in the sc treatment in 2017 and 2018, respectively (Figure 17 and Figure 18). The DRYMp values of pooled experiments of the respective populations and trial conditions (FGH and Field) are presented in supplementary Figure S8.

The analysis of variance of tolerance index values of each experiment (DRYMp) (Table 4) and combined across years (DRYMp) (Table 5) indicates that the tolerance indices were significantly affected by genotypes, treatment, year and trial condition (Field, FGH). In addition, analysis of variance across trial conditions indicated that tolerance levels varied from trial condition to trial condition as indicated by significant experimental by trial conditions interaction (supplementary Table S6). Furthermore, in population B, DRYMp values were different between long-term stress, early and late stress (Figure 17 and Figure 18). This indicates the need to take stress timing and duration into consideration for tolerance prediction. Therefore, drought tolerance prediction has to match the drought pattern in the target environment.

A


FGH
DRYM
0.50
0.40
0.30
0.20
0.10
0.00
-0.10
-0.20
-0.30
2015
2016
Field
DRYM
0.30
0.20
0.10
0.00
-0.10
-0.20
2015
2016
22497
858641
866296
869004
899440
899445
899446
899457
899460
899464
899484
899486
899491
899518
899519
899522
899530
899569
899584
899596
899620
899626
899646
899648
899659
899660
899663
899664
899665
899704
899708
899710
899717
899719
899732
899745
899748
899756
899788
899814
899815
899822
899831
899834
899847
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899891
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899925
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900024
900029
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Genotype

B

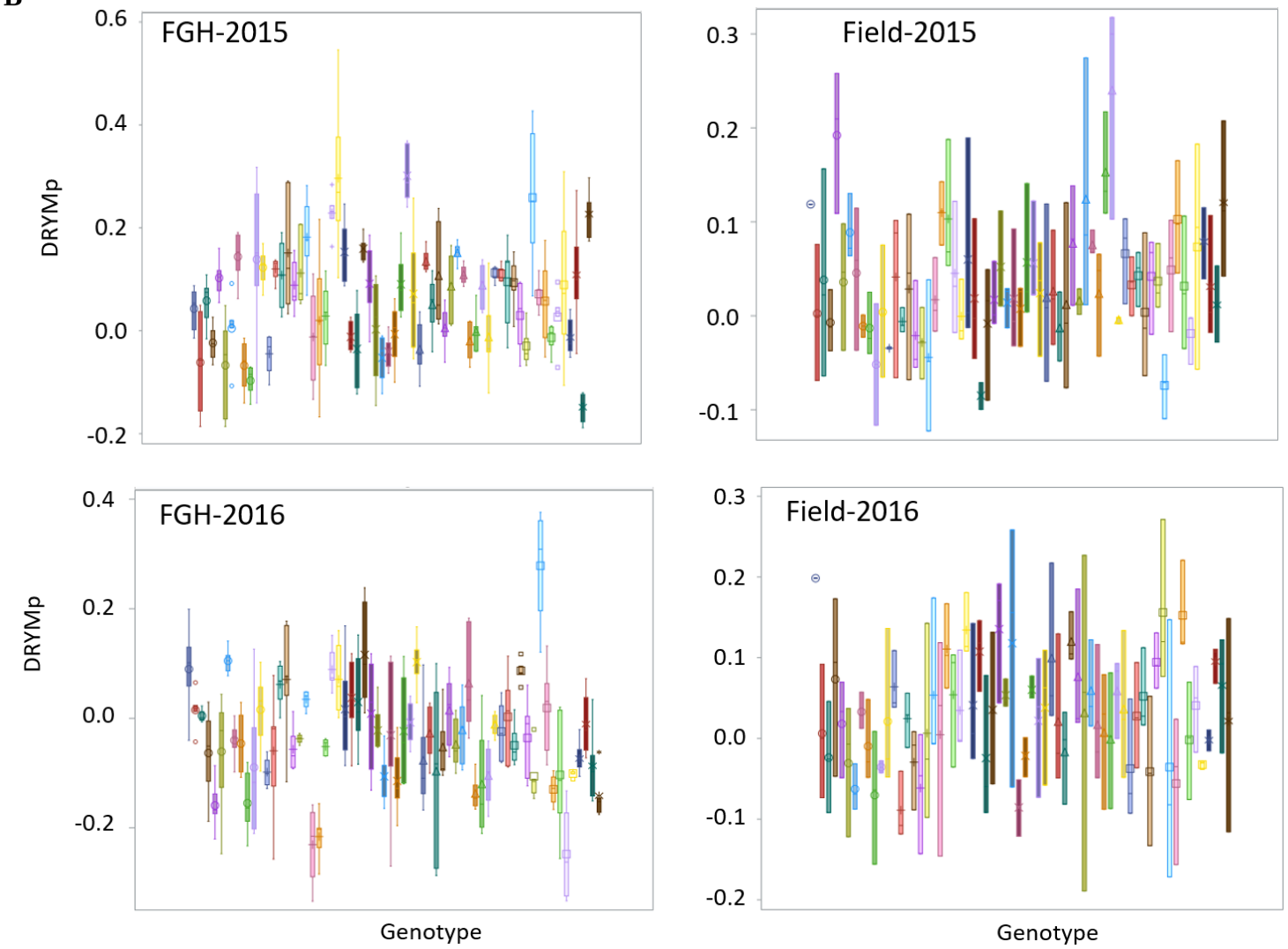
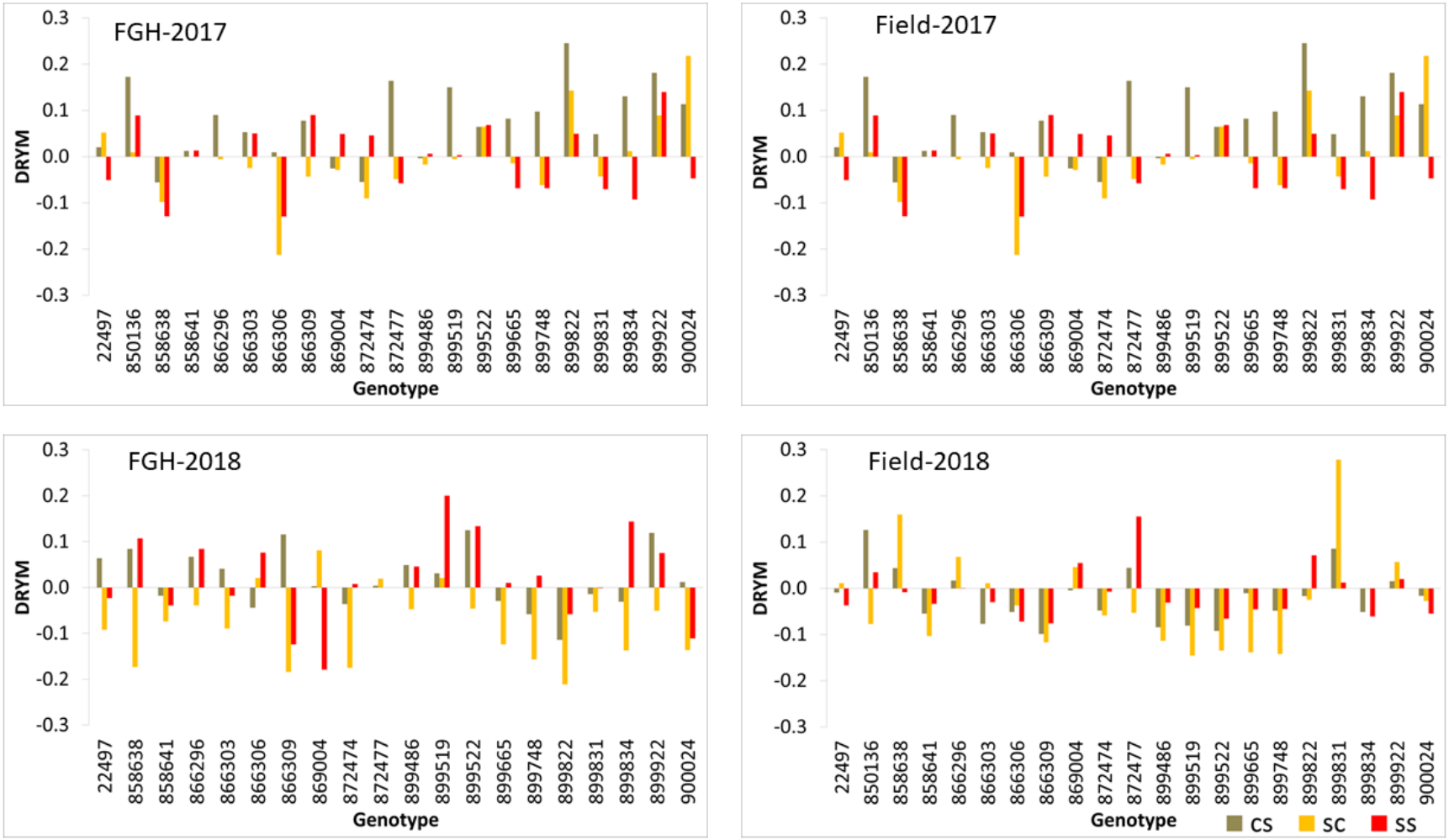


Figure 16. Median DRYMp values of population A genotypes in FGH and field experiments in 2015 and 2016 (Data from M. Haas). old figure (A) and new figure (B). Experimental design and number of replicates see Table 1. See the genotype code in supplementary Figure S11.

A


FGH-2017
Field-2017
FGH-2018
Field-2018
DRYM
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
22497
850136
858638
858641
866296
866303
866306
866309
869004
872474
872477
899486
899519
899522
899665
899748
899822
899831
899834
899922
900024
Genotype
CS
SC
SS

B

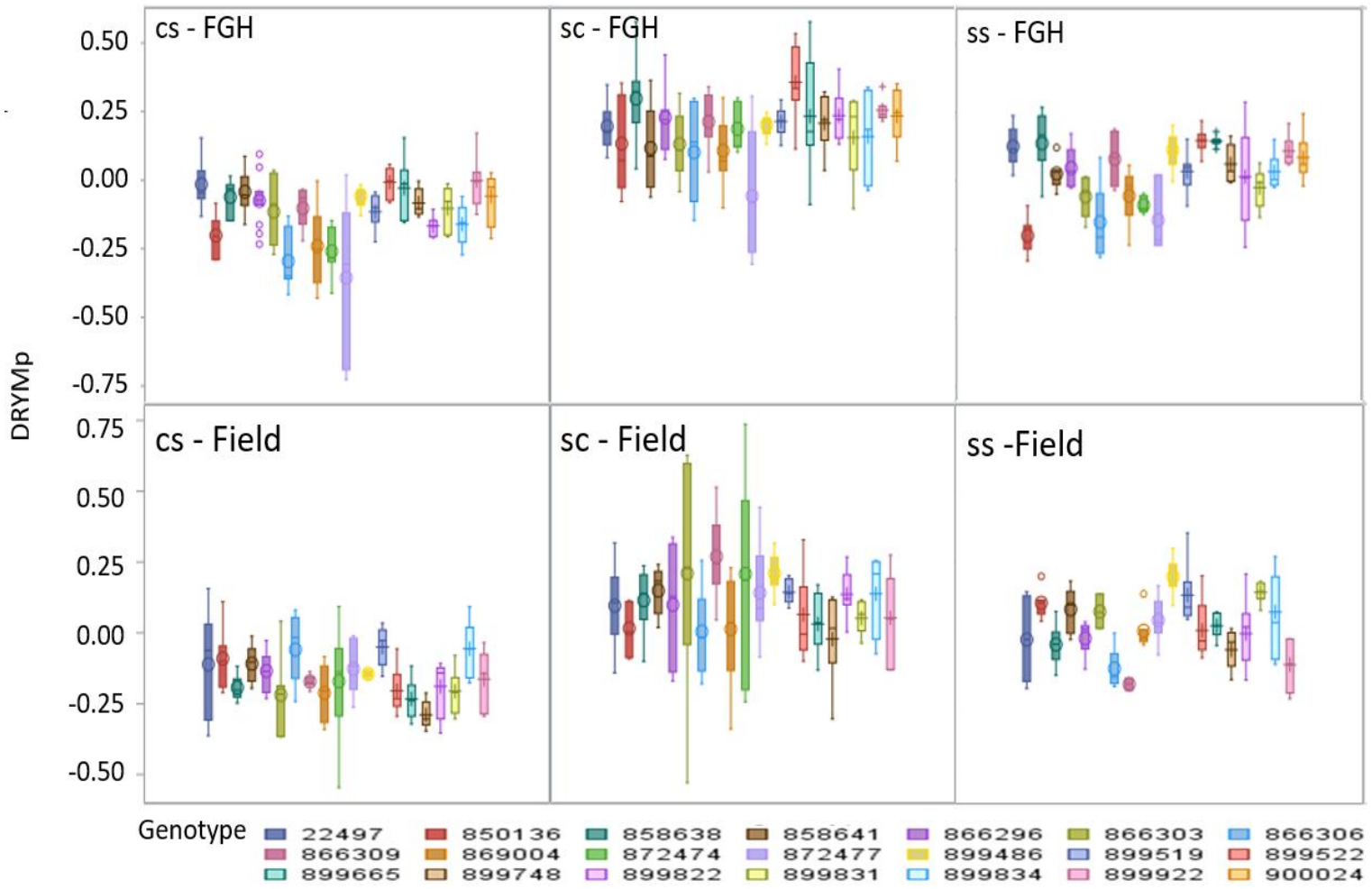


Figure 17. Median DRYMp values of population B genotypes determined in FGH (top) and field (bottom) experiments in 2017. The old figure (A) and new figure (B). Experimental design and number of replicates see Table 1.

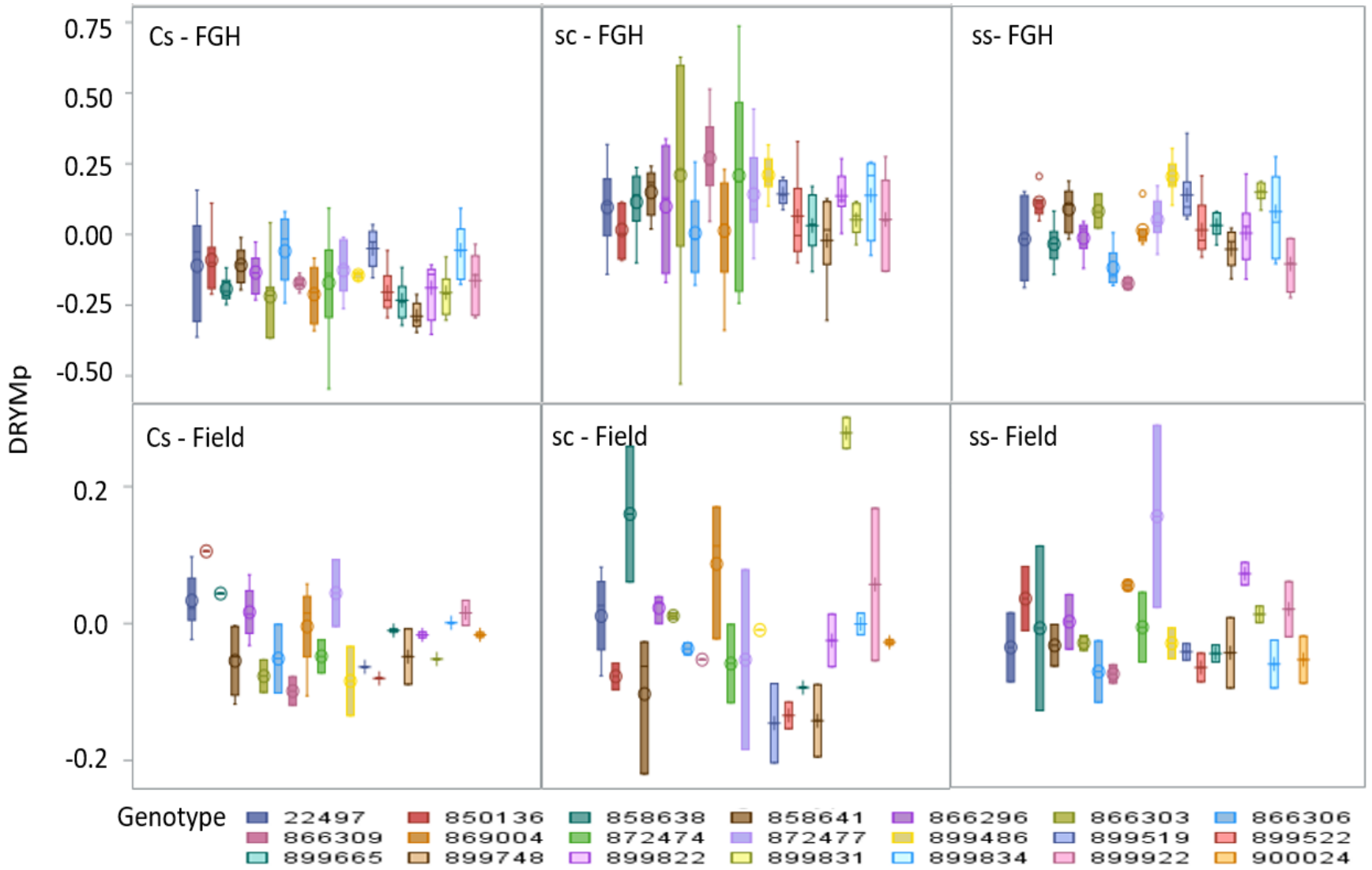


Figure 18. Median DRYMp values of population B genotypes determined in FGH (top) and field (bottom) experiments in 2018. The old figure (Figure 17 A). Experimental design and number of replicates see Table 1.

Table 4. ANOVA summary of the effect of treatment and genotype on DRYMp values calculated on the basis of data from single experiments. F values shown. Effects of model and genotypes were significant at 0.01 levels, except where ns is indicated. ns - nonsignificant at a p-value of 0.05. Pop-population.

Pop	Year	Treatment	Parameter	Model	Genotype
A	2015	ss	DF	64	63
		ss	DRYMp	8.86	8.98
A	2016	ss	DF	64	63
		ss	DRYMp	8.30	8.42
B	2017	cs, sc, ss	DF	21	20
		cs	DRYMp	6.57	6.88
		sc	DRYMp	2.81	2.95
		ss	DRYMp	7.49	7.84
B	2018	cs, sc, ss	DF	20	19
		cs	DRYMp	2.18	2.22
		sc	DRYMp	1.38^{ns}	1.43^{ns}
		ss	DRYMp	5.12	1.64

Table 5. ANOVA summary of the effect on genotype (G), treatment (T), year (Y), G×T, G×Y and G×T×Y on DRYMp calculated from starch yield data measured in 2015 and 2016 in population A and in 2017 and 2018 in population B under screenhouse and field condition. F-values and significance levels are shown. ** indicates significance at a level of 0.01, ns - nonsignificant at p-values of 0.05. Pop - population.

Pop	**Trial**	**Parameters**	**Model**	**G**	**T**	**G×T**	**Y**	**G×Y**	**G×T×Y**
B	FGH	DF	128	20	2	40	1	19	39
		DRYMp	9.98^{**}	7.83^{**}	397.91^{**}	1.79^{**}	26.74^{**}	5.54^{**}	1.78^{**}
B	Field	DF	88	20	2	40	1	20	
		DRYMp	1.62^{**}	2.13^{**}	4.20^{**}	0.89^{ns}	10.25^{**}	2.23^{**}	
A	FGH	DF	132	63			1	63	
		DRYMp	10.31^{**}	12.61^{**}			243.64^{**}	4.80^{**}	
A	Field	DF	129	63			1	63	
		DRYMp	1.82^{**}	2.16^{**}			0.52^{ns}	1.55^{**}	

3.3 Laser-scanner phenotyping

3.3.1 Laser scanner data quality

The plants were automatically phenotype for about five (2015), ten (2016), eight (2017) and seven weeks (2018), yielding 114 (2015), 217(2016), 221(2017) and 180 (2018) thousand data points. As an example, raw data of plant height is presented in Figure 19. At an early stage of phenotyping, data uniformity was low, presumably because of differences in plant establishment. In the last weeks of the observation period, shoots began to log and intermingle. In consequence, it was not possible to differentiate between the canopies of adjacent plants. Furthermore, plants logged before reaching the final height especially in population A (Figure 19, 2016 experiment). Therefore, short data intervals of 27 to 42 DFP (2015) and 29 to 44 (2016) DFP were used for fitting growth curve in population A. As an example, the window of long-term stress condition data of PH in 2015 experiment is presented in Figure 20.

From continuous data, different traits (maximum, mean, diurnal range, growth rate) was calculated for each canopy parameters to analyse their association with the tolerance index DRYM by Pearson correlation analysis.

3.3.2 Laser scanner parameters

Continuous measurement of plant parameters (see Materials and Methods) indicated that canopy features like leaf angle and plant height varied diurnally (see Figure 21) and with plant age (Figure 22). Additionally, Figure 22 indicates that some canopy parameters like plant height, leaf area and light penetration reached maximum value at a certain plant age, while others like LI and LA oscillated around some value throughout plant growth. Generally, continuous data on canopy parameters indicated that diurnal change as well as plant age should be considered during tolerance prediction.

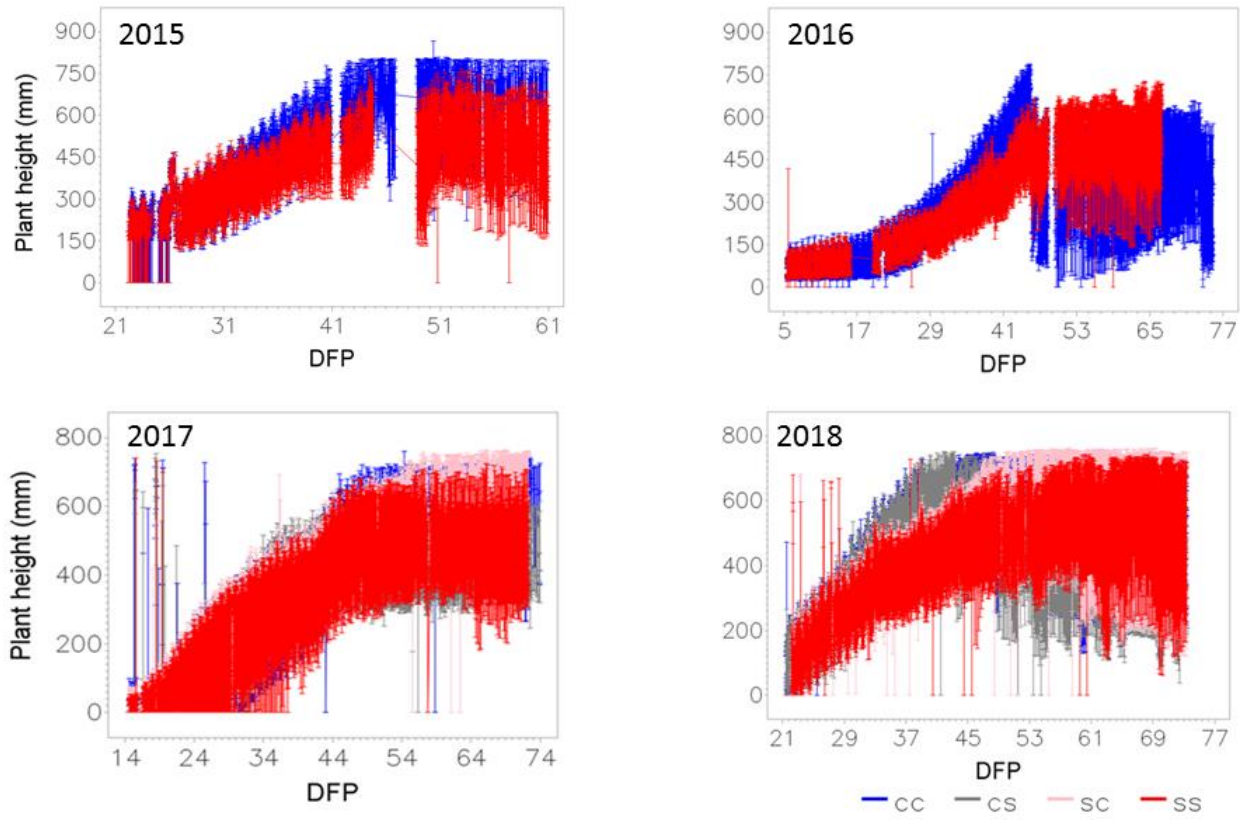


Figure 19. Raw data of plant height in different treatment condition in 2015-2018.

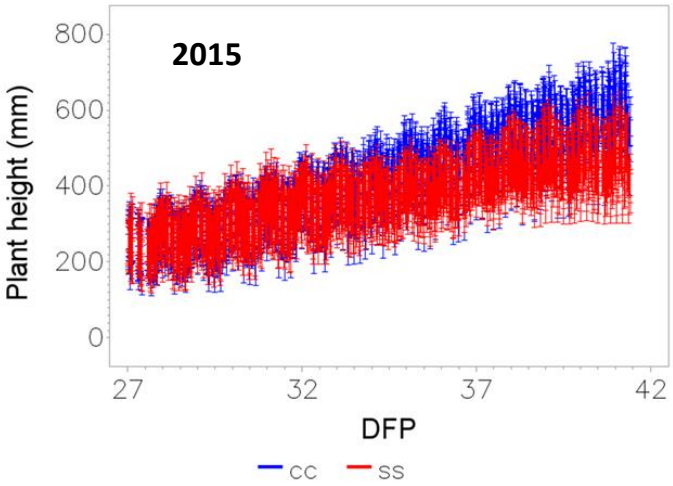


Figure 20. The age window of plant height data in optimal water supply (cc) and the long-term (ss) stress treatments used for further analysis in the 2015 experiment.

A

B

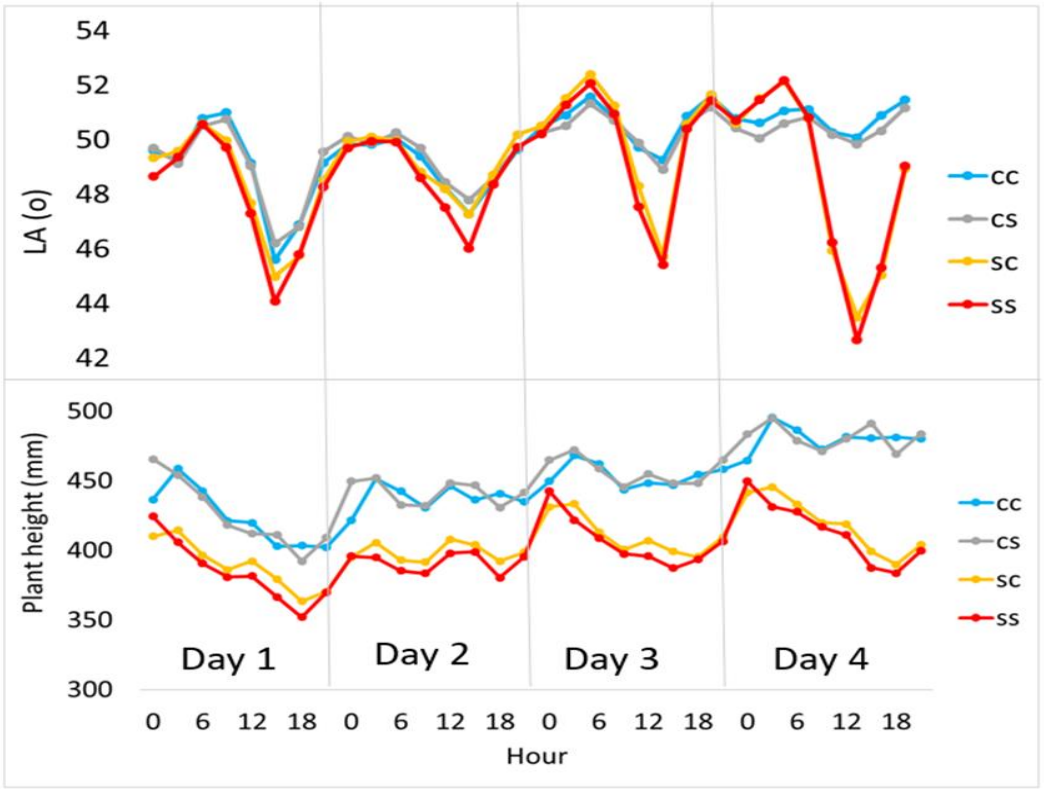


Figure 21. Mean diurnal change of leaf angle (°) (upper panel) and plant height (mm) (lower panel) of genotypes from 2 to 5 June 2019, old (A)and new (B). At this stage, the water supply under treatment cs was the same as under cc, the water supply under treatment sc was the same as under ss. The data from Phenospex (http://141.14.246.248/data/accessed on 20 June 2019).

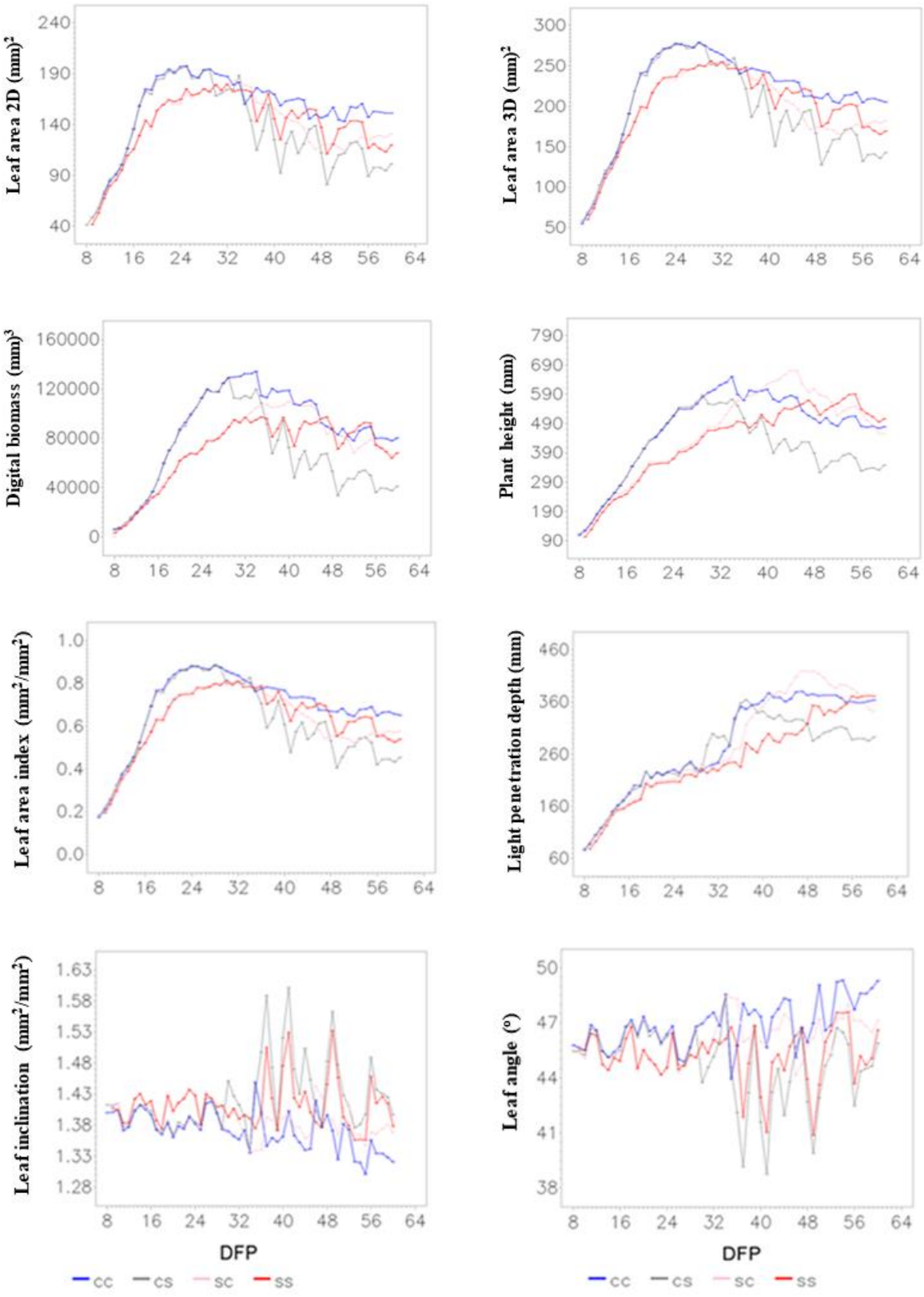


Figure 22. Traces of mean morphological trait values over all genotypes of respective treatment against plant age in 2018.

3.3.3 Descriptive statistics of canopy traits

Continuous laser scanner data indicated that some of the morphological parameters attained a maximum value at some point of the plant age and others oscillated around some value throughout the plant age. Therefore, the maximum and mean value was calculated for each canopy trait by calculating the maximum (PH, A2D, A3D, DB, LAI and LPD) and mean (LI and LA) for each replicate plant and subsequently calculating the mean value for each genotype, treatment and year (2015 to 2018). The data normality was checked by Shapiro-Wilk test and presented as a supplementary table (supplementary Table S15). Analysis of variance was done for the effects of genotype and treatment and their interaction on respective parameters. The summary tables of the ANOVA results are presented for data combined over all experiments of each population in Table 6 and each respective experiment in Table 7. All parameters determined in this study were affected by water stress. In population A, the responses of genotypes to water stress varied between genotype as indicated by the significant genotype by treatment interactions (Table 7). In population B, genotype by treatment interaction was observed on leaf area 3D and LAI (Table 7).

Table 6. ANOVA summary for combined data analysis over two experiments: The effect of genotype, treatment, year and their interaction on the maximum of plant height (PH), leaf area (2D and 3D), light penetration depth (LPD), leaf area index (LAI), digital biomass (DB) and the mean values of leaf inclination (LI) and leaf angle (LA) in 2015 and 2016 for population A and in 2017 and 2018 in population B. F-values and significance levels are shown. * - significant at a p-value of 0.05 and ns - nonsignificant at a p-value of 0.05. Values not assigned a * or ns were significant at p-value of 0.01.

Pop	Parameter	Model	G	T	Y	G×T	G×Y	G×T×Y
A	DF	255	63	1	1	63	63	64
	PH	26.84	16.14	5027.20	11.05	3.99	3.06	5.04
	A3D	13.29	17.21	770.47	718.06	2.04	2.08	8.57
	A2D	9.6	11.42	675.90	407.99	2.48	1.97	5.59
	LA	10.68	21.30	204.69	142.91	3.66	3.15	9.48
B	DF	163	20	3	1	60	19	60
	PH	12.31	13.20	295.68	643.32	1.12^{ns}	1.35^{ns}	1.93
	A3D	11.45	43.24	79.92	524.63	1.61	3.90	1.50
	A2D	11.91	38.27	110.34	631.14	1.51	4.52	1.18^{ns}
	LA	146.3	5.86	7.35	22958.3	2.32	4.64	2.29
	DB	5.99	6.16	176.42	0.34^{ns}	0.95^{ns}	1.68^{*}	3.88
	LAI	9.69	42.23	83.38	238.67	1.53	3.71	1.65
	LPD	48.01	9.19	51.40	7123.10	1.01^{ns}	2.14	1.82
	LI	12.15	28.88	245.81	35.72	1.67	6.91	5.91

Pop - population, G - genotype, T- treatment, Y-year

Table 7. ANOVA summary: The effect of genotype, treatment and their interaction on the maximum of plant height (PH), leaf area (2D and 3D), light penetration depth (LPD), leaf area index (LAI), digital biomass (DB) and the mean values of leaf inclination (LI) and leaf angle (LA) in 2015 -2018. F-values and significance levels are shown. * and ns are significant at p-value of 0.05 and not significant, respectively. Values not assigned by * or ns were significant at p-value of 0.01.

Pop	year	Parameter	Model	G	T	G × T
A	2015	DF	255	63	1	63
		PH	9.68	8.07	1662.92	3.73
		A3D	3.13	7.82	66.61	2.21
		A2D	4.05	12.15	31.34	1.98
		LA	3.76	10.87	23.11	2.28
A	2016	PH	15.13	9.24	2974.28	3.62
		A3D	4.86	5.27	689.46	2.11
		A2D	6.23	7.38	916.19	1.87
		LA	7.50	13.16	771.39	3.46
B	2017	DF	210	20	3	60
		PH	4.42	6.10	204.32	0.79^{ns}
		A3D	4.25	29.17	22.05	1.56
		A2D	4.18	26.01	45.05	1.47^{*}
		LA	1.44	4.28	2.47^{ns}	1.84
		DB	3.39	4.88	138.65	0.80^{ns}
		LI	4.12	28.34	22.56	1.49^{*}
		LI	5.10	19.54	139.87	1.60
		LPD	4.34	29.07	31.51	1.09^{ns}
B	2018	DF	203	19	3	57
		PH	3.42	9.42	98.76	1.51^{*}
		A3D	3.36	15.81	68.01	1.26
		A2D	3.31	15.51	67.31	1.28^{ns}
		LA	2.71	3.00	102.14	1.02^{ns}
		DB	5.78	18.05	208.56	1.30^{ns}
		LAI	3.45	16.00	70.56	1.38^{*}
		LI	5.14	21.03	147.60	1.14^{ns}
		LPD	1.93	3.44	39.23	1.03^{ns}

Pop - population, G - genotype, T - treatment, Y- year

The ANOVA, in which all data of population A or B were analysed together, indicated that the response of genotypes to water stress varied from genotype to genotype as well as between years as indicated by significant genotype x treatment x year interaction (Table 6).
In population A, the comparison of means (Figure 23) indicated that plants under ss conditions had relatively smaller maximum plant height and leaf area (2D and 3D) than plants under optimal water conditions. However, leaf angle was higher (more horizontal leaves) in the cc condition than ss condition. Data combined over years indicated that maximum plant height, leaf area and mean leaf angle were smaller in ss plants than in cc plants (supplementary Table S7).

In population B and both years, the comparison of means of maximum plant height, leaf area (3D and 2D) and digital biomass indicated that higher values were observed under optimal water condition than ss condition (Figure 24). On the other hand, LPD was higher under sc condition than under optimal condition and ss and cs conditions. The same was true for data combined over the years (supplementary Table S7). Data combined over the years indicated that LI was higher under ss than other treatments. Among the treatments, late stress (cs) resulted in the smallest maximum PH, DB and LPD in 2017 (Figure 24). In both years, maximum leaf area under late stress was higher than under early stress, indicating that plants had attained maximum values before the onset of stress. On the other hand, maximum plant height under cs was less than under sc stress. This indicated that different canopy parameters reached their biological maximum values at different times of the plant age. Therefore, considering different stress timing and stress duration may be important to predict tolerance.

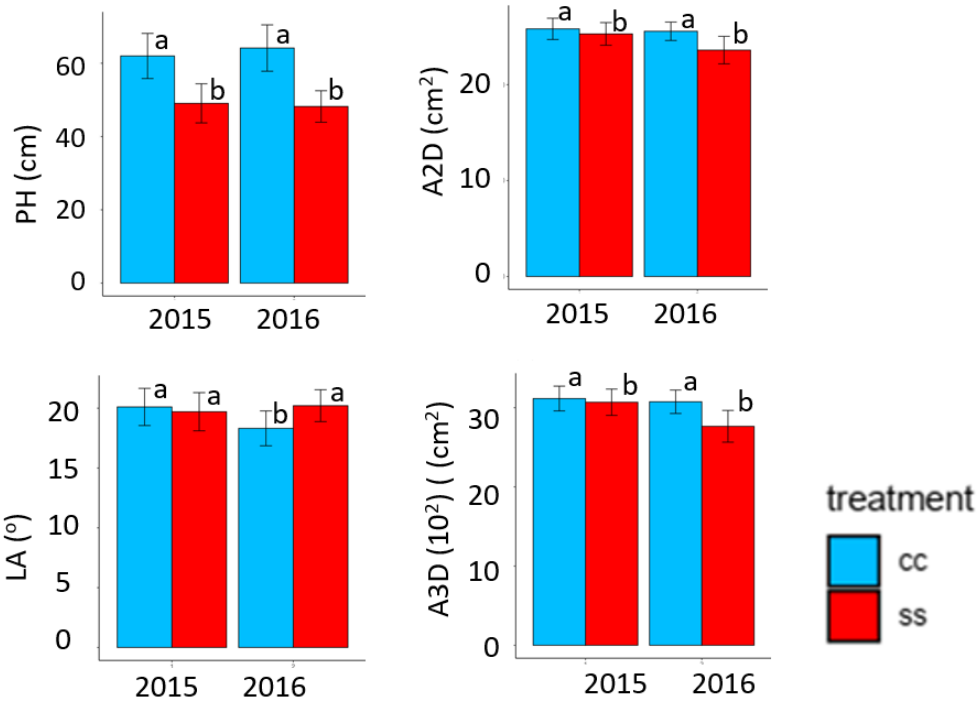


Figure 23. The maximum plant height (PH), maximum leaf area (A2D, A3D), and mean leaf angle (LA) in genotypes of population A under optimal water supply (cc) and long-term drought stress (ss) in FGH experiment 2015 and 2016. Means comparison was done by *regwq*. Means with the same letter were not significantly different at p-values of 0.05. Bars represent mean ± SD of replicates.

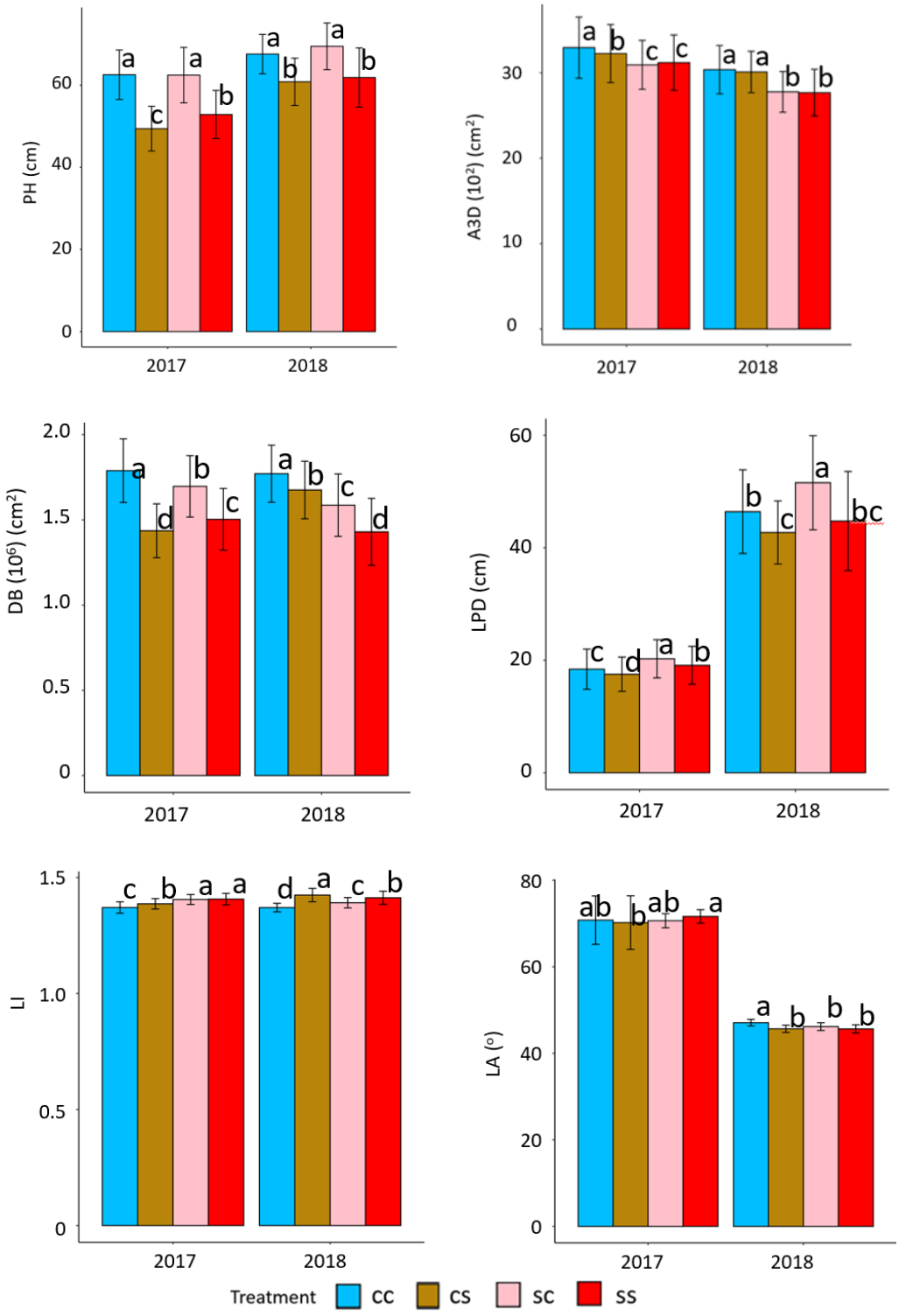


Figure 24. The maximum plant height (PH), maximum leaf area A3D, maximum light penetration depth (LPD), mean leaf angle (LA), mean leaf inclination (LI) and maximum digital biomass (ten thousand) in genotypes of population B under four different treatments in FGH experiments 2017 and 2018. Means comparison was done by *regwq*. Means with the same letter were not significantly different at p-values of 0.05. Bars represent mean ± SD of replicates.

The Pearson correlation coefficients between morphological traits measured under the different treatment conditions and DRYMp values predicted in respective treatment conditions are presented in Table 8. However, morphological traits measured cc conditions were correlated with DRYMp calculated in long-term stress condition.

In population B, light penetration depth was significantly and negatively correlated with DRYMp estimated in the respective treatment condition in 2017. In 2017, further negative correlations between DRYMp and morphological parameters were observed for maximum plant height under cs and ss conditions. On the other hand, mean leaf angles under ss and sc condition were positively correlated with respective DRYMp in 2017. Leaf area 2D under cs and sc were positively correlated with DRYMp values. In 2018, DRYMp was significantly correlated with leaf angle and leaf inclination in treatment ss. The Leaf angle also showed a positive significant correlation with DRYMp under cs condition. In data combined over years of population B, the mean LA in ss and sc conditions were positively correlated with average DRYMp in FGH treatments. Whereas the maximum LPD (in ss, cc and sc) and mean LI (in ss, cc and cs) were negatively correlated with average tolerance index in FGH experiments. Among all the parameters considered under ss conditions in population B, leaf angle showed the most consistent result thus leaf angle may be a promising predictor for drought tolerance under long-term stress conditions.

In population A, correlations between morphological parameters and DRYMp were either weaker or not significant in 2015 (Table 8). One of the probable reasons for lower correlation in population A was that plants lodged before they attained the maximum canopy height. In data combined over years in population A, the maximum PH in both optimal and long-term stress conditions were negatively correlated with average DRYMp in FGH conditions. Altogether, in population A, tolerance was related with shorter plant heights.

Altogether, in population A, tolerance was related with shorter plant heights (2016 and combined) and in population B experiments, tolerant genotypes showed more horizontal leaf in long-term stress than the susceptible genotypes.

Table 8. Pearson correlation coefficient between DRYMp calculated from starch yields of each experiment and treatment conditions and average FGH with maximum plant height (PH), leaf area (A2D and A3D), digital biomass (DB), leaf area index (LAI), light penetration depth (LPD) and the mean of leaf angle (LA) and leaf inclination (LI) for population A and population B of the same experiment measured under the conditions given for treatment. DF was 62 (2015 and 2016), 19 (2017) and 18 (2018). * and ** indicate significance at p-value of 0.05 and 0.01. T- treatment, com - combined over years. canopy parameters measured in optimal conditions were correlated with DRYMp of long-term stress conditions. Pop - population.

Pop	year	T	PH	A3D	A2D	LA	DB	LAI	LI	LPD
A	2015	cc	-0.21	0.14	0.19	-0.08				
		ss	-0.23	0.18	0.22	0.08				
A	2016	cc	**-0.26***	-0.15	-0.06	-0.05				
		ss	0.08	-0.13	-0.08	-0.15				
B	2017	cc	-0.30	0.32	0.31	-0.06	0.01	0.32	**-0.47***	**-0.51***
		cs	**-0.56****	0.39	**0.43***	0.03	-0.19	0.38	-0.22	**-0.64****
		sc	-0.41	0.39	**0.44***	**0.50***	0.27	0.39	-0.33	**-0.48***
		ss	**-0.48***	0.37	0.40	**0.52***	-0.07	0.39	-0.37	**-0.56****
B	2018	cc	-0.23	-0.01	0.01	0.39	-0.19	-0.02	-0.37	-0.37
		cs	-0.26	0.14	0.25	**0.49***	-0.04	0.12	-0.36	-0.26
		sc	0.13	0.02	0.06	0.17	0.35	0.02	-0.14	-0.17
		ss	0.05	-0.21	-0.10	**0.63****	0.10	-0.22	**-0.60****	-0.36
B	com	cc	-0.40	0.03	0.05	-0.23	-0.27	0.04	**-0.59****	**-0.49***
		cs	-0.33	0.11	0.12	0.00	-0.06	0.11	**-0.50***	-0.22
		sc	-0.30	0.13	0.20	**0.48***	-0.18	0.12	-0.29	**-0.51***
		ss	-0.42	0.05	0.10	**0.59****	-0.26	0.04	**-0.71****	**-0.62****
A	com	cc	**-0.51****	-0.04	0.11	-0.15				
		ss	**-0.31****	-0.01	0.09	-0.17				

Nb. Traits measured under cc conditions were correlated with DRYMp in ss conditions.

The difference between optimal water condition and respective stress treatment of maximum (PH, A2D, A3D, DB, LAI, LPD) and mean (LA and LI) were calculated then correlated with DRYMp of respective treatment and each experiment. In data combined over years, the difference between optimal water and stress values were correlated with average DRYMp of respective population in FGH conditions. In specific experiment condition, DRYMp values were significantly correlated with difference values between optimal and long-term stress of maximum PH (2015), mean LA and mean LI (2018).

In data combined over years of population B, the difference between optimal condition and long-term stress condition of maximum LPD and mean LI were positively correlated with tolerance (average DRYMp). In early stress condition, mean LA was negatively correlated with tolerance of average DRYMp. In population A, the difference between the optimal and long-term stress of maximum plant height was negatively correlated with average tolerance of population A in FGH. In both populations, the correlation values between difference values between optimal and stress conditions and tolerance index were not showed consistent values so that using values of canopy parameter difference between optimal and stress as predictive parameters may not be effective.

Table 9. Pearson correlation coefficients between difference values between optimal water and stress condition of maximum plant height (PH), leaf area (A2D and A3D), light penetration depth (LPD), digital biomass (DB), leaf area index (LAI) and mean leaf inclination (LI) and leaf angle (LA) with tolerance index (DRYMp) of each experiment. * and ** denote significance at 0.05 and 0.01 levels, respectively. T- treatment, com - combined over years of respective population (Pop).

Pop	Year	T	PH	A2D	A3D	LA	DB	LAI	LI	LPD
A	2015	ss	**-0.31***	-0.08	-0.12	0.05				
A	2016	ss	-0.22	0.01	-0.04	0.00				
B	2017	ss	0.27	-0.19	-0.12	-0.30	-0.05	-0.14	-0.08	0.11
B	2017	sc	-0.05	0.15	0.22	-0.27	-0.19	0.22	-0.34	-0.09
B	2017	cs	0.22	-0.17	-0.18	-0.32	-0.18	-0.18	-0.36	0.27
B	2018	ss	-0.04	0.14	0.18	**-0.64****	-0.15	0.16	**0.69****	0.20
B	2018	sc	0.12	-0.34	-0.31	-0.17	-0.19	-0.33	0.13	-0.02
B	2018	cs	-0.28	0.00	0.06	-0.06	-0.26	0.11	0.16	-0.18
B	Com	ss	0.25	-0.05	-0.03	-0.42	-0.03	0.02	**0.58****	**0.47***
		cs	-0.08	0.16	0.12	-0.32	-0.30	0.16	-0.23	-0.02
		sc	-0.22	-0.13	0.01	**-0.60****	-0.39	0.03	0.06	0.15
A	Com	ss	**-0.32***	0.01	-0.03	-0.01				

3.3.4 Initial linear growth rate

The initial change of morphological traits plotted against plant age was determined by fitting a linear regression model for each genotype and year in the data range, in which the respective trait increased approximately linearly, thus calculating the initial slope of the growth curve. This value is abbreviated as slope of the trait in the subsequent text.

In population A, the initial slope of leaf area 2D and leaf area 3D were significantly correlated with tolerance level in 2015 but not in 2016 (Table 10). In the 2015 experiment, tolerant genotypes showed relatively higher slope of leaf area than susceptible genotypes.

In population B, DRYMp was positively correlated with the slope of leaf inclination under cc and ss condition in 2017 (Table 10). Slopes of plant height under cc and cs condition were negatively correlated with DRYMp. In addition, the slope of digital biomass under cs and the slope of light penetration depth were negatively correlated with DRYMp. In 2018 under cc condition, the slope of leaf angle was negatively correlated with DRYMss values. On the other hand, leaf inclination slope was positively correlated with DRYMss values. Thus, low leaf angle slopes and high leaf inclination slopes under cc condition were associated with high tolerance of genotypes. Since leaf inclination slope was consistently correlated with DRYMp under cc condition in both years, it may be potential canopy parameters that can be measured under well-watered conditions to predicted drought tolerance in potato. Generally, in population B, lower initial slopes of plant height under cc and higher slope of leaf inclination under cc or ss condition were associated with drought tolerance.

Generally, in both populations, the correlations of growth rates and DRYMp of genotypes were mostly not consistent, which implies that DRYMp prediction from growth rate determined in small experiments could be difficult.

Table 10. Pearson correlation coefficients between slope of linear regression of plant height (PH), leaf area (A2D and A3D), leaf angle (LA), digital biomass (DB), leaf area index (LAI), leaf inclination (LI) and light penetration depth (LPD) on plant age determined under different treatments with DRYMp of respective experiment. df was 62 (2015 and 2016), 19 (2017) and 18 (2018) experiment. * and ** denote significance at 0.05 and 0.01 level, respectively. T - treatment.

Pop	year	T	PH	A3D	A2D	LA	DB	LAI	LI	LPD
A	2015	cc	-0.12	**0.39****	**0.38****	-0.22				
		ss	0.07	**0.48****	**0.49****	-0.27				
A	2016	cc	-0.16	0.15	0.16	-0.05				
		ss	0.16	0.19	0.21	-0.03				
B	2017	cc	**-0.46***	0.15	0.14	-0.32	-0.27	0.15	**0.49***	-0.32
		cs	**-0.46***	0.14	0.11	-0.23	**-0.47***	0.14	0.31	**-0.62***
		sc	-0.42	0.30	0.26	0.05	-0.18	0.30	0.24	-0.16
		ss	-0.41	0.06	0.03	-0.09	-0.31	0.06	**0.56****	-0.38
B	2018	cc	-0.11	0.35	0.32	**-0.47***	0.03	0.32	**0.50***	-0.19
		cs	-0.14	0.01	0.02	-0.14	-0.03	0.00	0.10	-0.17
		sc	0.09	-0.21	-0.15	0.10	0.13	-0.16	-0.18	-0.24
		ss	0.02	0.09	0.09	-0.27	-0.05	0.12	-0.13	-0.40

-1 1

3.3.5 Polynomial growth model

In the polynomial model, the morphological parameters and DFP were used as independent and dependent, respectively. In population A in ss condition, linear coefficients and quadratic coefficients of leaf area (2D and 3D) were negatively and positively correlated with tolerance index of the same experiment in the 2016 experiment, but not in the 2015 experiment (Table 11). In population B, the linear coefficients for plant height were negatively correlated with DRYMss and long-term stress conditions in 2017 (Table 12). Under cs conditions, linear coefficients for leaf area index and light penetration depth were negatively correlated with tolerance index in 2017. The other significant correlation was observed between DRYMp values and linear coefficients for digital biomass and LPD in 2018 under cc condition (Table 12).

In population B, the correlation between DRYMp and quadratic coefficient values were mostly non-significant. The only significant correlation was observed between DRYMp and quadratic coefficient values for plant height (2017) and light penetration depth (2018) measured under cc condition (Table 12).

In both populations, the result indicates that correlation between linear and quadratic coefficients with DRYMp were not consistent between years. Thus, predicting of tolerance from these parameters may not be possible.

Table 11. Pearson correlation coefficients between linear and quadratic coefficients of polynomial functions of population A of plant height (PH) and leaf area (A2D and A3D) with DRYMp of the respective experiment. df was 62. * and ** denote significance at 0.05 and 0.01 level, respectively.

Experiment	Treatment	Linear coefficient			Quadratic coefficient		
		PH	A2D	A3D	PH	A2D	A3D
2015	cc	-0.23	0.24	0.22	0.22	-0.20	-0.18
	ss	-0.18	0.16	0.13	0.17	-0.11	-0.09
2016	cc	-0.08	-0.23	-0.22	-0.02	0.21	0.20
	ss	-0.24	**-0.36****	**-0.34****	0.20	**0.32****	**0.29***

Table 12. Correlation coefficients between linear and quadratic coefficients of polynomial functions of population B of plant height (PH), leaf area (A2D and A3D), digital biomass (DB), leaf area index (LAI), and light penetration depth (LPD) with DRYMp calculated from starch yields in the respective experiment. df was 19 and 18 in 2017 and 2018 experiments. * and ** denote significance at 0.05 and 0.01level, respectively.

Experiment	Treatment	Linear coefficient					
		PH	A2D	A3D	DB	LAI	LPD
2017	cc	**-0.46***	0.22	0.20	-0.39	-0.10	-0.11
	cs	-0.40	0.00	0.12	-0.05	**-0.51***	**-0.52***
	sc	-0.39	0.27	0.28	-0.30	-0.11	-0.10
	ss	**-0.45***	0.20	0.20	-0.32	-0.24	-0.24
2018	cc	-0.12	0.02	0.01	**-0.47***	-0.22	**0.53***
	cs	-0.17	-0.11	-0.13	-0.20	-0.06	0.19
	sc	0.06	-0.25	-0.29	-0.13	-0.29	0.11
	ss	0.02	-0.25	-0.22	-0.35	-0.24	0.36
		Quadratic coefficient					
2017	cc	**0.44***	-0.16	-0.14	0.39	-0.07	-0.06
	cs	0.37	0.08	-0.05	0.06	0.38	0.39
	sc	0.35	-0.19	-0.20	0.31	-0.01	-0.03
	ss	0.42	-0.06	-0.06	0.33	0.10	0.09
2018	cc	0.16	0.06	0.05	0.45	0.31	**-0.56***
	cs	0.14	0.20	0.23	0.18	0.04	-0.28
	sc	-0.04	0.25	0.32	0.40	0.32	-0.15
	ss	0.03	0.35	0.37	0.42	0.38	-0.36

3.3.6 Diurnal leaf movement

Leaf movements caused diurnal oscillation of morphological parameters estimated from laser scanner records. The time windows used for this analysis were between 26-41 (2015), 29-44 (2016), 14-72 (2017) and 21-73 (2018) DFP. From continuous laser scanner records, the mean of the daily range was calculated for each morphological parameter, genotype and treatment condition and correlated with DRYMp (Table 13).

In population A, mean PH ranges in control and stress condition were negatively correlated with tolerance index in 2015 but not in 2016 experiment. In combined data over both experiments on population A indicated that the range of leaf angle (LA) and leaf area (A2D and A3D) under ss condition were negatively (LA) and positively (leaf area) correlated with mean DRYMp values of both experiment (Table 13). Ranges for plant height measured under control conditions were negatively correlated with average DRYMp values of the genotypes calculated from both experiments.

In population B in 2018, the means of the daily ranges for plant height (PH), leaf angle (LA), leaf inclination (LI) and light penetration depth (LPD) measured under ss treatment condition were correlated with DRYMp of the same experiment. The correlation coefficient indicated that the tolerance index was negatively correlated with PH, LI and LP and positively correlated with leaf angle (Table 13). Thus, genotypes that showed higher diurnal variation of leaf angle were tolerant. However, these correlations were not found in 2017 experiments. In combined data over both population B experiments, leaf angle and leaf inclination in sc condition were negatively correlated with average DRYMsc values in two-year experiments.

In both years, the correlation values between the range of leaf movement with tolerance index were not consistent, indicating that using the range of leaf movement measured in small experiments as predictive parameter for the drought tolerance index may not be effective. In summary, the range of leaf movement was a poorer predictor of DRYMp values than the maximum/mean value of morphological traits.

Table 13. Correlation coefficients between daily ranges of plant height (PH), leaf area (A2D and A3D), leaf angle (LA), digital biomass (DB), leaf area index (LAI), leaf inclination (LI) and light penetration depth (LPD) with DRYMp of the respective experiment. DF was 62 (2015 and 2016), 19 (2017), 18 (2018), combined over year was 126 (population A) and 39 (population B). * and ** denote significance at 0.05 and 0.01 levels, respectively. T - treatment, com - combined over years of respective population. Pop - population.

Pop	year	T	PH	A2D	A3D	LA	DB	LAI	LI	LPD
A	2015	cc	**-0.47****	-0.23	-0.23	-0.04				
		ss	**-0.34****	-0.22	-0.21	-0.06				
	2016	cc	-0.24	-0.20	-0.23	-0.05				
		ss	-0.17	-0.19	-0.21	-0.06				
A	com	cc	**-0.34****	-0.03	-0.02	0.22				
		ss	0.13	**0.20***	**0.20***	**-0.20***				
B	2017	cc	0.06	0.07	0.10	0.06	0.04	0.10	-0.16	-0.08
		cs	0.14	0.25	0.26	0.01	0.18	0.25	-0.03	0.03
		sc	-0.14	-0.08	-0.06	-0.14	-0.09	-0.06	-0.26	-0.21
		ss	0.02	0.09	0.11	-0.06	0.05	0.11	-0.18	-0.12
B	2018	cc	-0.07	0.01	0.19	0.21	0.09	0.19	-0.21	-0.24
		cs	-0.07	0.04	-0.05	0.33	-0.05	-0.05	-0.31	-0.11
		sc	-0.12	-0.04	-0.12	0.06	0.07	-0.10	-0.07	-0.09
		ss	**-0.46***	-0.16	-0.22	**0.64****	-0.36	-0.21	**-0.65****	**-0.50***
B	com	cc	0.04	0.04	0.07	0.00	0.04	0.07	-0.01	-0.08
		cs	0.18	0.05	0.06	0.31	0.16	0.07	0.31	0.05
		sc	-0.06	0.38	0.36	**-0.58****	0.22	0.35	**-0.59****	-0.08
		ss	-0.07	0.03	0.04	0.01	-0.03	0.04	-0.02	-0.16

3.3.7 The association of daily canopy structure with tolerance

In this study, daily mean values of leaf area (2D and 3D), plant height, digital biomass, leaf angle, leaf inclination, leaf area index and leaf inclination were correlated with DRYMp of the same experiment. There were measurement gaps in 2015 (46-48 DFP) and 2018 (46-49 DFP). The correlation was plotted against plant age (Figure 25, Figure 26 and Figure 27).

In population A under long-term stress conditions, leaf area (2D and 3D) correlated positively and leaf angle correlated negatively with DRYMp of the specific experiment (Figure 25). The correlation values were mostly significant at reproductive stage; however, the correlations were relatively weak.

In population B under long-term stress conditions in 2017, daily mean values of leaf area (2D and 3D), leaf inclination (LI) and leaf angle (LA) measured after 46 DFP were positively correlated with DRYMp of the respective experiment, whereas light penetration depth (LPD) and plant height (PH) were negatively correlated with DRYMp (Figure 26). In 2017 under late stress conditions, DRYMp values were positively correlated with daily mean values of leaf area (A3D and A2D) and LAI after 46 DFP, but negatively correlated with mean daily values of LPD (Figure 26).

In 2018 in long-term stress conditions, daily mean values of leaf angle (LA) and leaf area (A2D and A3D) were positively correlated with DRYMp values, whereas light penetration depth (LPD) and leaf inclination (LI) were negatively correlated with DRYMp values of specific experiments. The correlation values were affected by plant age after 52 DFP, especially the correlation between leaf area, LPD and DRYMp values (Figure 27).

In both populations, tolerant genotypes had a higher leaf area (A3D and A2D) and lower light penetration depth (LPD) (population B) under stress conditions. The association of canopy parameters and tolerance index was closest in the reproductive stage (since flower initiation), especially in ss treatment conditions (Figure 26 and Figure 27).

Generally, among the traits considered in this study, leaf area (2D or 3D), leaf angle and light penetration depth looked promising to predict water stress tolerance under long-term stress conditions especially when determined in the reproductive stage. Therefore, tolerance prediction in ss stress could be improved from canopy parameter phenotyping since flower initiation to maturity.

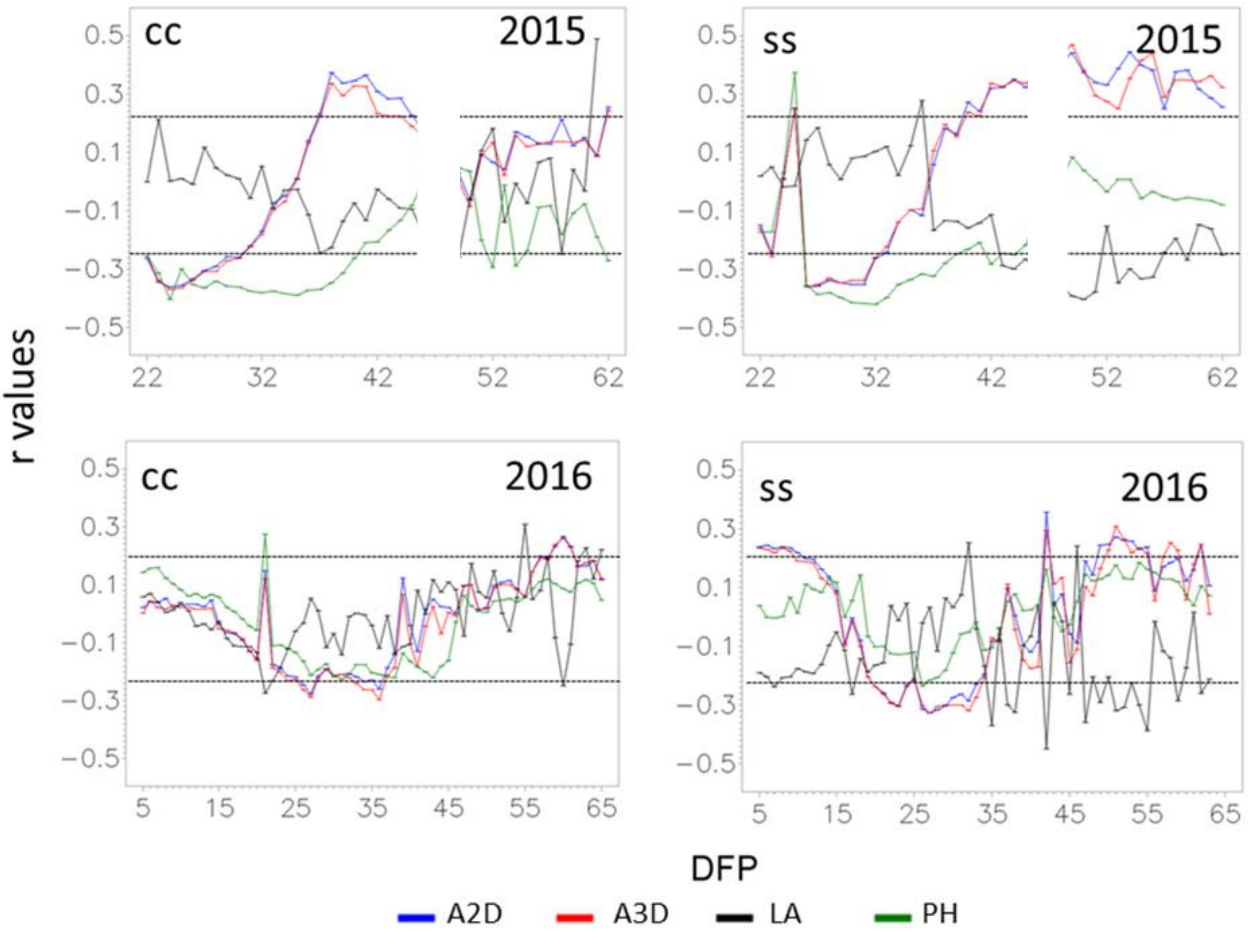


Figure 25. The Pearson correlation coefficients between the daily means of morphological traits measured in treatment cc and ss of experiment 2015 or 2016 and DRYMp of the same experiment depicted against plant age. DF was 62 in 2015 and 2016 experiments. Significance threshold at a p-value of 0.05 is +/- 0.25 and indicated by horizontal broken lines. Measurement gaps (46-48 DFP) in 2015 experiment present as a gap.

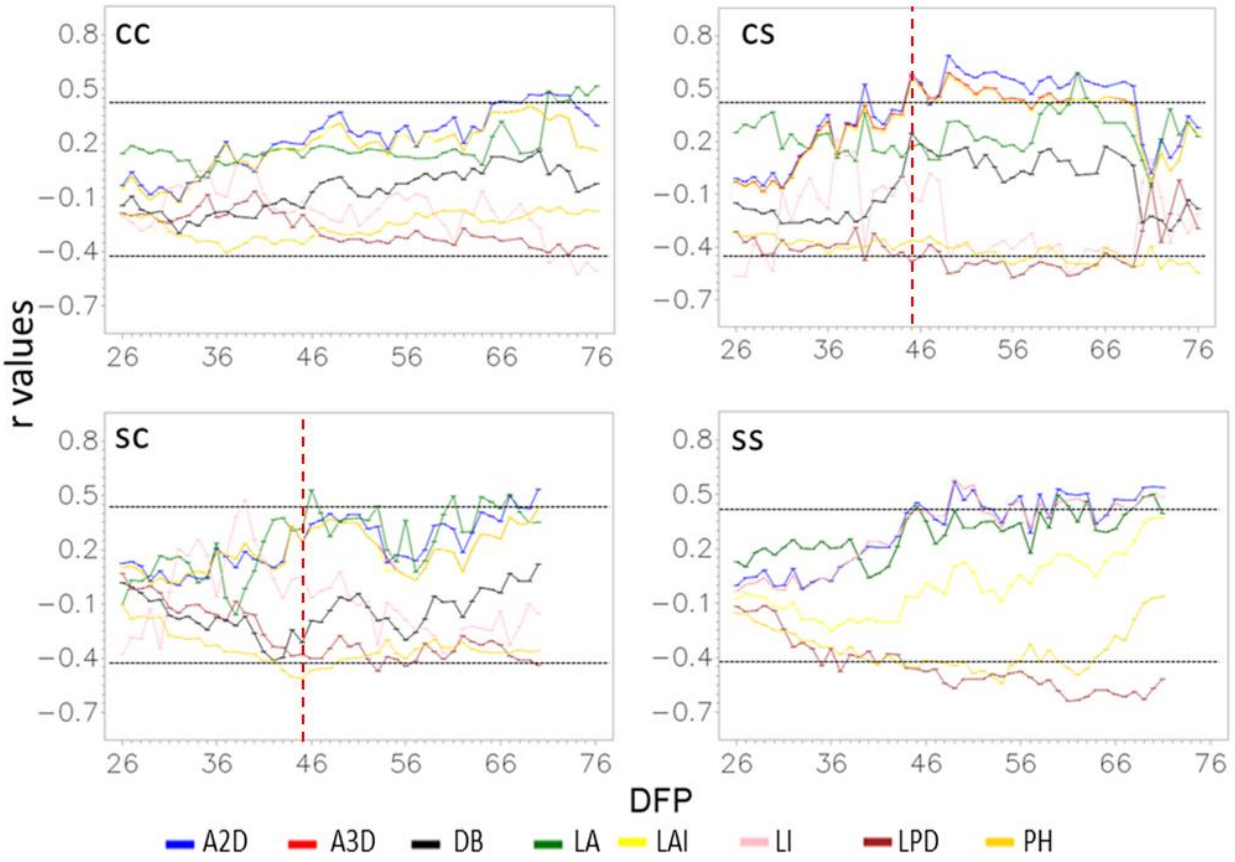


Figure 26. The Pearson correlation between the daily means of morphological traits measured in treatment cc, cs, sc and ss of experiment 2017 and DRYMp of the same experiment depicted against plant age. DF was 19. Significant threshold at p-value of 0.05 is +/- 0.43 and indicated by horizontal broken lines. Treatments switch date is indicated by the red vertical broken line**.**

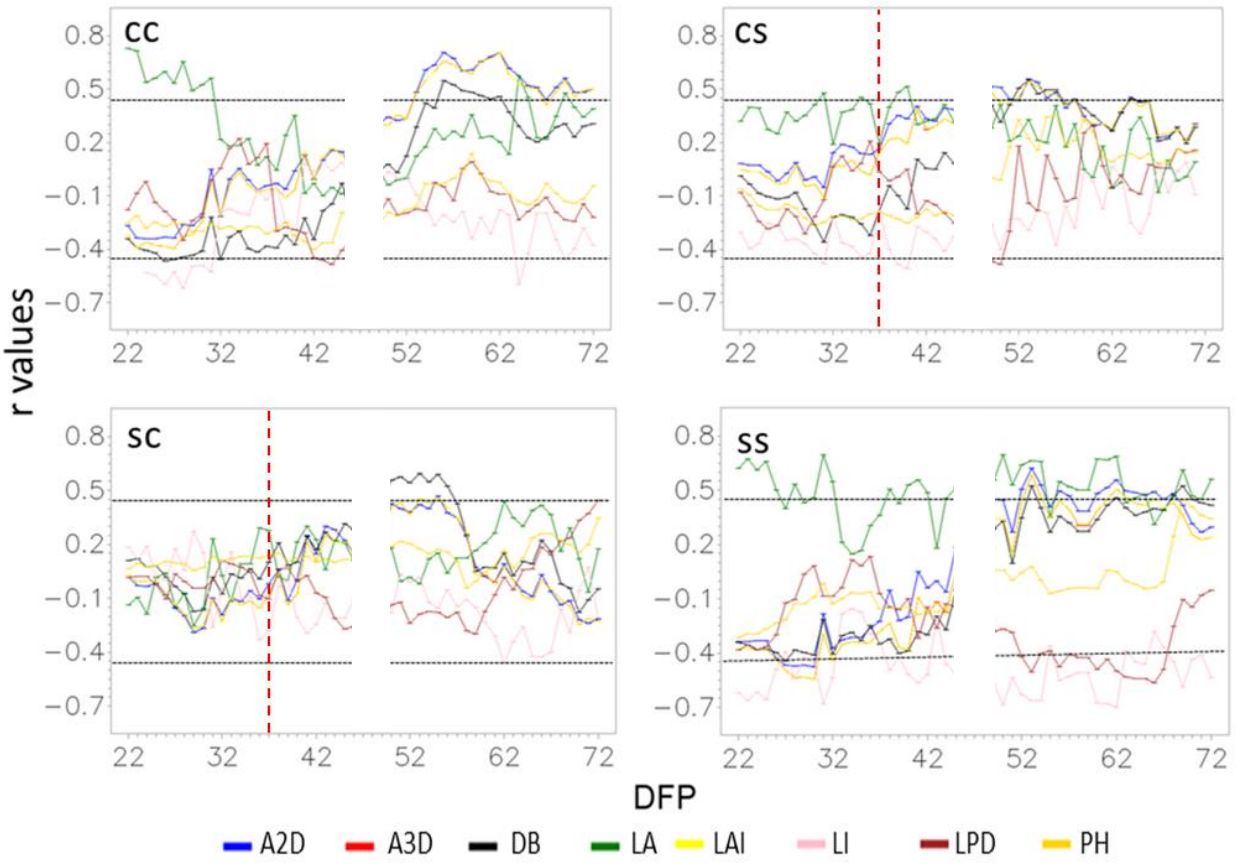


Figure 27. The Pearson correlation between the daily means of morphological traits measured in treatment cc, cs, sc and ss of experiment 2018 and DRYMp of the same experiment depicted against plant age. Significant threshold at a p-value of 0.05 is +/- 0.45 and indicated by horizontal broken lines. DF was 18. Treatments switch date is indicated by the red vertical broken line. Measurement gaps (46-49 DFP) in 2018 experiment present as a gap.

3.4 Tolerance prediction from single morphological parameters

In this case, genotype DRYMp values were predicted from maximum (PH, A2D, A3D, DB, LAI, and LPD) and mean (LI, LA and CTD) morphological and physiological parameters measured from the same experiment of respective treatment (Table 14). In population B under long-term stress condition, tolerance was predicted from either of the following parameters: LA and LI. In 2017 experiment, tolerance was predicted from different parameters and treatment conditions. For example, under cc, cs and ss conditions, tolerance was predicted from LPD. In addition to LPD, in ss and cs conditions tolerance was predicted from PH. Tolerance prediction under sc conditions was not effective in population B. However, tolerance prediction in population A was only possible from LA under ss condition in 2016.

Generally, in population B, simple linear regression output indicated that DRYMp values could be linearly predicted from different canopy traits, but the model R^2 values were relatively low (Table 14). This indicated that tolerance levels of genotypes are not fully explained by any one of the single canopy parameters considered in this study.

Table 14. DRYMp predicted from each canopy traits (mean or maximum) of which were measured in the same experiment and treatment condition. * and ** were significant at 0.05 and 0.01 p-values. Model R^2 values were shown. Parameters measured in cc condition was used to predicted tolerance in long-term stress conditions.

Treatment	Parameter	Year					
		2016		2017		2018	
		Intercept	R^2	Intercept	R^2	Intercept	R^2
cc	LPD			-0.002*	0.18		
	LI			-2.668*	0.19		
cs	LPD			-0.002**	0.30		
	PH			-0.002*	0.24		
ss	LPD			-0.002*	0.29		
	LI			-2.368*	0.15	-3.011**	0.47
	LA	-0.043*	0.11	0.052**	0.27	0.089**	0.44
	PH			-0.002*	0.22		

3.5 Canopy temperature and canopy temperature depression

3.5.1 Surface temperature

Surface temperature was measured in population B experiments by infrared thermosensors (IRT), which where mounted on the Fieldscan system. The movement of the Fieldscan system was controlled by the software that controlled the laser scanner measurements. The raw data of IR thermosensor measurements of one scanning cycle is shown in Figure 28. The IRT was programed at 3 s scanning interval. The Fieldscan carrier device moved at a speed of 32 mm/s during the forward movement whilst the laser scanner measurements were recorded. Fieldscan moved about three times as fast during the reverse movement between the measurements cycle (Figure 28). After eight scanning and reversing movements, the Fieldscan remained in the parking position for 32 min before moving forward for the next measurement. Figure 28 illustrates that the plant canopy was much cooler than the metal barcodes that were positioned between the plant blocks and ground between the blocks without plants and in the parking position. The plant canopy temperature data were attributed to the plant metadata based on the time stamp of the IRT data and the time stamp of the laser scanner data. The time stamp was corrected for plant position and the time lag of 47 s,

which was caused by the physical distance between the IRT sensors and the laser scanner sensors on the Fieldscan (see Material and Methods and Figure 7).

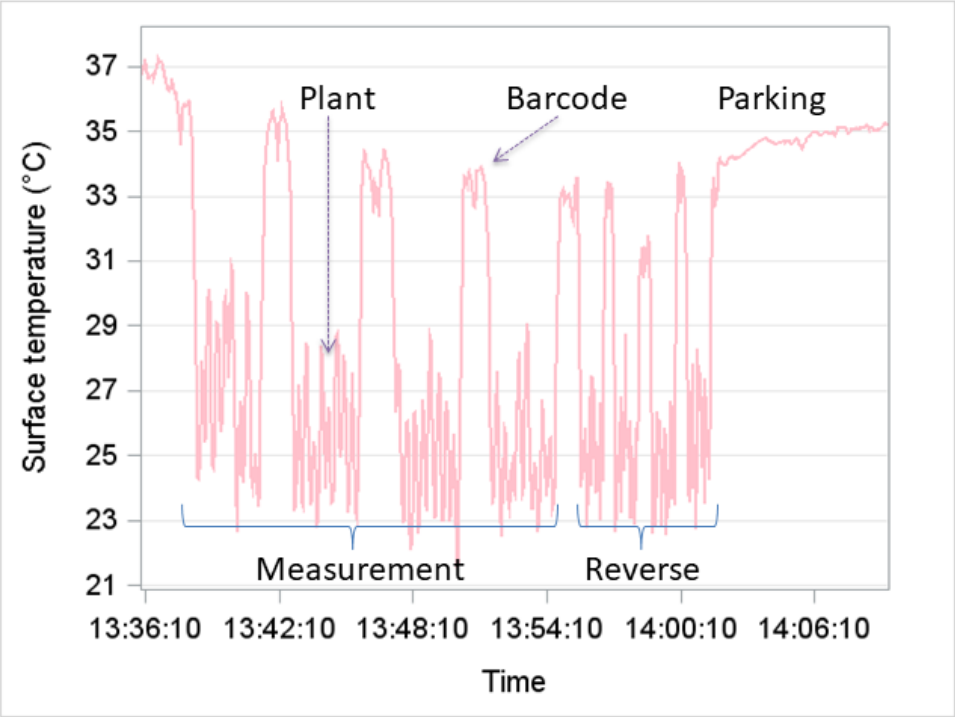


Figure 28. Surface temperature measurements during one movement cycle of the Fieldscan in the early afternoon of 15 May 2018. The temperatures for the plant canopy, the metal surfaces of the barcodes and the ground in the parking position. Fieldscan moved with 32 mm/s during measurement and reversed in the parking position with a speed of 82 mm/s.

3.5.2 IRT data quality

Three values of canopy temperature were measured for each plant every 30 min between 27 to 73 DFP (2017) and 24 to 74 DFP (2018). *Proc gplot* and *proc sql* were run in SAS (Version 9.4, SAS) to check for outliers and data uniformity (supplementary Table S15). If the difference between a CT and average CT of a genotype per hour per day was less or greater than two standard deviations away from the mean, the value was considered an outlier.

3.5.3 Effect of drought on mean canopy temperature (CT) and canopy temperature depression (CTD)

In this study, canopy temperature (CT) was observed for nearly seven weeks in the genotypes of population B in 2017 and 2018. CTD was calculated as canopy temperature minus air temperature. Analysis of variance was done on the effects of genotype, treatment, time and their interaction on mean CT and mean CTD (Table 15). For each experiment condition as well as combined over

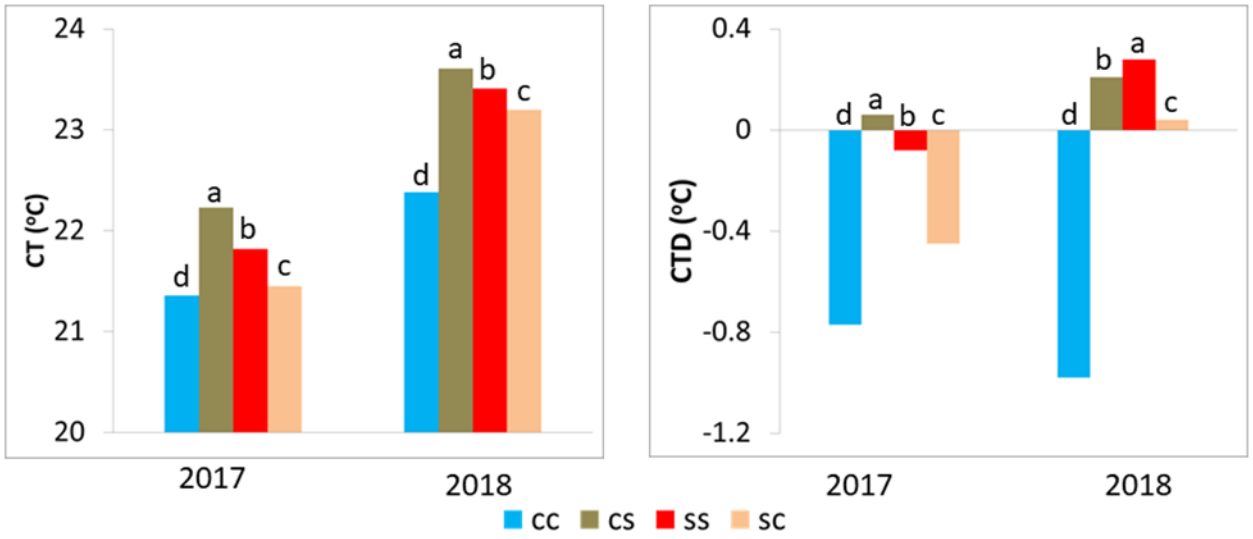
seasons, CT and CTD were significantly affected by water stress. The responses of genotypes to water stress varied from genotype to genotype as indicated by the significant genotype × treatment interactions (see Table 15). Furthermore, the response of genotypes varied from day to day and between years as indicated by the significant genotype x date x treatment interaction and genotype x treatment x year interactions (Table 15). This indicated that the interpretation of CT measurement depends on additional information on environmental conditions and developmental effects.

The comparison of means (Figure 29) indicated that stress after flower initiation (cs) affected CT and or CTD more than stress before flowering (sc). Mean CT of late stress (cs) plants was even higher than CT of long-term stress plants. This may be because of the shock after switching treatments or may be because of a higher rate of senescence, especially in lower leaves. Mean CT of genotypes in respective experiments and treatment conditions (Figure 30) are presented below. Mean separation of CT and CTD of genotypes in respective experiments and treatment conditions are presented as supplementary table (supplementary Table S12).

Table 15. ANOVA summary: The effect of genotype, treatment, day, hour and their interaction on daily mean CT and CTD in 2017 and 2018. F-values are shown. All main and interaction effects were significant at p values of 0.01. Where exp - experiment, G - genotype, T - treatment, D - day, H - hour, P - parameter, Y- year, com - combined over years.

Exp	P	Model	G	T	D	H	GT	GD	GTD	Y	GY	GTY
2017	DF	3809	20	3	45	23	60	900	2751			
	CTD	41	79	2122	1718	1778	31	2	7			
	CT	214	26	784	4721	25867	10	1	3			
2018	DF	3789	19	3	49	23	57	931	2700			
	CTD	55	36	4339	2320	1747	47	2	10			
	CT	225	18	2172	3819	27869	23	1	4.9			
com	DF	4449	20	3	53	23	60	1052	3151	1	19	60
	CTD	67	55	5037	2800	3269	22	3	11	1014	40	68
	CT	222	17	1537	2412	35065	6	4	5	19219	12	20

A


24
23
22
21
20
CT (°C)
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b
c
d
a
b
c
d
2017
2018
0.4
0
-0.4
-0.8
-1.2
CTD (°C)
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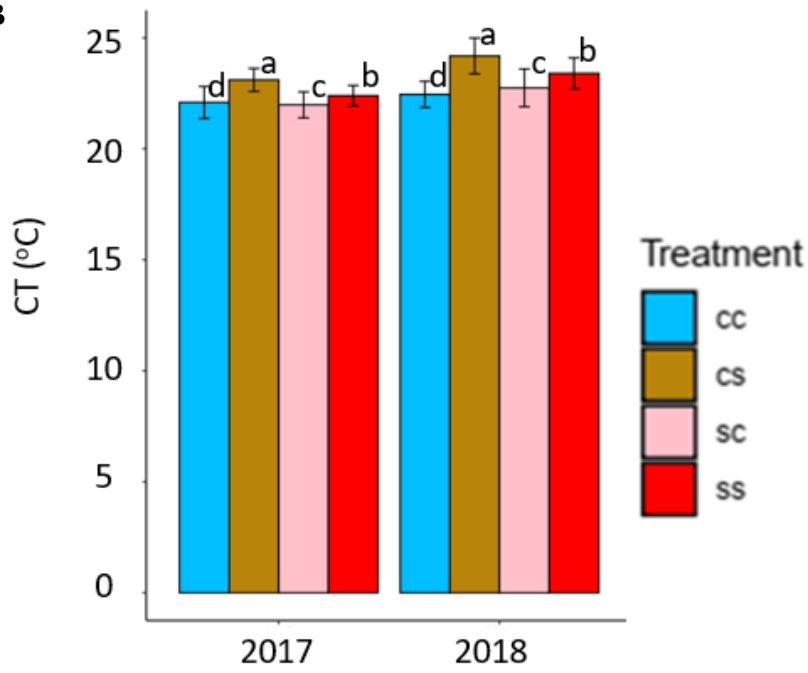
a
b
c
b
a
c
d
2017
2018
cc
cs
ss
sc

B


25
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CT (°C)
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d
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b
2017
2018
Treatment
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ss

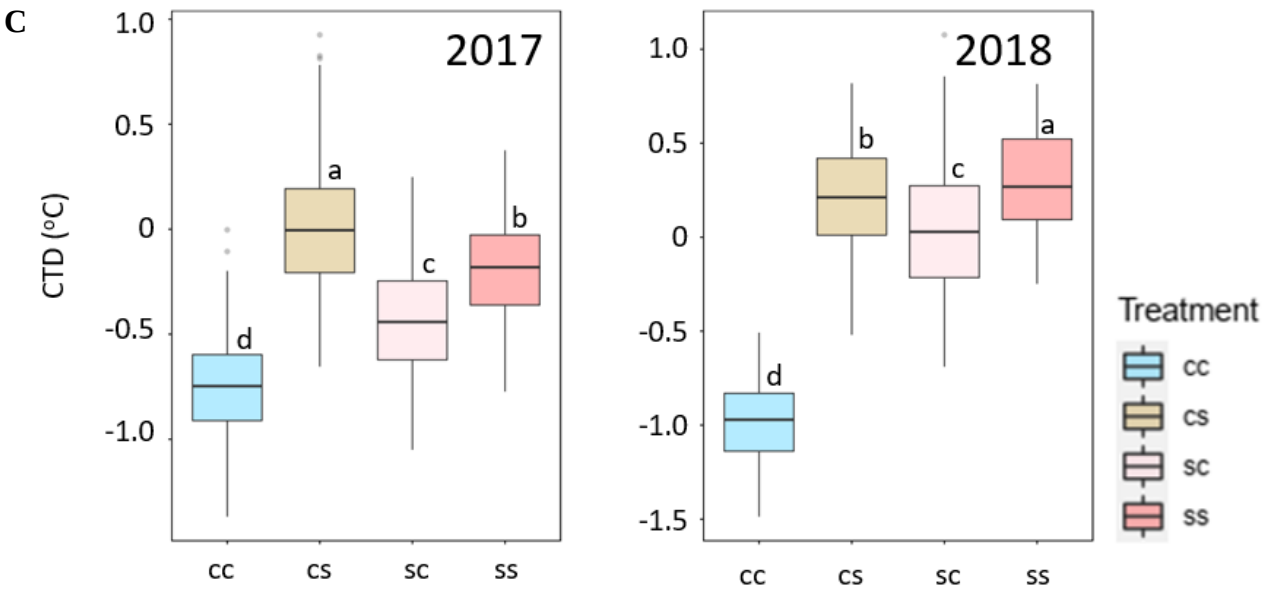


Figure 29. Mean CT (upper) and CTD (lower) between 48-70 DFP (2017) and 50-70 DFP (2018) of the respective FGH experiment. Old (A) and new (B and C). Mean separation was done by *regw test* and treatments assigned by the same letter are not significantly different at p-values of 0.01. Bars represent mean ± SD of replicates.

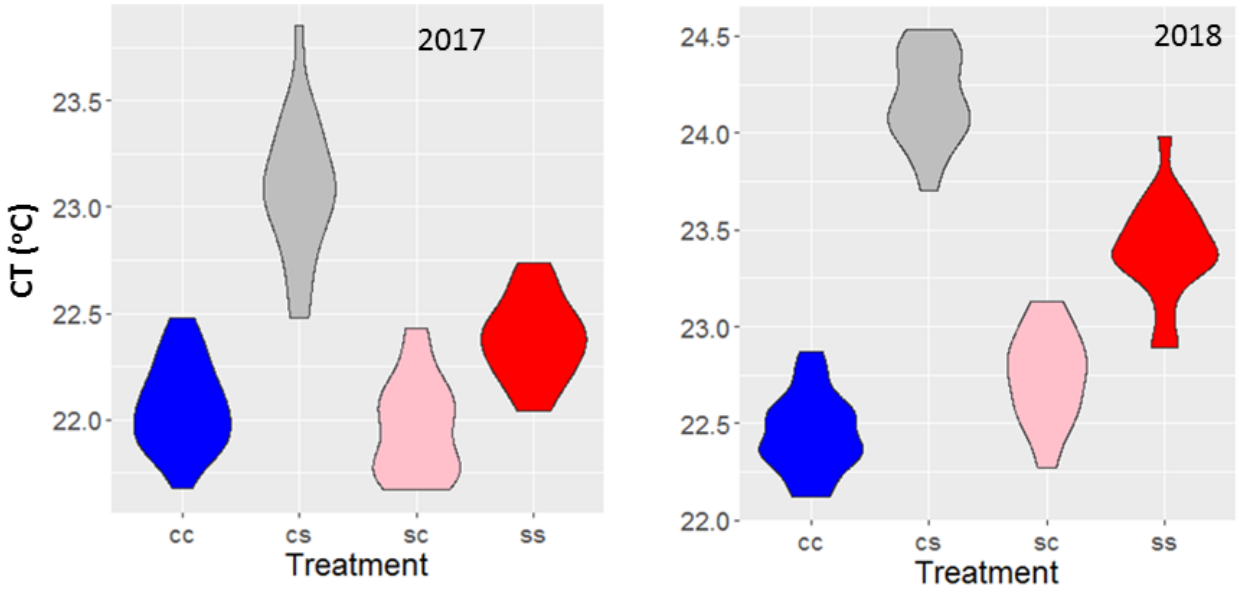


Figure 30. Distribution of genotypic means of canopy temperature (CT) between 48-70 DFP (2017) and 50-70 DFP (2018) in 2017 and 2018 FGH experiments**.**

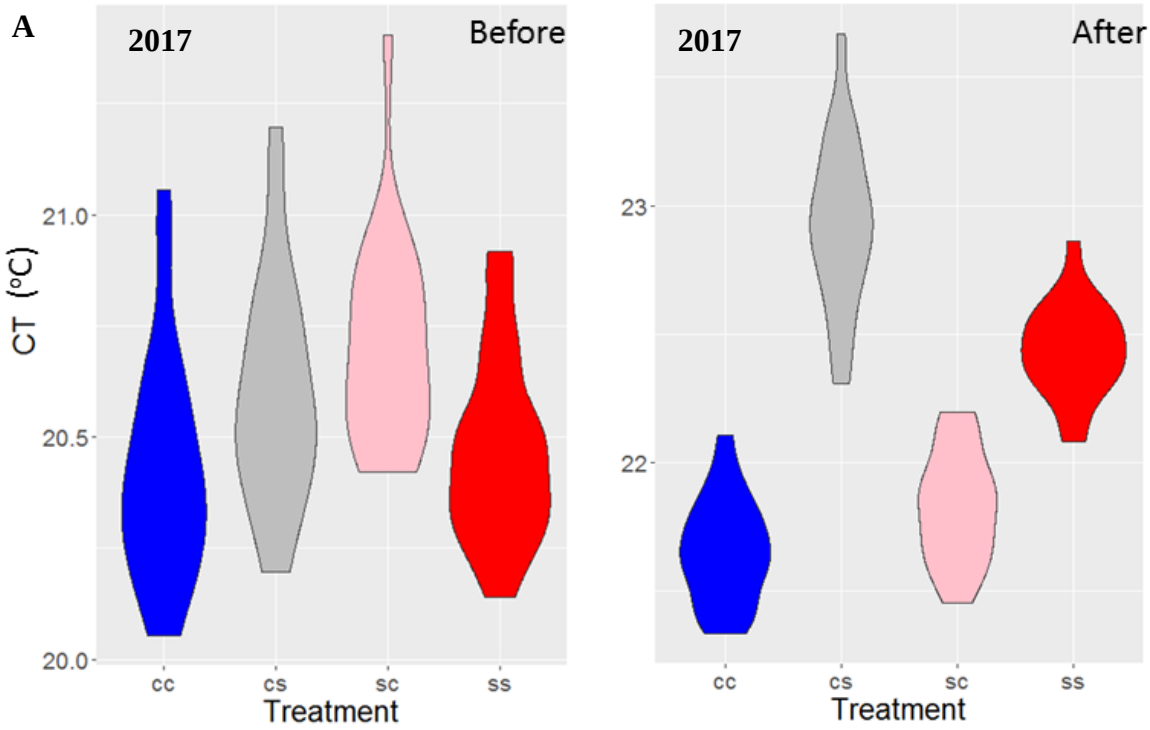
3.5.4 Mean CT and CTD before and after treatment switch

As indicated in Materials and Methods of this study, there were treatment switches from optimum to stress in cs block and from stress to optimum in sc block at 45 (2017) and 38 (2018) days from planting. Based on the treatment switch date, data sets were grouped in before and after treatment switch. Mean CT and CTD for each genotype were calculated for both before and after treatment switch data sets of each experiment. In both years, mean CT (Figure 31) and mean CTD (Figure 32) of plants after treatment switch were higher in stress blocks than in plants under optimal water condition. After treatment switch, CT in cs blocks was higher than mean CT in long-term stress blocks. In the before-treatment switch data set, mean CT and CTD was different between optimal (cc or cs) and stress block (ss or sc) plants in 2018, but not in the 2017 experiment. Relatively cooler air temperature in 2017 experiment, especially for the first seven days after water stress initiation, may play its part in reducing the variation between treatments. The means separation of CT and CTD of respective treatments in before-treatment and after-treatment switch data are presented as Figure (see Figure 31 and Figure 32).

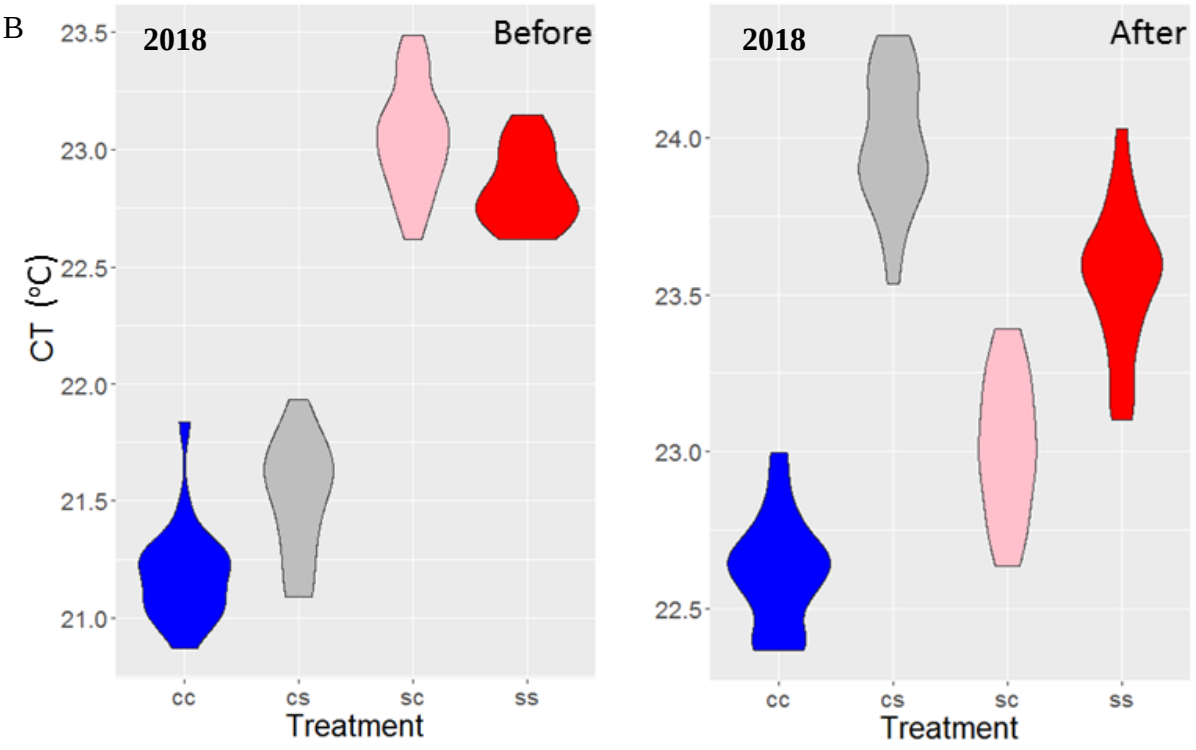
The summary tables of the ANOVA results are presented in Table 16. Both CT and CTD before and after treatment switch were significantly affected by water stress. Furthermore, CT and CTD of genotypes varied between days and treatments as indicated by significant interaction between genotype x treatment x day. This indicates that CT and CTD for drought tolerance should be measured in specific ages of the plant and under specific environmental conditions.

Table 16. ANOVA summary: The effect of genotype, treatment, day, hour and their interaction on CT and CTD before and after switch treatments in 2017 and 2018. F-values are shown. All main and interaction effects were significant at p values of 0.01. ns - nonsignificant at p value of 0.05. G - genotype, T - treatment, D - day, H - hour, P - parameter, Y- year and GS - treatment interval before (B) and after (A) switch of treatments between cs and sc treatments.

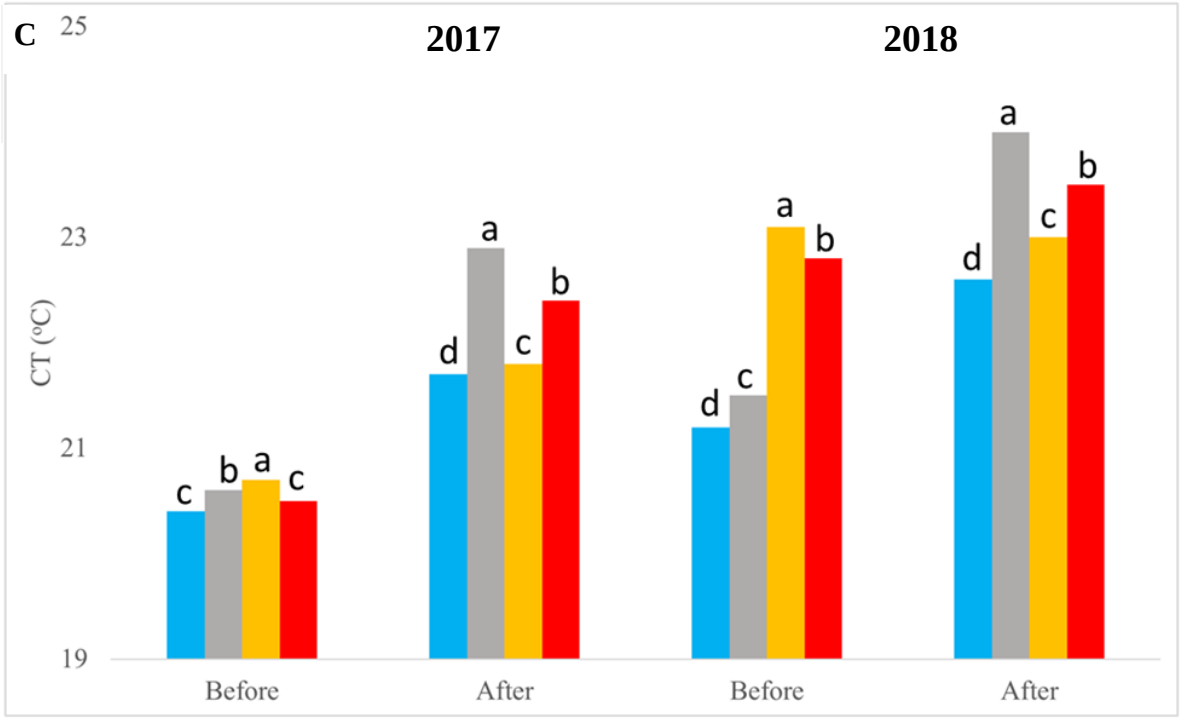
Year	GS	P	Model	G	T	D	H	G×T	G×D	G×T×D
2017	A	DF	2465	20	3	28	23	60	560	1764
		CT	190	21	1318	3675	15481	8	0	2
		CTD	34	63	3753	1433	826	25	1^{ns}	5
	B	DF	1373	20	3	16	23	60	320	924
		CT	234	15	588	5605	10280	4	1^{ns}	1^{ns}
		CTD	39	31	820	1269	851	9	2	4
2018	A	DF	2629	19	3	32	23	57	608	1880
		CT	207	21	2538	3899	17538	13	0^{ns}	4
		CTD	40	46	5527	1551	778	27	1^{ns}	8
	B	DF	1189	19	3	16	23	57	304	760
		CT	312	8	970	3689	12924	7	1^{ns}	4
		CTD	67	13	1180	2203	1025	12	2	7



B


2018
Before
2018
After
CT (°C)
23.5
23.0
22.5
22.0
21.5
21.0
24.0
23.5
23.0
22.5
cc
cs
sc
ss
Treatment

C


2017
2018
CT (°C)
25
23
21
19
c
b
a
c
d
a
c
b
d
c
a
b
d
a
c
b
Before
After
Before
After

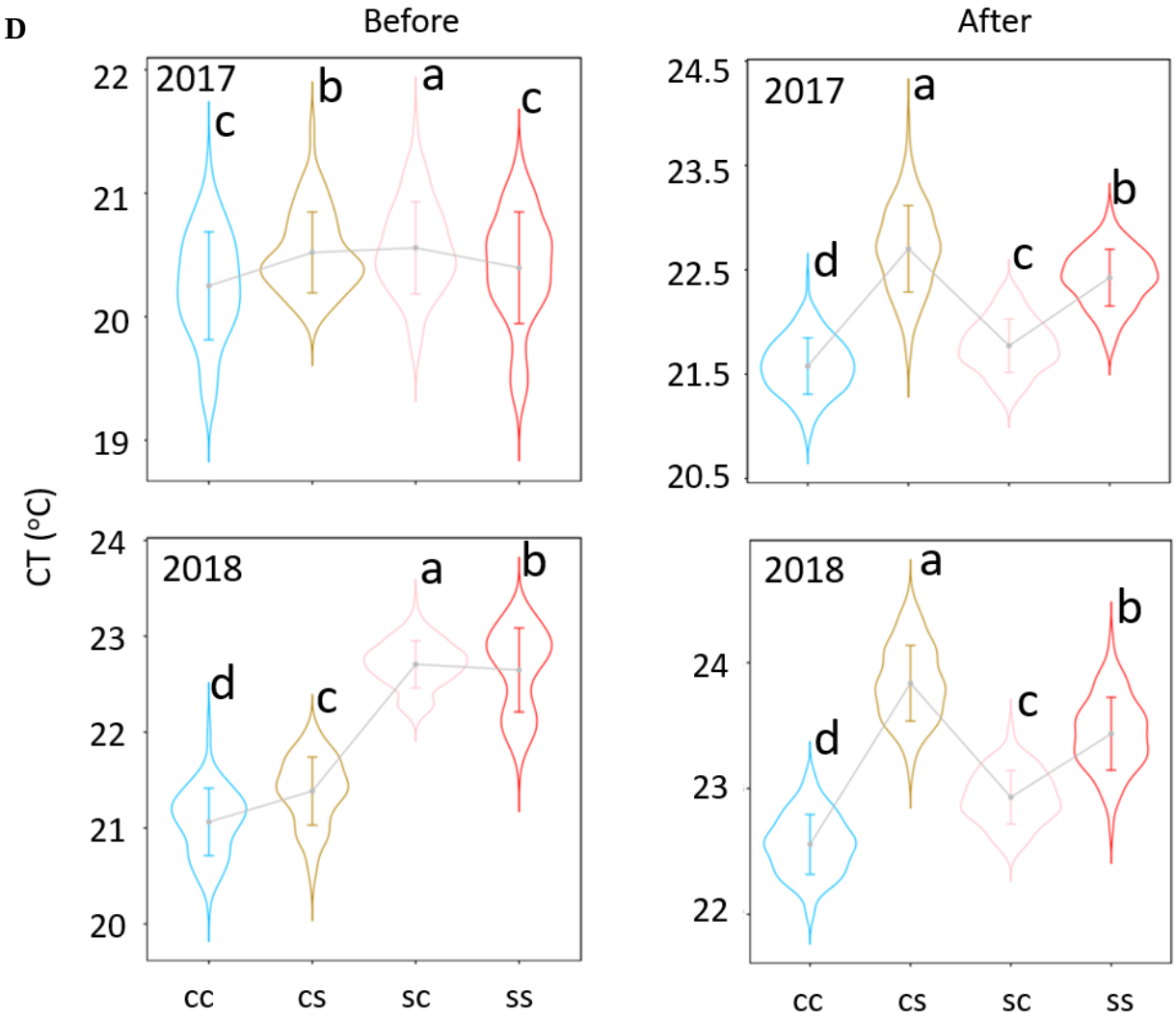
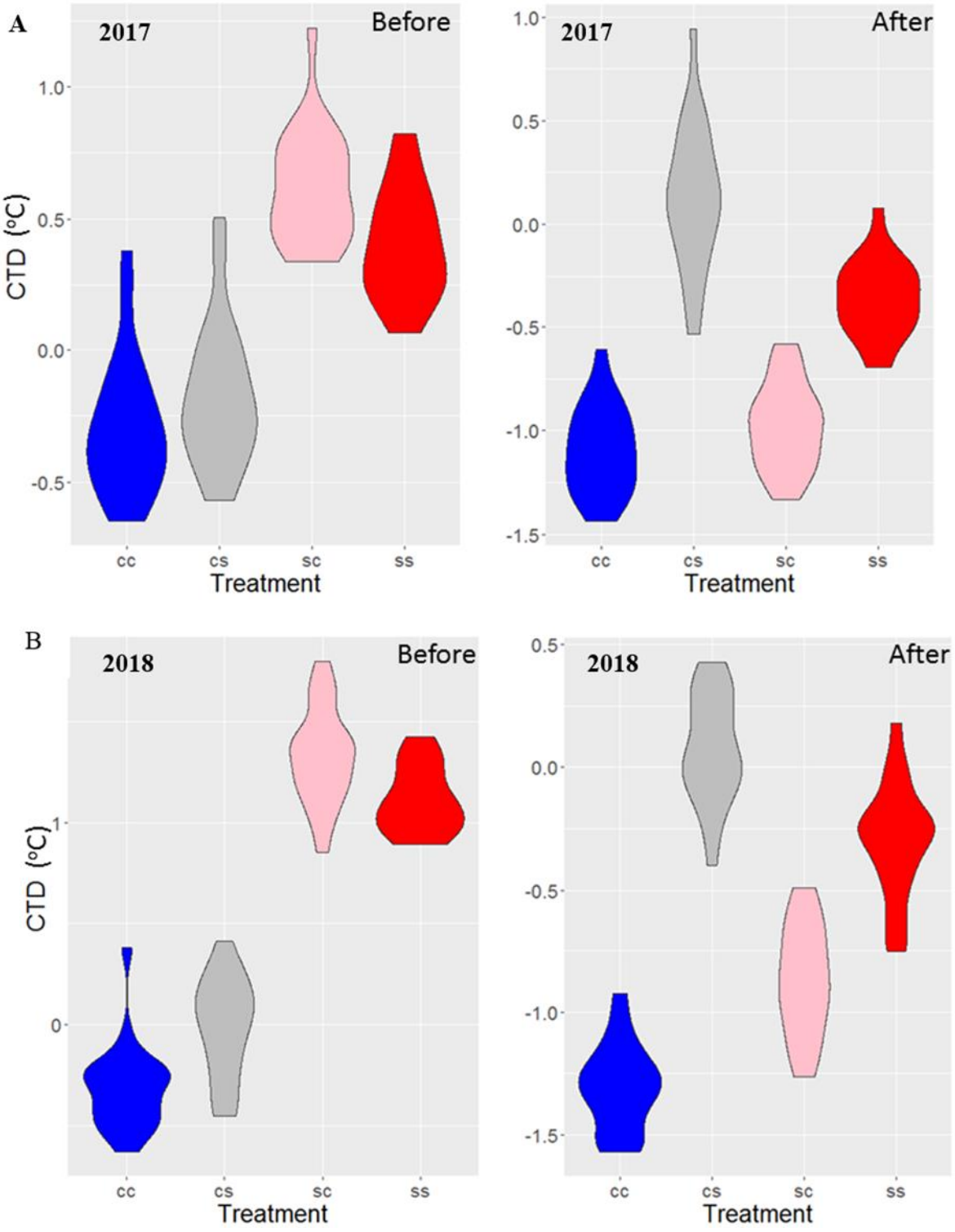


Figure 31. Distribution of genotypic means and Mean separation of CT before (left) and after (right) treatment switch in population B, 2017 (top) and 2018 (bottom). Old figures: A (2017), B (2018) and C (2017(left) and 2018(right)) and new figure D. Means comparison was done by *regwq test.* Means followed by the same letter are not significantly different at p value of 0.01. Bars represent mean ± SD of replicates.


A
2017
Before
2017
After
CTD (°C)
Treatment
cc
cs
sc
ss
B
2018
Before
2018
After
CTD (°C)
Treatment

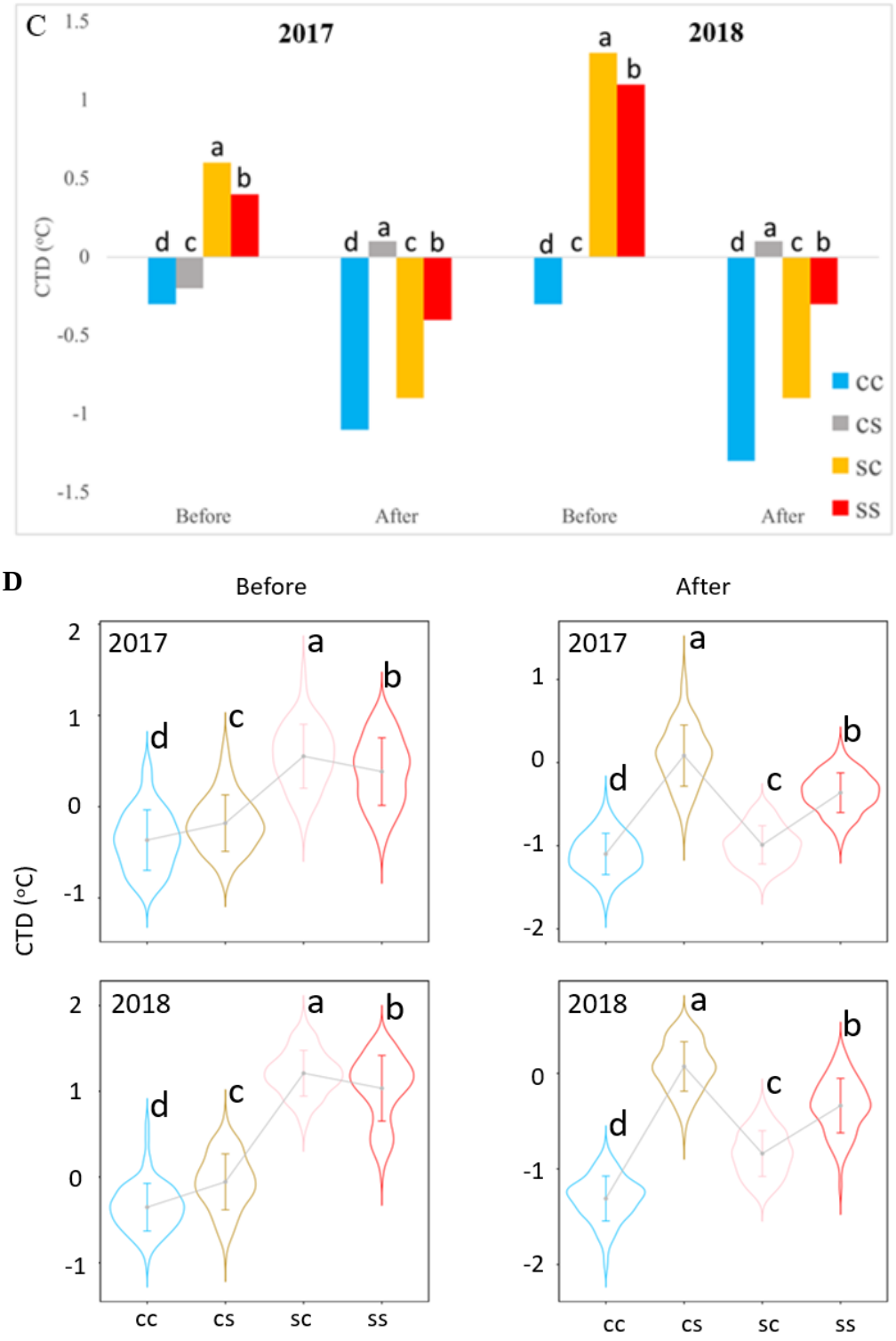


Figure 32. Distribution of genotypic means and Mean separation of CTD before (left) and after (right) treatment switch in population B, 2017 (top) and 2018 (bottom). Old figures: A (2017), B (2018) and C (2017(left) and 2018 (right)) and new figure D. Means comparison was done by *regwq test.* Means followed by the same letter are not significantly at p value of 0.01. Bars represent mean ± SD of replicates.

3.5.5 Diurnal change of canopy temperature and CTD

In both experiments, diurnal changes of CT were clearly observed. As an example, one of the parent's CT before (36 and 38 DFP) and after treatment switch (54 and 59 DFP) in 2018 are presented in Figure 33. In all treatments, maximum values of CT were observed between 8:00 and16:00. Higher values were recorded in stressed plants before flowering in treatment ss and sc and after flowering in ss and cs (Figure 33). In line with (Olufay *et al.* 1993), canopy temperature in stress blocks rises faster before noon than in plants under optimal water.

3.5.6 CT and CTD change over growth period

Daily mean CT and CTD of all genotypes are depicted against plant age in Figure 34. The CT (A and B) and CTD (C and D) varied from day to day during plant development. Both before and after the treatment switch, daily mean CT and CTD was higher under stress conditions than under optimal water conditions.

A few days after the treatment switch in 2017, the CT of cs plants was much higher than in the plants of other treatments (Figure 34 A). This suggests that plants in cs treatment were highly shocked when switched from optimal water supply to water stress condition. For several days after the reduction of the water supply, the loss of leaf turgor was frequently visible on cs plants.

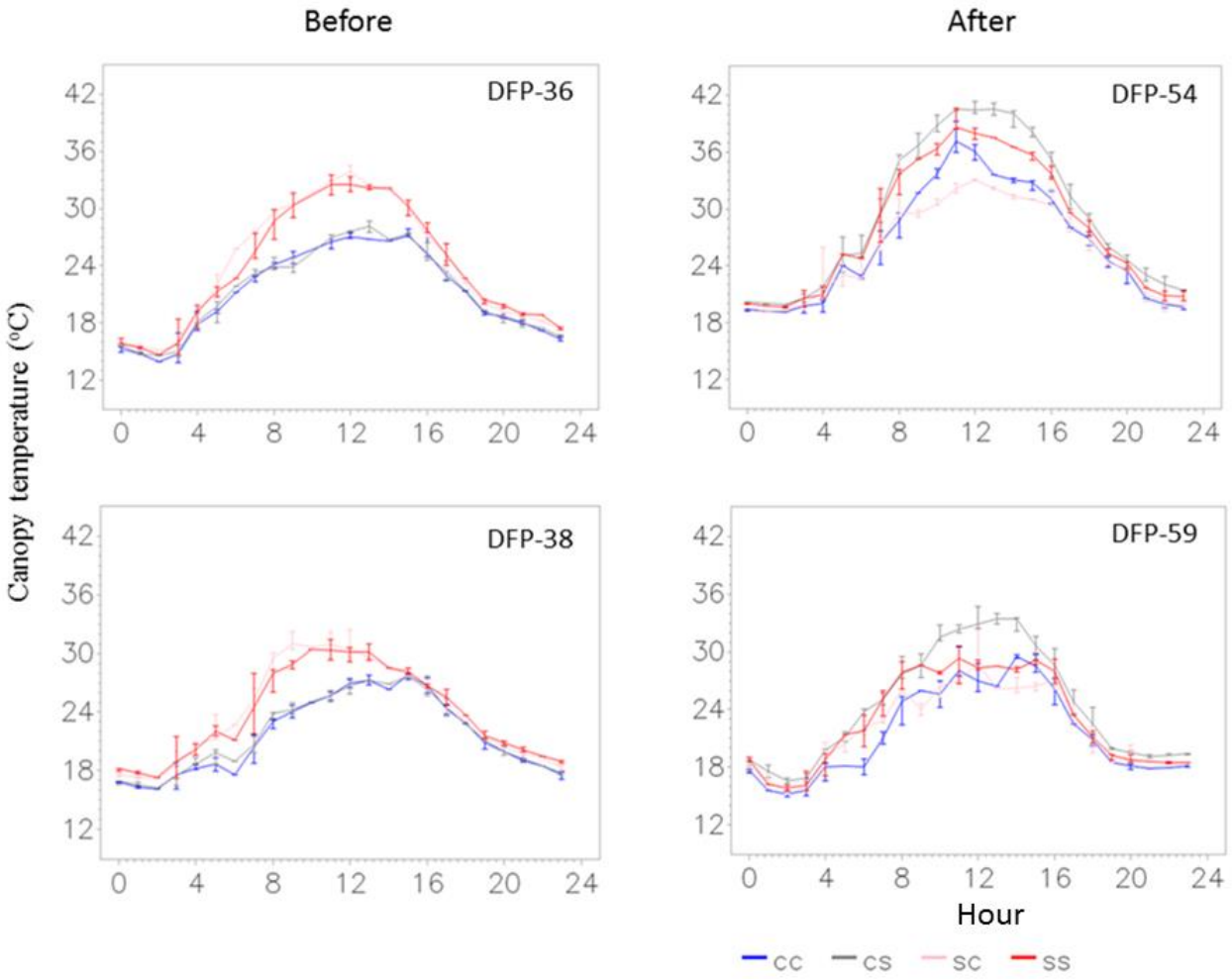


Figure 33. Diurnal variations in the mean CT of genotype 858641 before (day 36 and 38) and after (Day 54 and 59) treatment switch in four different treatments in 2018. Bars represent mean ± SE of seven replicates.

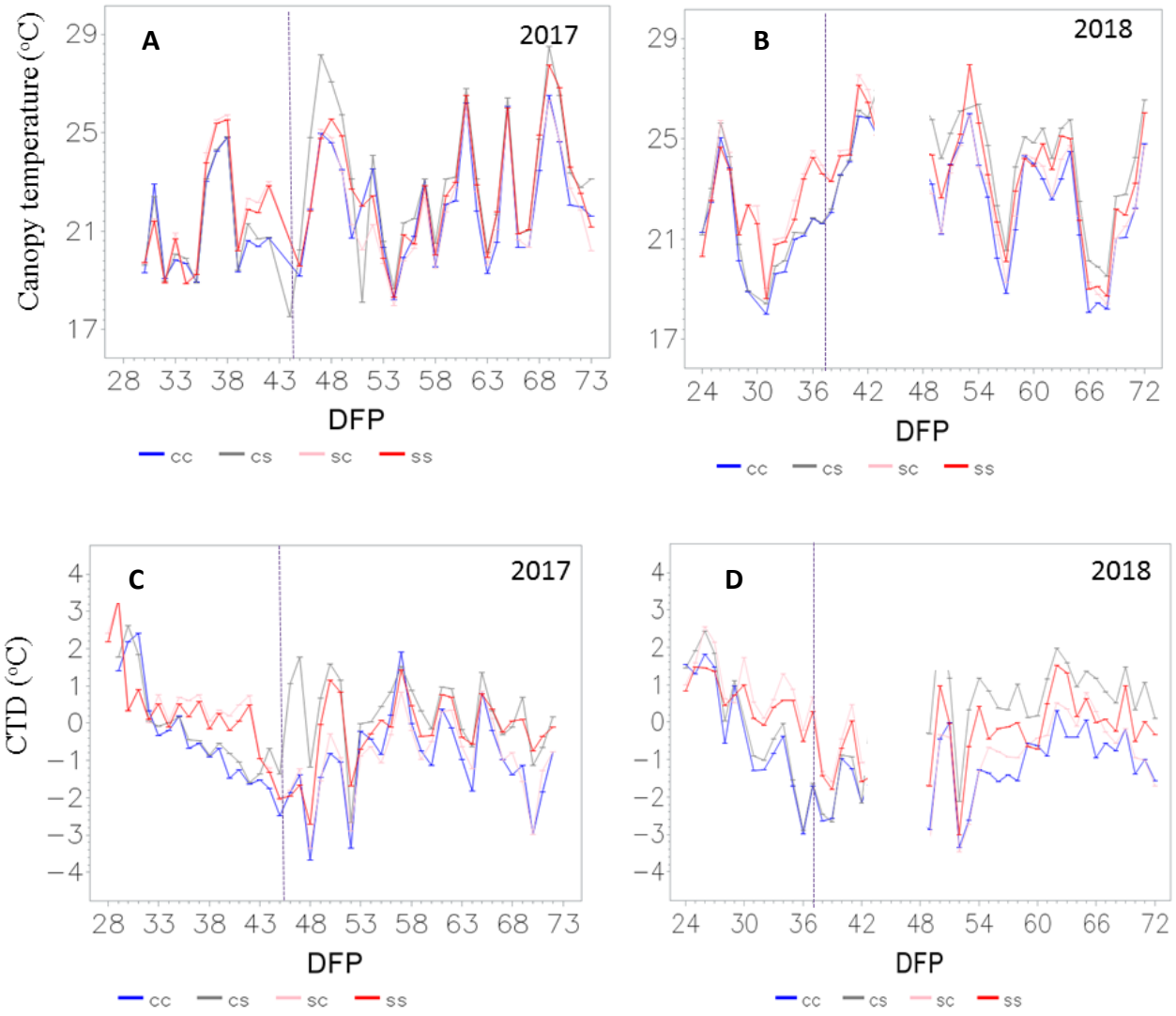


Figure 34. Daily mean canopy temperature (A and B) and CTD (C and D) of all genotypes plotted against days from planting in 2017 and 2018. Treatments switch date is indicated by a vertical broken line.

3.5.7 Relation between CTD and DRYMp

Pearson correlations were calculated to determine the relationship between daily means of CTD of each genotype and the genotype's DRYMp calculated from the starch yield data of the same experiment and stress conditions (late, early or long-time stress). Since the tolerance index cannot be calculated for optimal water conditions, CTD in cc conditions was correlated with DRYMp calculated under ss conditions. Correlation coefficients are presented in Figure 35 and Figure 36. In both experiments, CTD under ss conditions correlated significantly with DRYMp especially during tuber bulking (Figure 35). Calculating the Pearson correlation for CTD values in different

intervals of the diurnal cycle, the best the correlations between CTD and DRYMp were found during the afternoon in the 2017 experiment (Figure 36). Significant correlation coefficient values were also found for CTD measured in the afternoon of 2018. Therefore, to improve the predictability of drought tolerance from CTD, CT should be measured in the afternoon.

In summary CTD was correlated with drought tolerance (DRYMp) of genotypes under water stress conditions, especially during tuber bulking and when CT was measured in the afternoon.

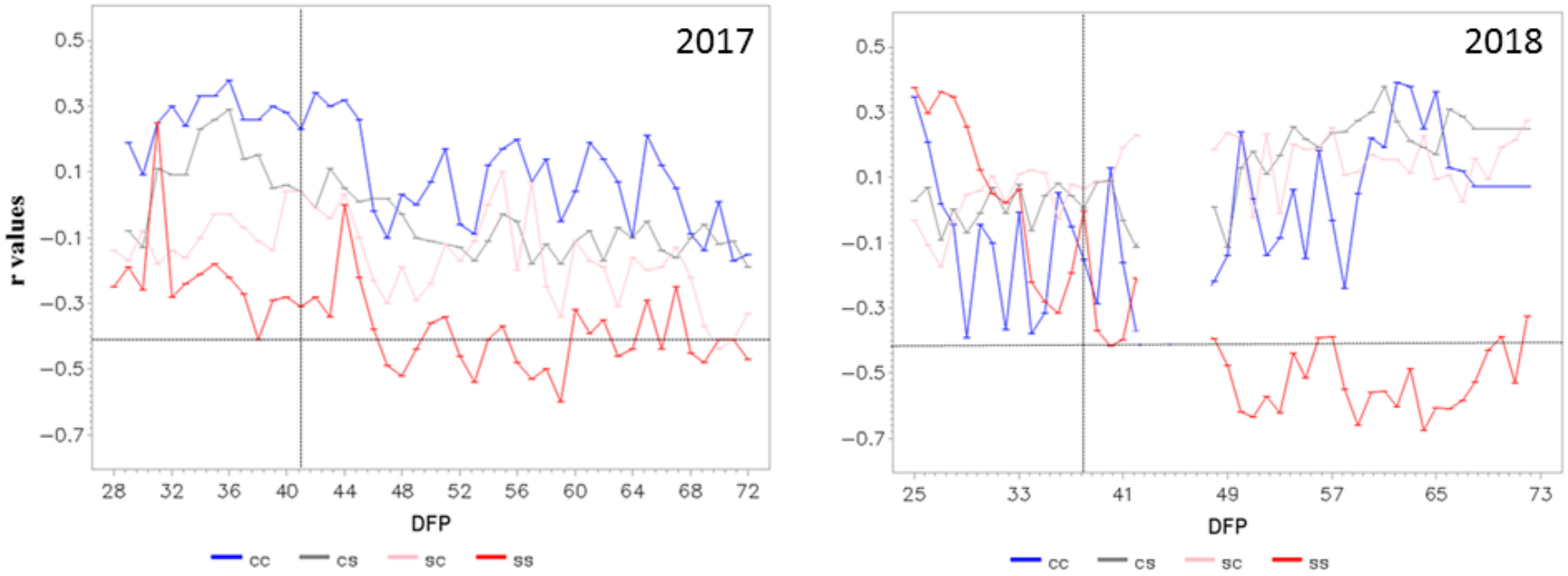


Figure 35. Pearson correlation coefficient values for correlation between DRYMp calculated from starch yields of the same experiment, respective treatment and daily mean CTD of respective treatment plotted against days from planting. df was 19 (2017) and 18 (2018). The CTD under optimal water conditions was correlated with DRYMp values under long-term stress conditions. Vertical and horizontal broken lines represent treatment switch date and lower significance threshold of the correlation coefficient (p=0.05).

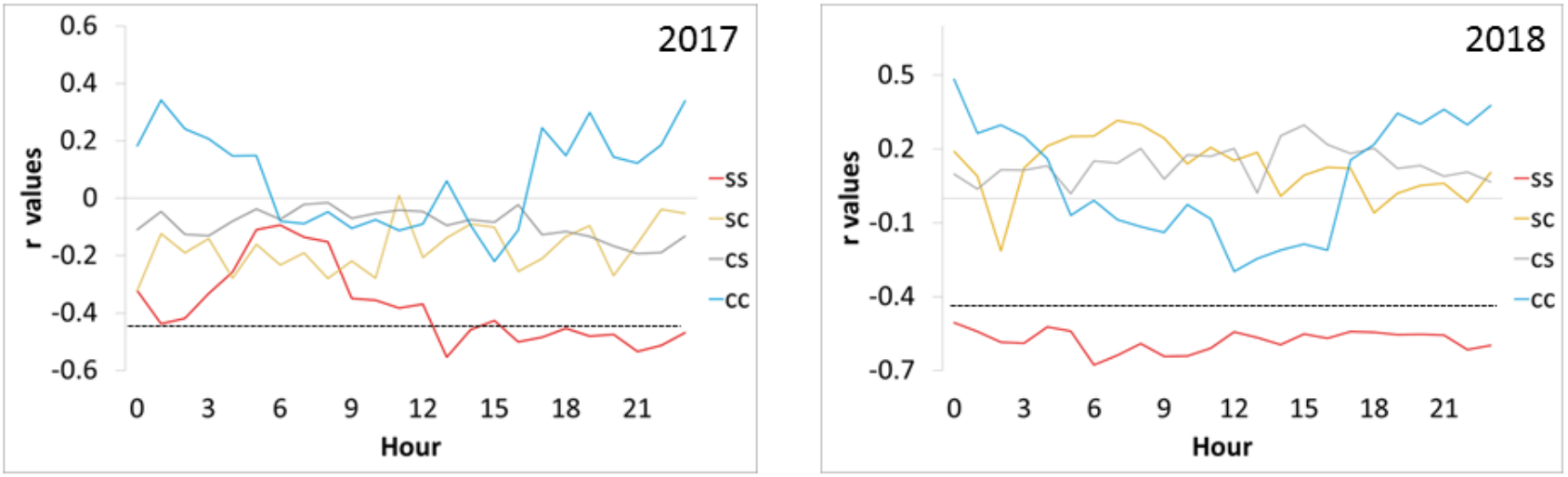


Figure 36. Pearson correlation coefficients for correlation between DRYMp calculated from starch yields of the same and hourly CTD in tuber filling stage. df was 19 (2017) and 18 (2018). The CTD under cc condition was correlated with DRYMp value under ss condition. Lower significance threshold (p = 0.05) indicated by horizontal broken line.

3.5.8 Mean CTD of tolerant and sensitive group

Based on DRYMp values of the same experiment and long-term stress conditions, genotypes were grouped into the tolerant (DRYMp > 0) and the sensitive (DRYMp < 0) group (Sprenger *et al.* 2015). The mean daily CTD of the tolerant group was plotted against selected plant age (Figure 37). Tuber filling stage was selected as suggested by our study. The CTD of the tolerant group was smaller than that of the sensitive genotype (Figure 37), but the difference between tolerance groups was small. The mean CTD (7 hr to 21 hr) of a selected tolerant and a selected sensitive genotype and the difference between them are plotted against plant age in Figure 38. The difference between the highest (sensitive) and lowest (tolerance) CTD of genotypes was between 0.5 to 1.5 ºC in 2017 and between 1 to 2 ºC in 2018 (Figure 38). This means genotypes in the tolerant group may keep stomata open for longer times and keep the canopy cooler than the sensitive group.

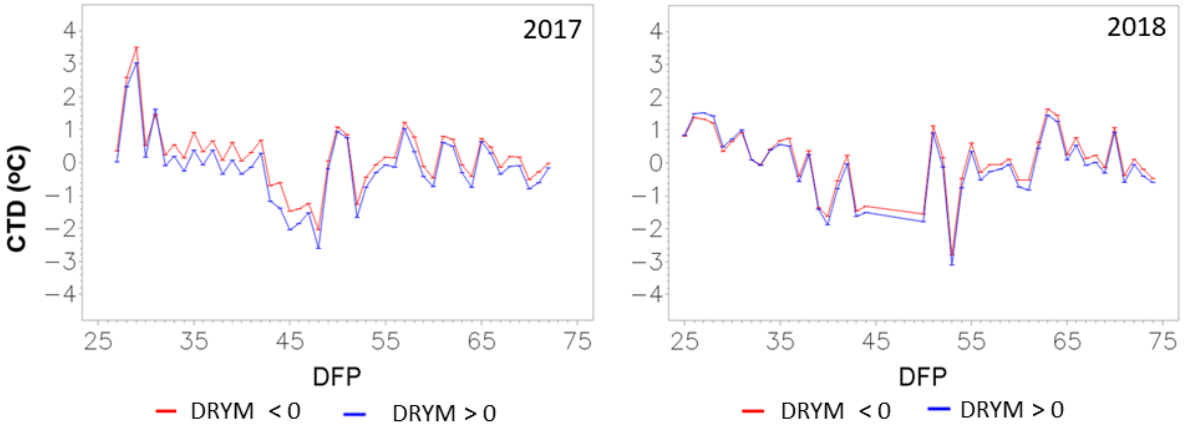


Figure 37. Mean CTD for the group of tolerant (DRYMp > 0, blue) and sensitive (DRYMp < 0, red) genotypes under stress conditions (ss) plotted against the measurement day after planting.

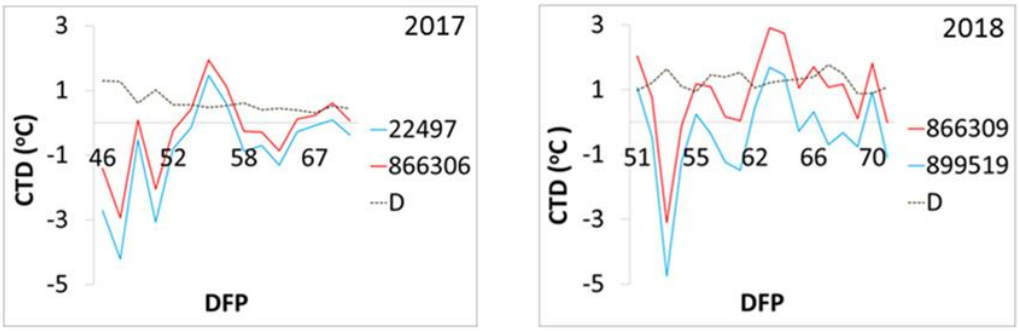


Figure 38. Mean CTD of the tolerant genotype with the lowest CTD values (DRYMp > 0, blue) and the sensitive genotype with the highest CTD values (DRYMp < 0, red) genotype plotted against plant age in selected growth stage, ss treatment in 2017 and 2018. Broken brown line (D) indicates daily CTD difference between the two genotypes.

3.5.9 CTD rank and tolerance group

Genotypes were grouped in to tolerant (DRYMp >0) and sensitive (DRYMp < 0) based on relative starch yield of the same experiment. To select genotypes based on CTD values, CTD values of the respective season were ranked in ascend orders (see Table 17). If we select the top 50% genotypes of each year, the efficiency of selection of tolerant genotypes was more than 80%. This implies that most tolerant genotypes maintained cooler canopies under stress conditions. However, some tolerant genotypes (e.g 899748) showed higher CTD values than the median of the distribution. On the other hand, some susceptible genotype (900024 in 2018 experiment) showed lower CTD. This could be because different tolerance mechanism could exist in the studied genotypes.

Table 17. Rank and CTD value of genotypes and their tolerance group (DRYMp >0 and DRYMp <0). Where T - tolerant and S - sensitive

Season	**2017**			**2018**		
Rank	**genotype**	**CTD**	**Tolerance**	**genotype**	**CTD**	**Tolerance**
1	900024	-1.41	T	899519	-0.47	T
2	22497	-1.40	T	872477	-0.45	--
3	899922	-1.35	T	899665	-0.30	T
4	866296	-1.31	T	900024	-0.13	S
5	899522	-1.31	T	22497	-0.07	S
6	899486	-1.30	T	858638	-0.05	T
7	858638	-1.29	T	899486	-0.03	T
8	899519	-1.28	T	899522	-0.03	T
9	899834	-1.23	T	866296	-0.01	T
10	899822	-1.18	T	899834	0.00	T
11	850136	-1.15	S	899922	0.05	T
12	899831	-1.11	S	899831	0.07	S
13	872477	-1.05	S	858641	0.12	S
14	866303	-1.03	S	866306	0.12	T
15	899748	-0.99	T	872474	0.16	T
16	858641	-0.99	T	899748	0.20	T
17	869004	-0.97	S	866303	0.22	S
18	899665	-0.93	T	899822	0.33	S
19	866309	-0.91	T	869004	0.38	S
20	872474	-0.86	S	866309	0.61	S
21	866306	-0.76	S			

3.5.10 Heatmap correlation between CTD and DRYM

Mean CTD values were calculated from tuber filling plant age intervals and afternoon diurnal time range intervals. The DRYMp was calculated for each specific experiment and treatment condition, DRYMs were calculated from all FGH experiments and all field experiments. The Pearson correlation heat map was produced for correlations between DRYMp values (specific experiment, all FGH experiment and all field experiment) and mean CTD over selected growth stages and time intervals is presented in Figure 39 and Figure 40. In FGH conditions, CTD in ss treatment was better correlated with DRYM values of respective season and DRYM values of ss condition. Correlation values between CTD in FGH and DRYM values in field condition were mostly nonsignificant, which implies that drought tolerance of genotypes varied from experimental condition to experimental condition (FGH and field). This indicated that tolerance predicted in FGH condition may not be helpful in field experiment conditions and vice versa. The result is also supported by non-significant correlation between SY in field and in FGH conditions (supplementary Table S5).

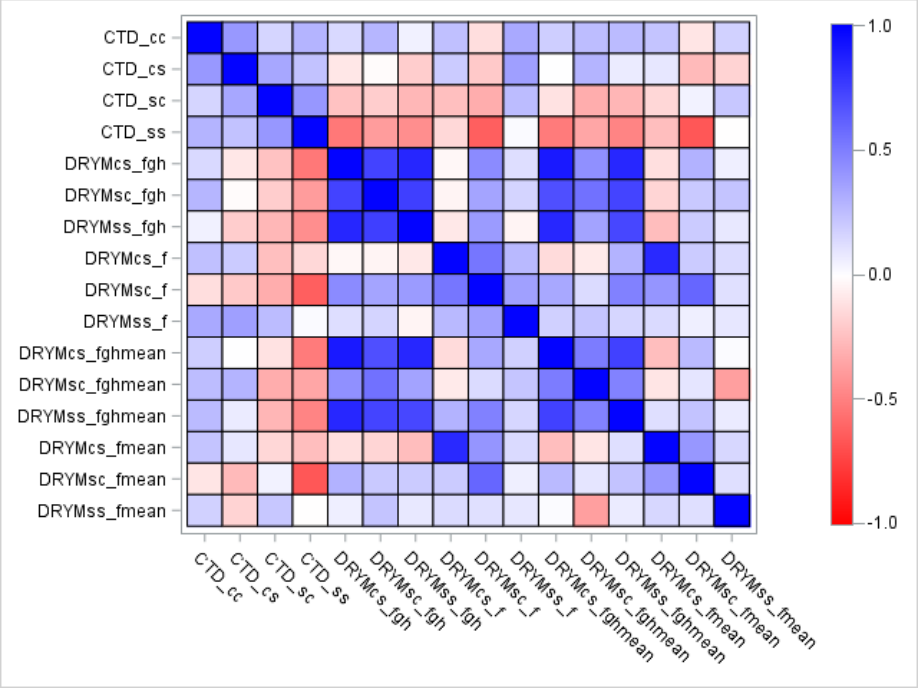


Figure 39. Pearson correlation heatmap of CTD measured in 2017 FGH experiment and DRYMp values. CTD values were measured from tuber filling plant age. DRYMcs, DRYMsc and DRYMss were DRYMp values in cs, sc and ss treatments, respectively. CTD_cc, CTD_cs, CTD_sc and CTD_ss were CTD of respective treatments. f - field trial. fmean and fghmean - DRYMp over field and FGH trial, respectively in 2011-2018.

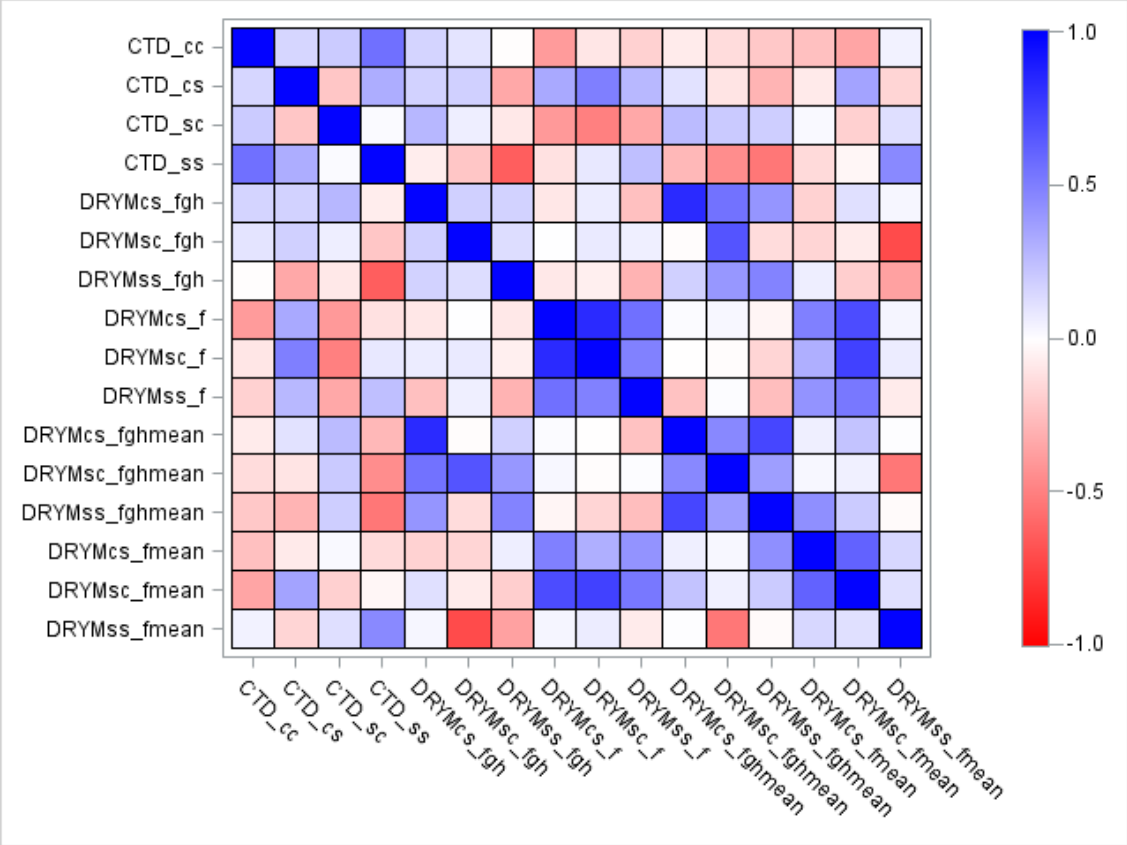


Figure 40. Pearson correlation heatmap of CTD measured in 2018 FGH experiment and DRYMp values. CTD values were from tuber filling plant age. DRYMcs, DRYMsc and DRYMss were DRYMp values in cs, sc and ss treatments, respectively. CTD_cc, CTD_cs, CTD_sc and CTD_ss were CTD of respective treatments. f - field trial. fmean and fghmean - mean DRYM over field and FGH trial, respectively in 2011-2018.

3.5.11 Polynomial model to catch diurnal canopy temperature change

The hourly mean CTD data (5 to 20:00) in ss treatment and selected growth stage was predicted from time of the day by using second degree polynomial. Then Pearson correlation was run between DRYMp of the same experiment with quadratic and the linear coefficients. The correlation coefficients for the correlation between DRYMp and the linear regression coefficients were -0.45* (2017) and -0.48* (2018), and 0.43* (2017) and 0.48* (2018) for the quadratic coefficient. Thus, tolerance was related with slow change of CT. Diurnal CT change results in a graph that opened downward (see sample graph Figure 41) or attained maximum value. To estimate the maximum, x-value for the maximum was calculated as liner coefficient / (2 x quadratic coefficient). Then the maximum value is calculated by applying the original polynomial function to this x-values. The estimated maximum values were correlated with the DRYMp of the same experiment. In the 2018 experiment, the estimated maximum values were significantly and

negatively correlated (r = -0.52*) with DRYMp values, as expected inverse relationship between CTD and tolerance index. However, this result was not confirmed by the 2017 experiment.

Altogether, linear coefficients, quadratic coefficient and estimated maxima of a polynomial regression of CTD on hour are potential predictors for drought tolerance.

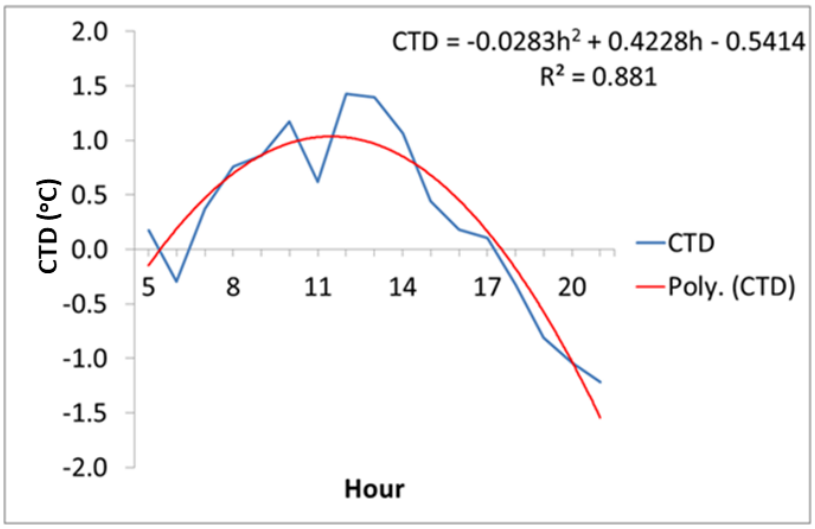


Figure 41. Mean CTD of all genotypes in (5:00 to 20:00) and fitted polynomial line ss condition in 2018.

3.5.12 Diurnal time range selection by LASSO model

In population B, CTD measured in afternoon showed better correlation with tolerance index than CTD measured before noon (Figure 36). For this case, DRYMp of the same experiment was predicted by using LASSO model from hourly mean CTD measured in the selected growth stage under long-term stress conditions. The LASSO model was selected, because CTD of consecutive hours were mostly high correlated and it was planned to select a robust diurnal hour to measure CT. Among the diurnal time ranges, the LASSO model selected 16:00, 14:00 and 11:00 hours in the 2017 dataset and 10:00 and 8:00 hours in the 2018 dataset (Figure 42). The progress of average square errors of training and test datasets of the LASSO model is presented in Figure 43. The summary statistics and parameter selection are presented as supplementary table (see supplementary Table S13 and supplementary Table S14). Even though the selected hours differed in two experiments, hours were in line with different reports (Balota *et al.* 2007; Karimizadeh 2011; Hirut *et al.* 2017; Thapa *et al.* 2018), which identified CT measurements between late morning to afternoon as suitable predictors for drought tolerance.

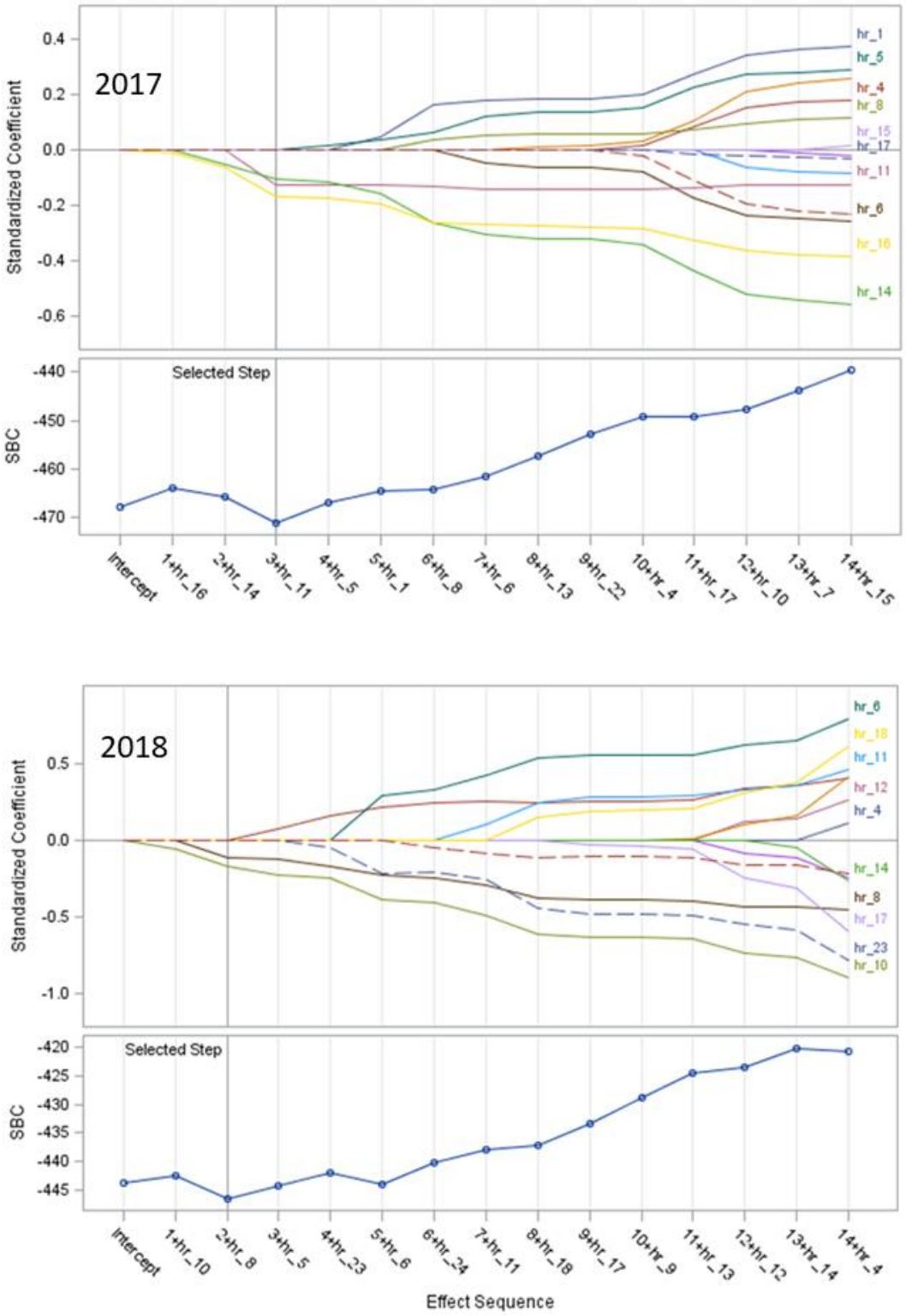


Figure 42. Coefficient progression and parameter selection by the LASSO model in 2017 and 2018 experiments. hr - hour, numbers before + sign represented parameters order in the model. SBC - Schwarz Bayesian information criterion.

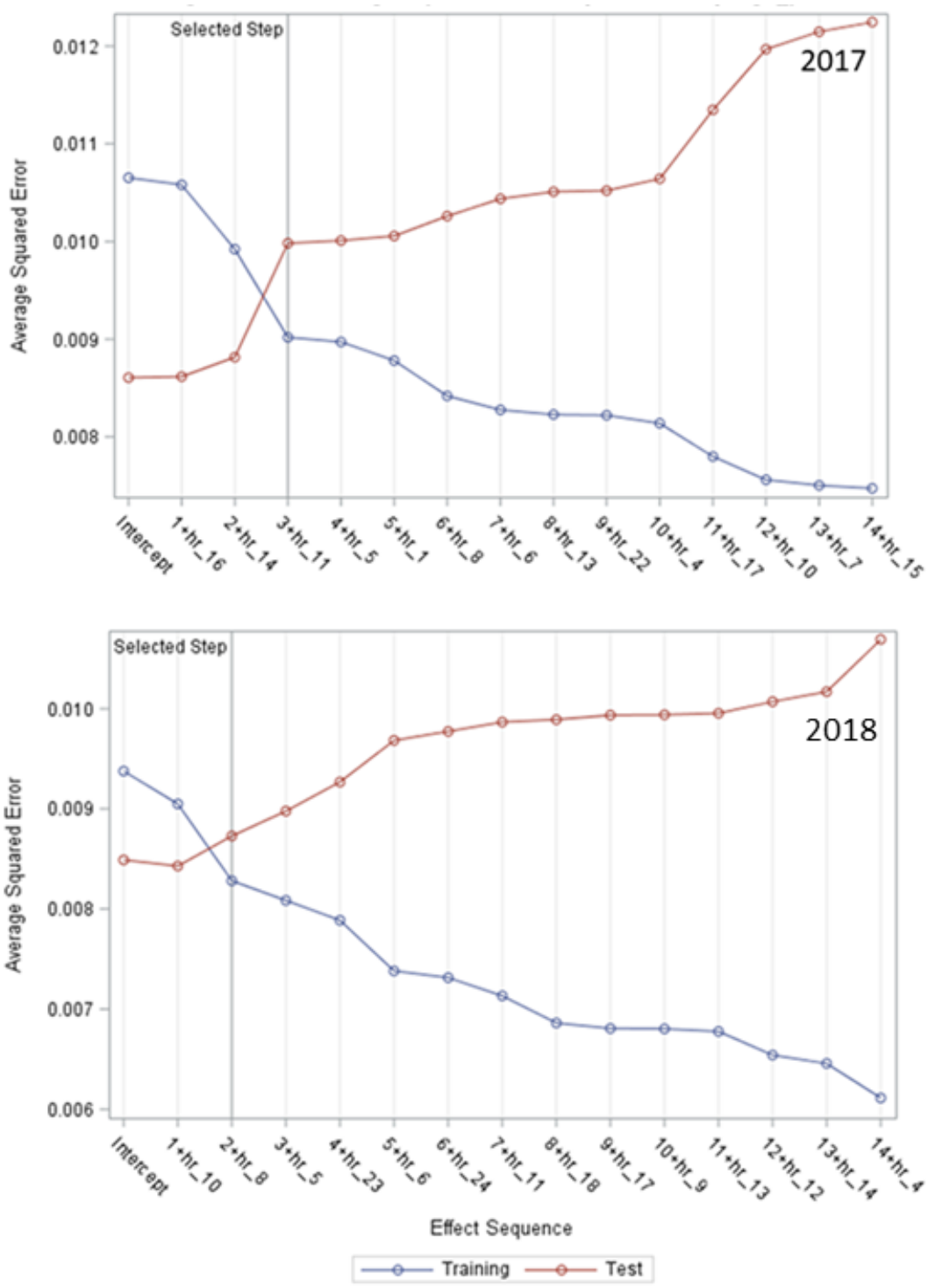


Figure 43. Progress of average square errors of training and test datasets of LASSO model. hr - hour, numbers before + sign represented parameters order in the model.

3.5.13 CTD as predictive parameter for DRYMp and starch yield (SY)

In ss condition, CTD values measured in reproductive plant stage (Figure 35) and afternoon time range (Figure 36) showed better correlation with DRYMp calculated from the data of the same experiment. In this case, the mean CTD values from selected plant stage and diurnal time intervals were correlated with DRYMp of each experiment. In both experiments, mean CTD was significantly correlated with DRYMp and SY of the respective experiment. The correlation coefficients between DRYMp and mean CTD were -0.52* (2017) and -0.64** (2018) and between SY and mean CTD were -0.50* (2017) and -0.56* (2018). In the next part, DRYMp and SY were linearly predicted from mean CTD (Figure 44). Linear regression models for both relationships were significant and the ANOVA R^2 values are shown in the figure below. This indicates that mean CTD under ss conditions could be one of the potential predictive parameters for DRYMp value (Figure 44 A, C) and SY (Figure 44 B, D). Generally, this study indicated that CTD was one of the potential parameters for screening potato genotypes for drought tolerance.

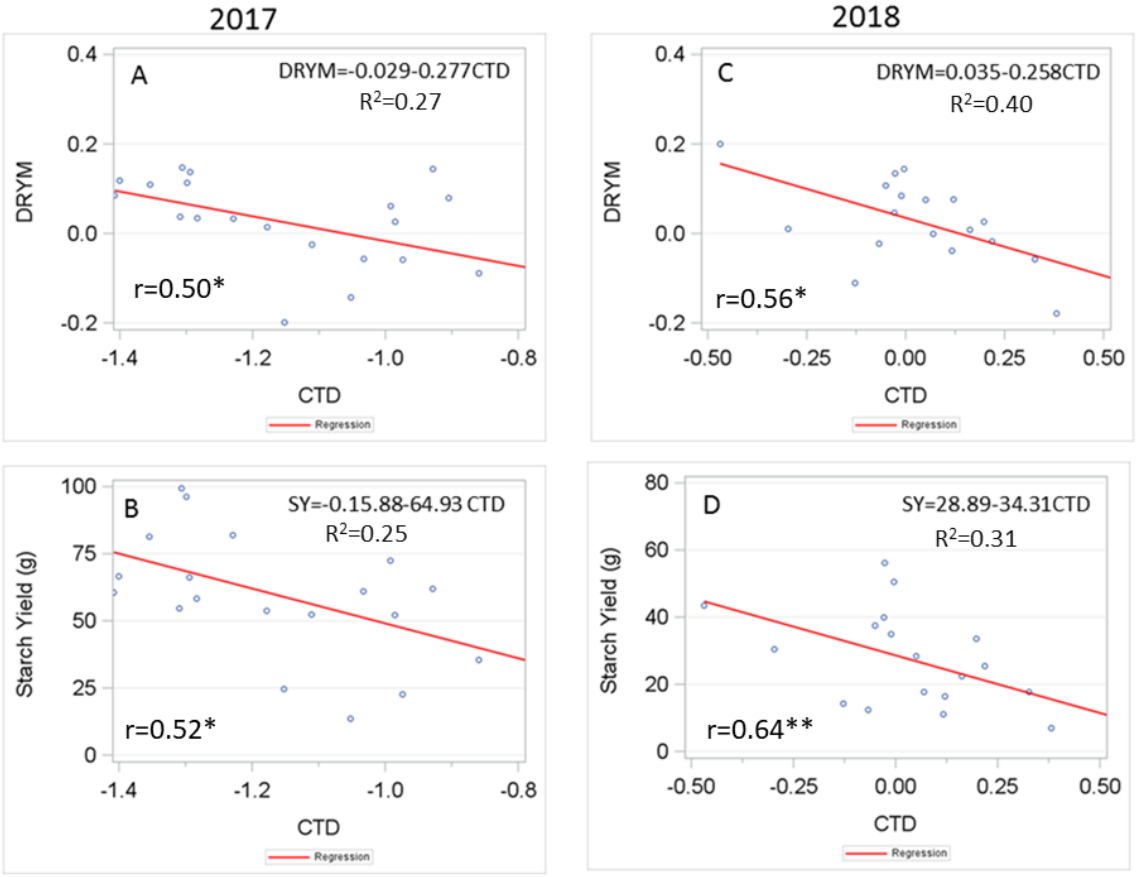


Figure 44. Relationship between DRYMp (A, C) and Starch yield (SY, B, D) and mean CTD of genotypes in ss treatment grown under FGH in 2017 (A, B) and 2018 (C, D).

3.6 Principal component analysis

Ahead of predicting tolerance from multiple predictive parameters in a single model, data exploration and visualization was done by Principal Component Analysis (PCA). PCA is a technique used to emphasize variation and bring out strong patterns in a dataset (Pitchaikani and Lipton 2017). As explained before, PCA focused on traits measured on long-term stress conditions. The standardize data of mean (LA, LI and CTD) and maximum (PH, Leaf area, DB, LPD and LAI) values were used for this analysis.

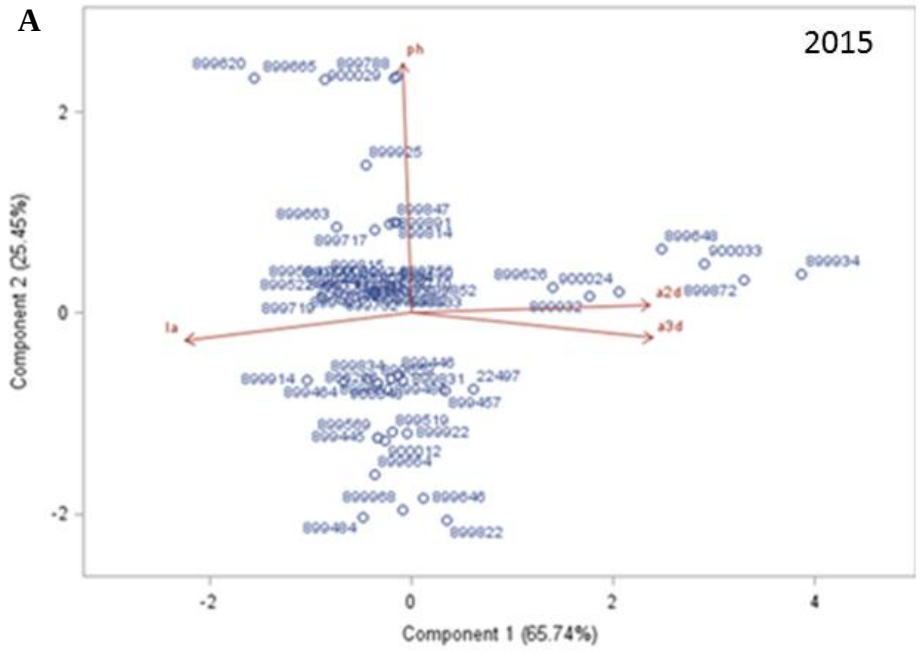
In population A, two components accounted for 80% (2015) and 81% (2016) of the total variance (Figure 45) and the Eigenvalues are presented in supplementary Table S8. The first component had a high positive loading of the variable leaf area (2D and 3D) and high negative loading of the variables LA (see supplementary Table S9). This component illustrates the genotypic variation of leaf area and leaf angle. The second eigenvector has high positive loadings of PH and a smaller positive loading of leaf area of genotypes. LA loading was negative but the values were higher (absolute value) in the 2015 experiment than in the 2016 experiment. This component seems to visualize the variation in plant height. In population A, leaf area 2D and leaf area 3D were very close to each other in the biplot (Figure 45). This indicated the presence of a high correlation between leaf area 2D and leaf area 3D.

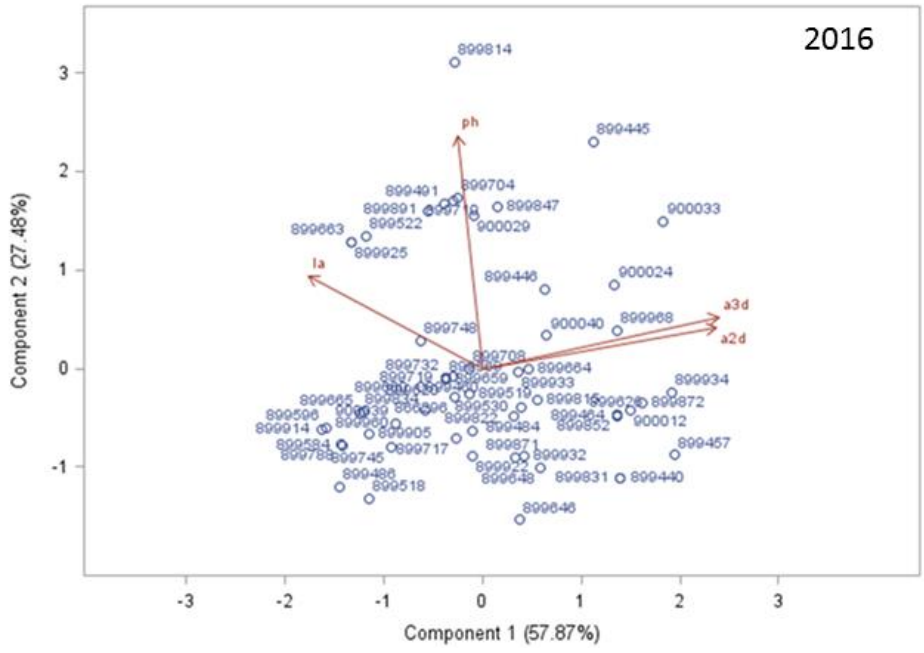
In population B, two components account for 76% (2017 experiment) and 64% (2018 experiment) of the total variance (Figure 46) and three components explain 88% (2017 experiment) and 86% (2018 experiment) (Figure 47). Each subsequent component contributes less than 10 percent (Figure 47). The eigenvalues (supplementary Table S8) of the PCA vectors for each trial indicated that two or three components provided a good summary of the data (Figure 47).

The first component has high positive loadings of the variables leaf area, LA and LAI and high negative loadings of the variables LPD and PH (supplementary Table S9). This component seems to illustrate the leaf area and LAI. The second eigenvector has high positive loadings of the variables DB and PH (2017 experiment) and CTD and LI (2018 experiment) and higher negative loadings of LA (2018 experiment). There is also a small positive loading of CTD in the 2017 experiment. In 2018, this principal component seems to represent LA, CTD and LI, while during 2017 it represented DB and PH. In both experiments, leaf area (3D and 2D) and LAI were to clustered together. In 2018 experiment, the parameters formed three close groups (Figure 46). The

first group contained leaf area and LAI, the second group contained CTD and LPD, PH and LI. The third group was LA. In 2017 experiment, CTD close to LPD. This suggests a high correlation between some parameters, especially between leaf area 3D, leaf area 2D and LAI. Therefore, multicollinearity should be considered when selecting multiple predictive parameters in a tolerance prediction model.

A





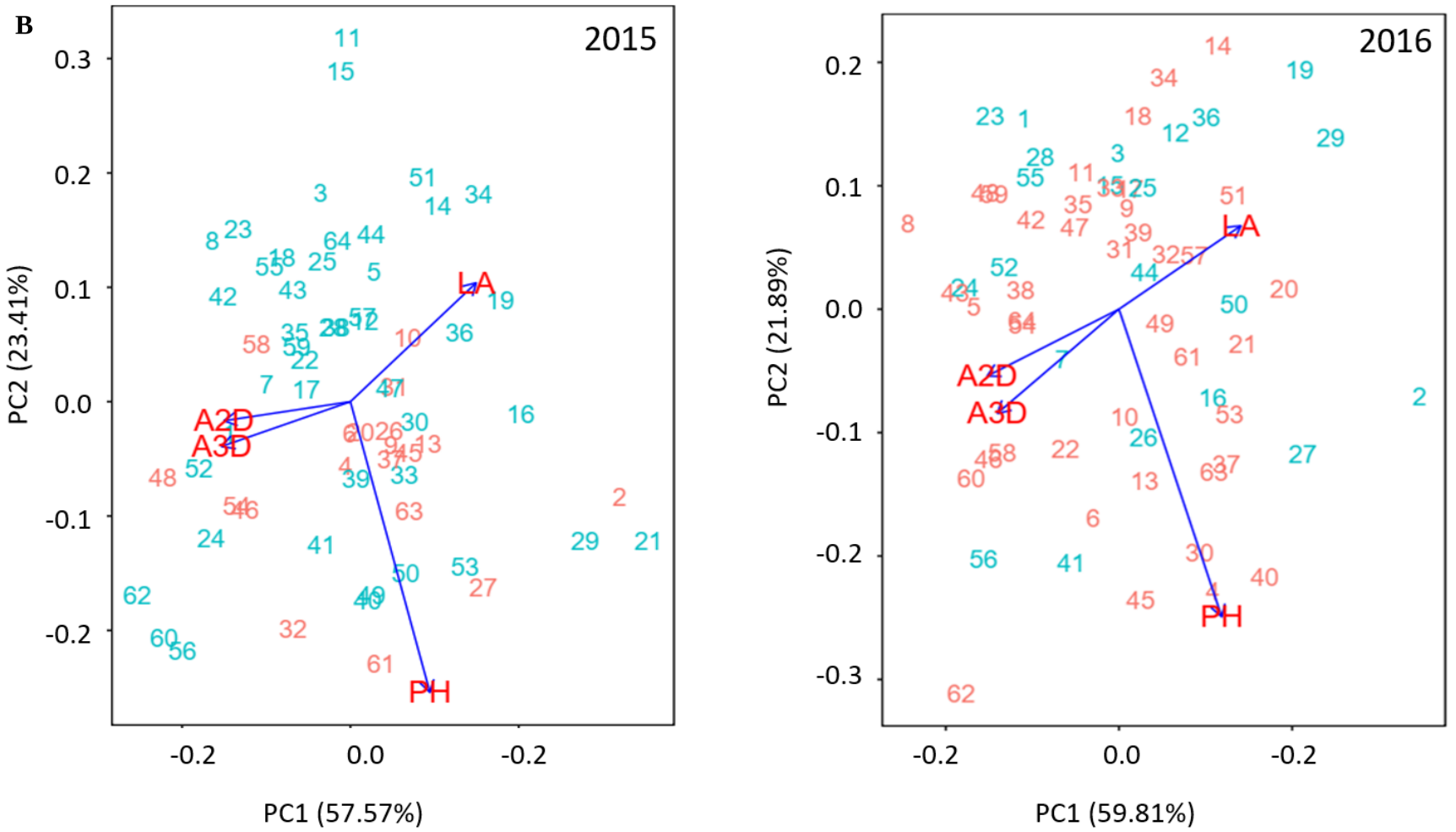
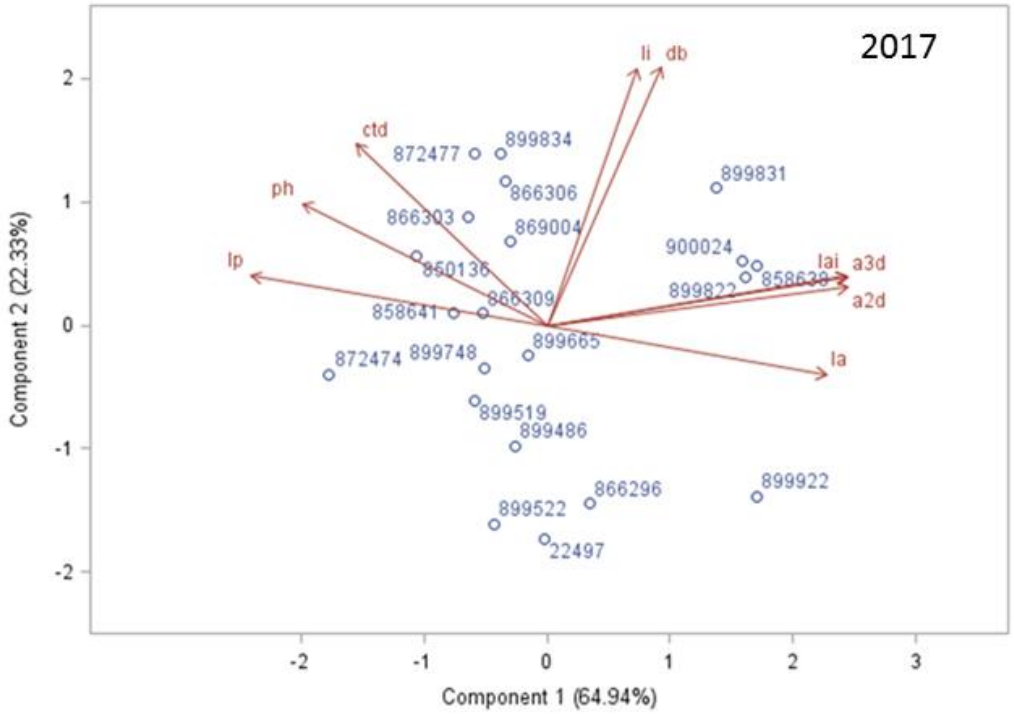
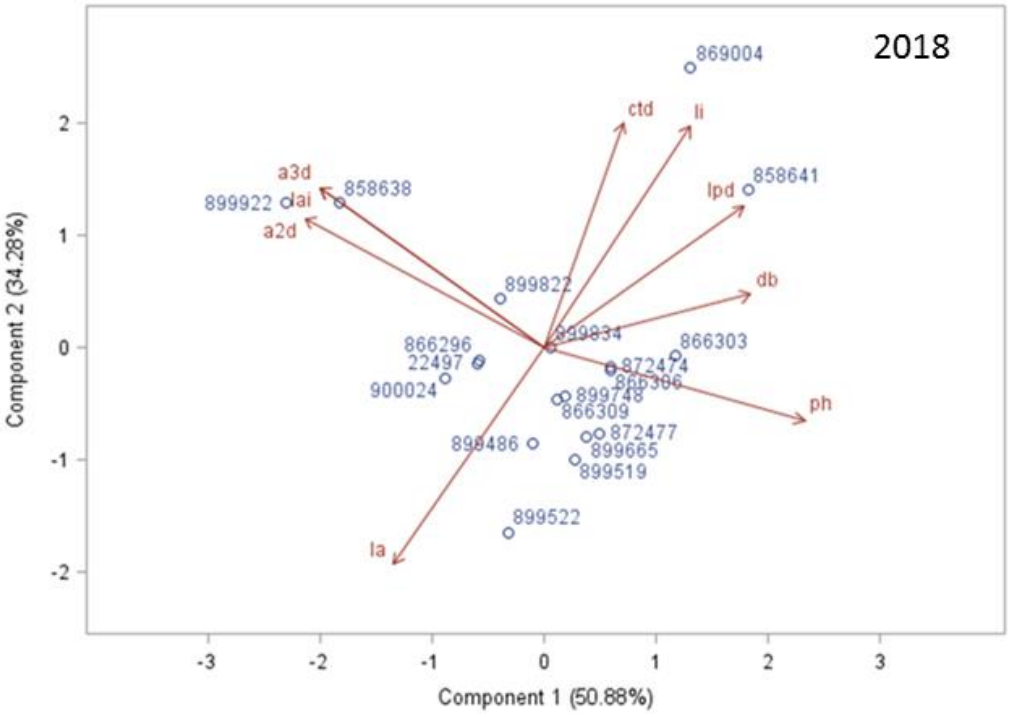


Figure 45. Biplot display for PCA component 2 versus PCA component 1 for canopy parameters measured in long-term stress condition in 2015 and 2016. Old figure (A) and new figure (B). Numbers are genotype identifiers (see supplementary Table S2). DRYMp > 0 highlighted with blue and DRYMp < 0 highlighted with red. For loadings see supplemental table 8.

A


2017
li
db
ctd
899834
872477
899831
ph
866306
866303
869004
900024
lai
a3d
lp
860136
858638
899822
866309
a2d
858641
899665
872474
899748
la
899519
899486
866296
899922
899522
22497
Component 2 (22.33%)
Component 1 (64.94%)


2018
869004
ctd
li
a3d
858638
858641
899922
lai
lpd
a2d
899822
db
866296
899834
866303
22497
872474
900024
866306
899748
866309
ph
872477
898486
899665
899519
899522
la
Component 2 (34.28%)
Component 1 (50.88%)

B

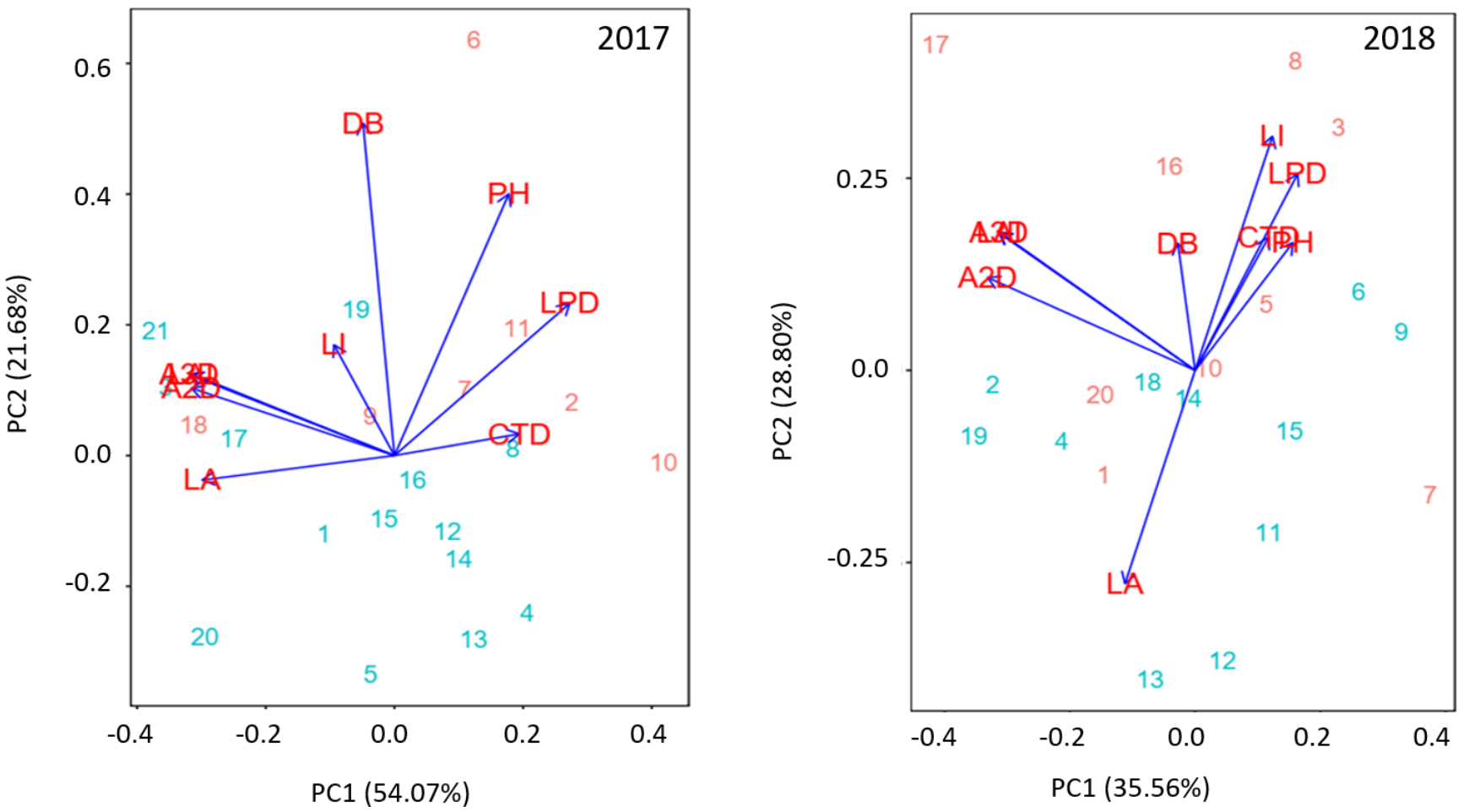


Figure 46. Biplot display for PCA component 2 versus PCA component 1 for canopy parameters measured in long-term stress condition in 2017 and 2018. Old figure (A) and new figure (B). Numbers are genotype identifiers (see supplementary Table S3). DRYMp > 0 highlighted with blue and DRYMp < 0 highlighted with red. For loadings see supplemental table 8.

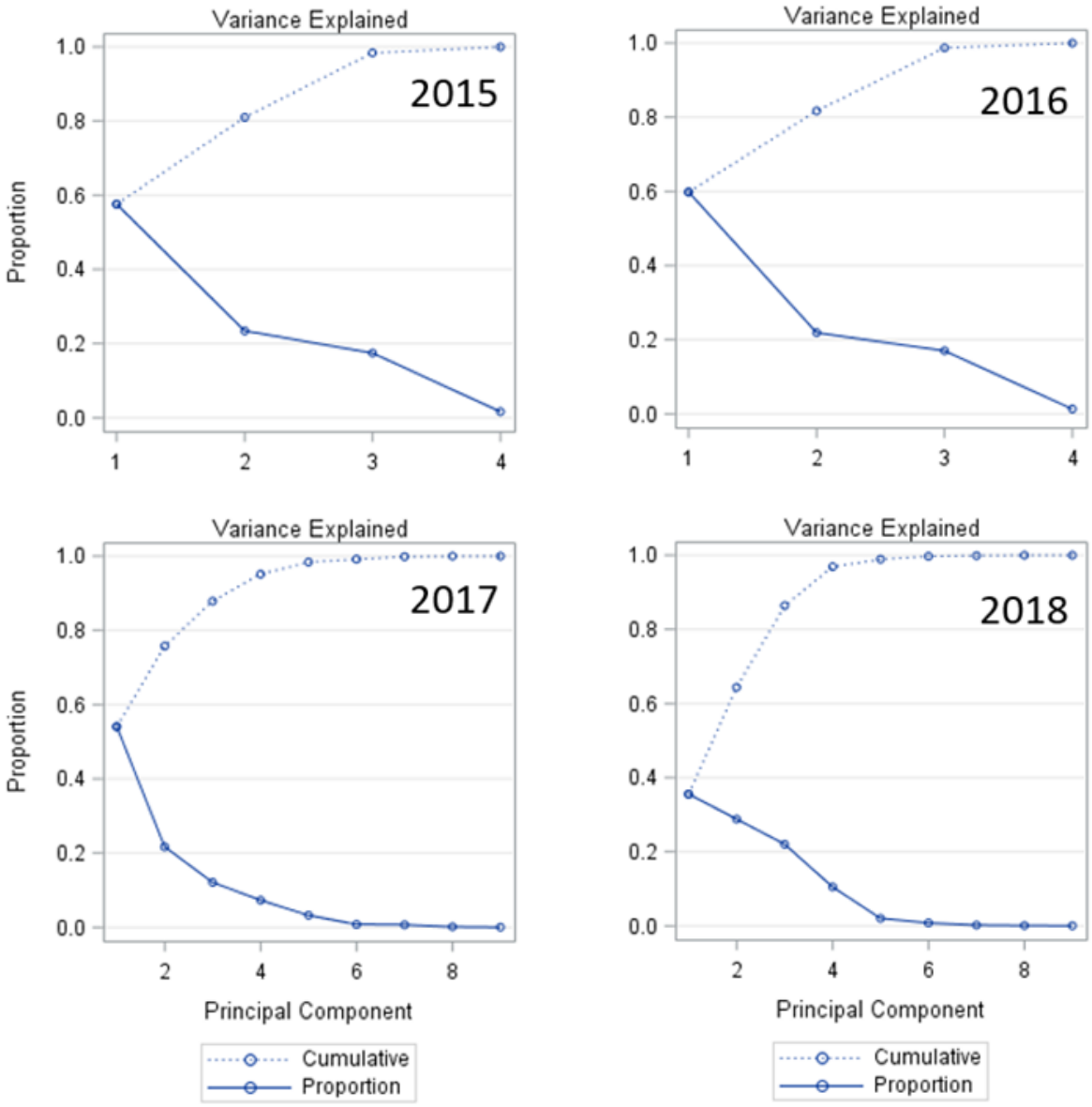


Figure 47. Variance explained by each principal component under long-term drought stress condition in 2015 to 2018

3.7 Clustering

The goal of clustering is to identify patterns or groups of similar objects within a dataset of interest (Madhulatha 2012). In this study, the closeness of two clusters was determined by Euclidean distance. Genotype tolerance levels (DRYMp) of the same experiment were color coded (blue-tolerant and red-sensitive) for each experiment and - combined over experiments - for each of the two populations.

In population A (2016 experiment), 18 out of 20 genotypes in the top (cluster V) and 12 out of 15 genotypes in the lower cluster (cluster I) were tolerant. The middle cluster contained around 50% susceptible genotypes (15 out of 28 genotypes) (Figure 49). However, this distribution was not observed in the 2015 experiment (Figure 48). In combined data from both experiments of population A, the lower cluster (I) contained more susceptible genotypes than the top cluster (supplementary Figure S9).

In population B, in the 2017 experiment, clustering result indicated that most of the tolerant genotypes clustered in the lower (cluster I) of the cluster (9 out of 12 genotypes) and upper (cluster IV) (four out of five genotypes) clusters (Figure 50). In 2018, susceptible genotypes were overrepresented in the top (cluster IV) (three out of four genotypes) and tolerant genotypes were overrepresented in the bottom (7 out of 11 genotypes) clusters (cluster I and cluster II) (Figure 50). In the cluster analysis on combined data of both experiments, seven out of nine genotypes in the upper cluster (cluster II -V) were susceptible, but only one in the lower cluster (cluster I) (supplementary Figure S10).

Altogether, the clustering analysis indicated that the cluster analysis on canopy data resulted in groups, in which either tolerant or sensitive genotypes were overrepresented. This indicated that canopy parameter values varied between tolerant and susceptible genotypes. Thus, tolerance prediction from canopy parameters may be possible especially in population B.

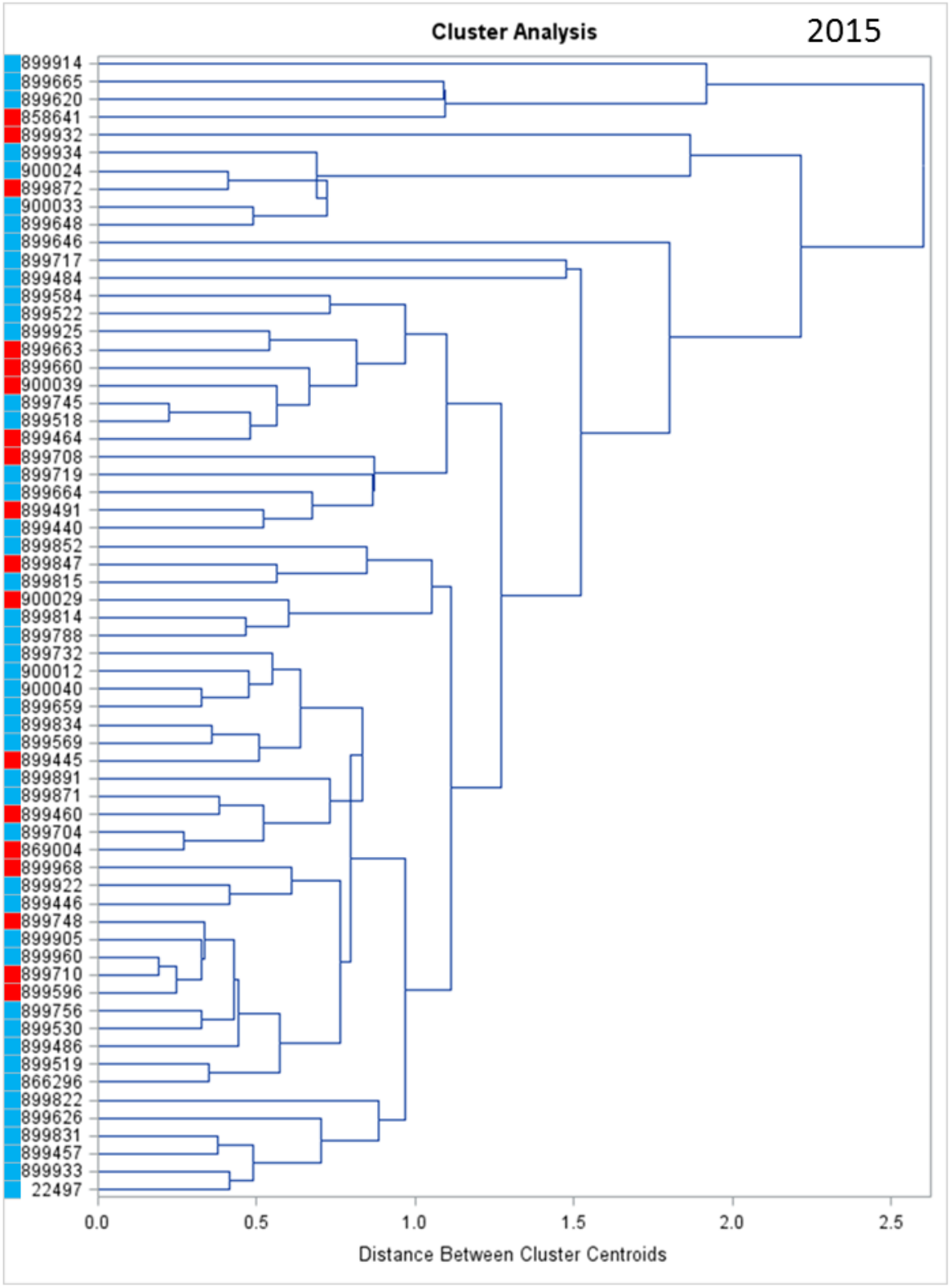


Figure 48. Cluster analysis for canopy parameters under ss treatment in 2015. Blue and red bars in front of the genotype codes represent the DRYMp values greater than zero and less than zero, respectively.

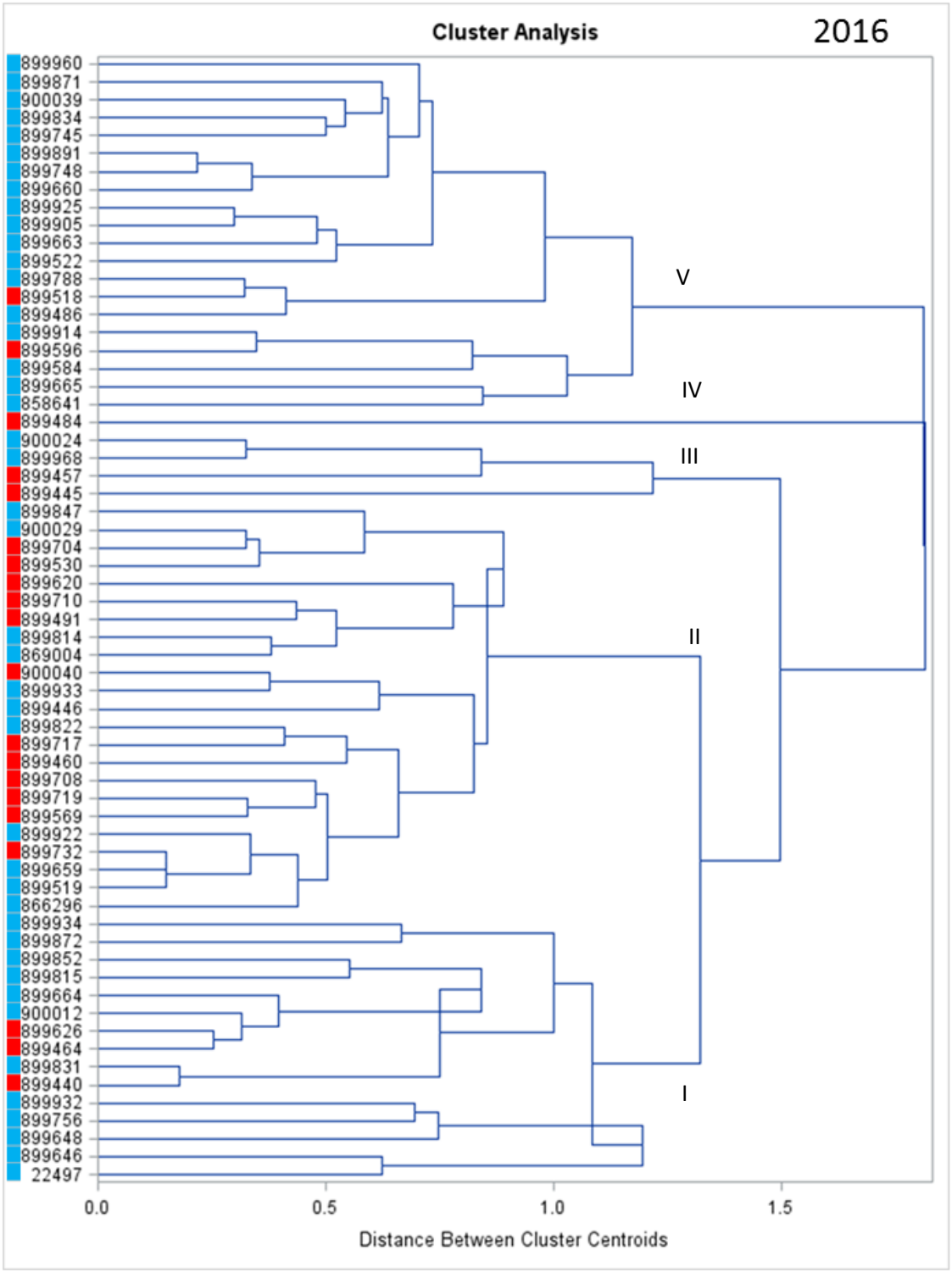


Figure 49. Cluster analysis for canopy parameters under ss treatment in 2016. Blue and red bars in front of the genotypes code represent the DRYMp values greater than zero and less than zero, respectively**.** The cut point of the cluster was 1.25.

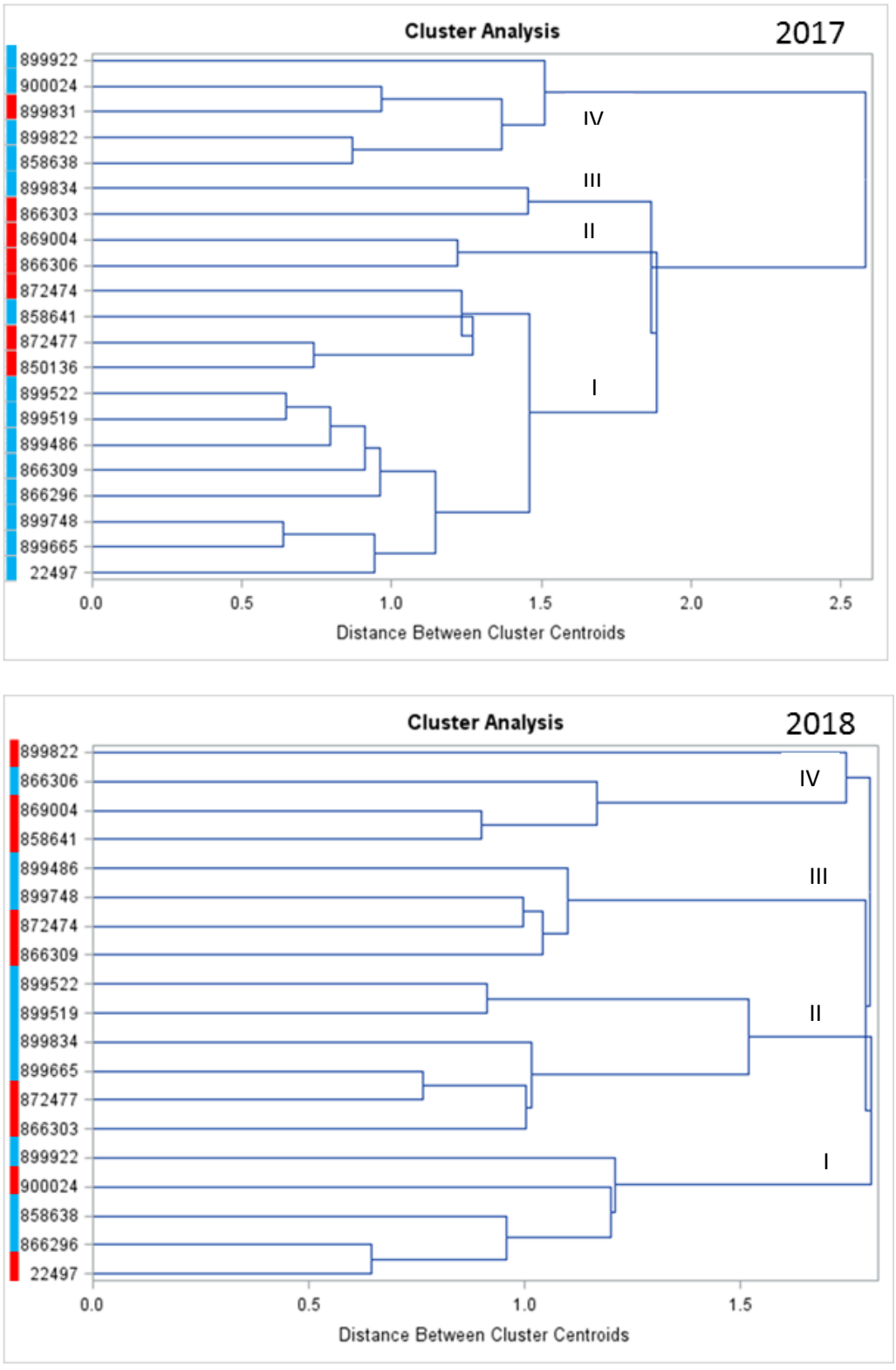


Figure 50. Cluster analysis for canopy parameters under ss treatment in 2017 (top) and 2018 (bottom). Blue and red bars in front of the genotypes code represent the DRYMp values greater than zero and less than zero, respectively. The cut point of the cluster was 1.7.

3.8 Decision tree

Decision tree is an advanced method for partitioning sets of items into classes (Nisbet *et al.* 2009). In this study, partitioning of genotypes in two classes of tolerance to long-term stress conditions was done for each experiment as well as combined over experiments for each population (Figure 51 and Figure 52 and Figure 53). Tolerance class was separated based on DRYMp (each experiment) and DRYMp (combined over experiments) index, tolerant (DRYMp > 0) and sensitive (DRYMp < 0). The final model fit statistics are listed in supplementary Table S10 and the variable importance are shown in supplementary Table S11 for each experiment and combined over experiments of respective populations.

The tree classification model was not effective in the separate experiments of population A. However, in data combined over years, the final tree model was generated with 4 nodes. The top and lower nodes were partitioned by A3D and PH, respectively (Figure 51). Leaf A3D greater than or equal to 304468 (node 2) resulted in a class that contained 70 % tolerant genotypes. The class with leaf A3D less than 304468 (node 2) and PH less than 436 (node 3) contained 66 % tolerant genotypes. The class with leaf A3D less than 304468 (node 1) and PH greater than or equal to 436 (node 4) contained 58 % sensitive genotypes. The classification result indicated that tolerance may be predicted from PH and leaf area. Tolerance was related to shorter plant height and higher leaf area.

In population B, the tree classification model was effective in the separate experiments and in data combined over the years. In the 2017 experiment, the final model contained eight nodes (Figure 52). Nodes were determined by LP and LI. LP less than 218 (node 1) and LI less than 1.42 (node 3) contained 89 % tolerant genotypes. LP less than 218 (node 1) and LI greater than or equal to 1.42 (node 4) and LP less than 178 (node 7) contained 81 % tolerant genotypes. LP less than 218 (node 1) and LI greater than or equal to 1.42 (node 4) and LP greater than or equal to 178 (node 8) contained 73 % sensitive genotypes. The other node identifying the sensitive group was node 2. Node 2 indicated that LP greater than or equal to 218 contained 75 % sensitive genotypes.

In the 2018 experiment, the population was partitioned by LI and the final model contained two nodes (Figure 52). LI less than 1.43 (node 1) contained 64 % tolerant genotypes. However, LI greater than or equal to 1.43 (node 2) contained 90 % sensitive genotypes (0.90).

In data combined over the years of population B, the final tree model was generated with 6 nodes. The nodes were partitioned by LI (node 1 and node 2), LP (node 3 and node 4) and DB (node 5 and node 6) (Figure 53). LI greater than or equal to 1.43 (node 2) contained 76 % sensitive genotypes. LI less than 1.43 (node 1) and LP less than 207 (node 3) contained 88 tolerant genotypes. LI less than 1.43 (node 1) and LP greater than or equal to 207 (node 4) and DB less than 40230178 (node 5) contained 81 % tolerant genotype. LI less than 1.43 (node 1) and LP greater than or equal to 207 (node 4), DB greater than or equal to 140230178 (node 6) contained 54 % sensitive genotypes.

In both populations, the output of the decision tree indicated that a tree model with only 2 or 4 leaves provides a high degree of accuracy for classification. Generally, leaf area and PH (population A) and LI, LPD and PH (population B) were the most important parameters to differentiate between sensitive and tolerant genotypes. Therefore, considering these parameters during phenotyping for drought tolerance may be helpful.

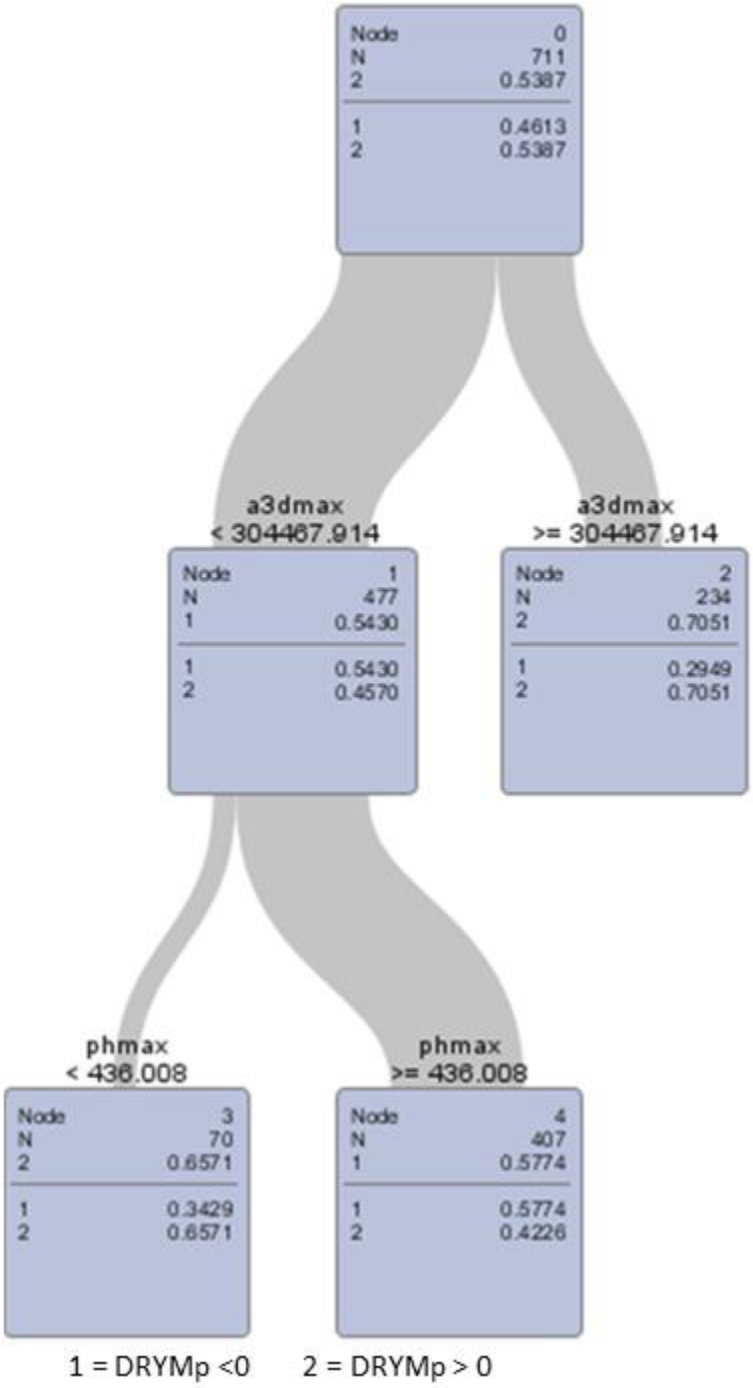


Figure 51. Decision tree model on canopy parameters from genotypes cultivated under long-term stress conditions in 2015 and 2016. Where 1 - sensitive group (DRYMp < 0), 2 - tolerant group (DRYMp > 0), a3dmax - maximum leaf area 3D, phmax - maximum plant height.

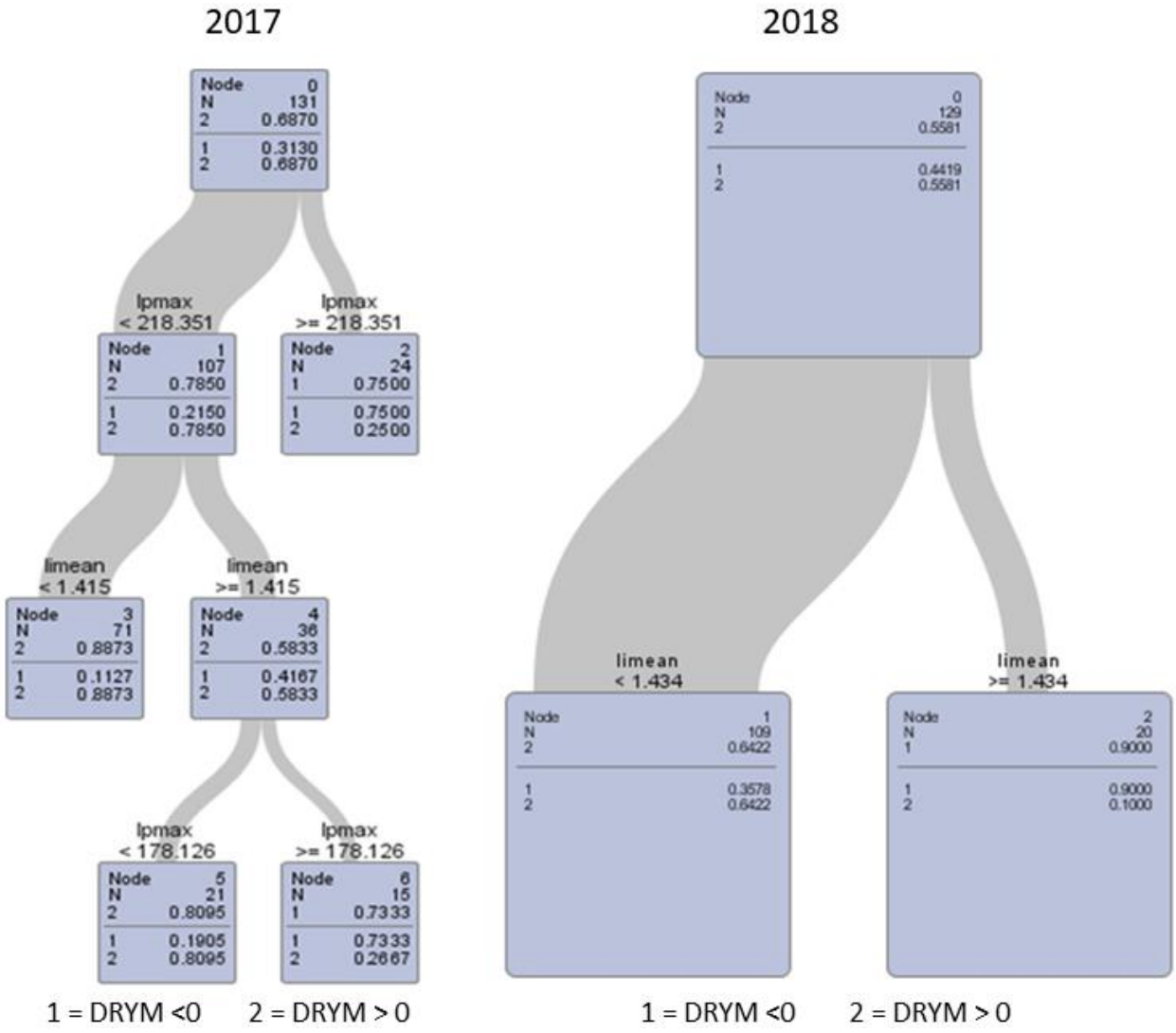


Figure 52. Decision tree model on canopy parameters from genotypes cultivated under long-term stress conditions in 2017 or 2018. Where 1 - sensitive group (DRYMp < 0), 2 - tolerant group (DRYMp > 0), lpmax - maximum light penetration depth, limean - mean leaf inclination.

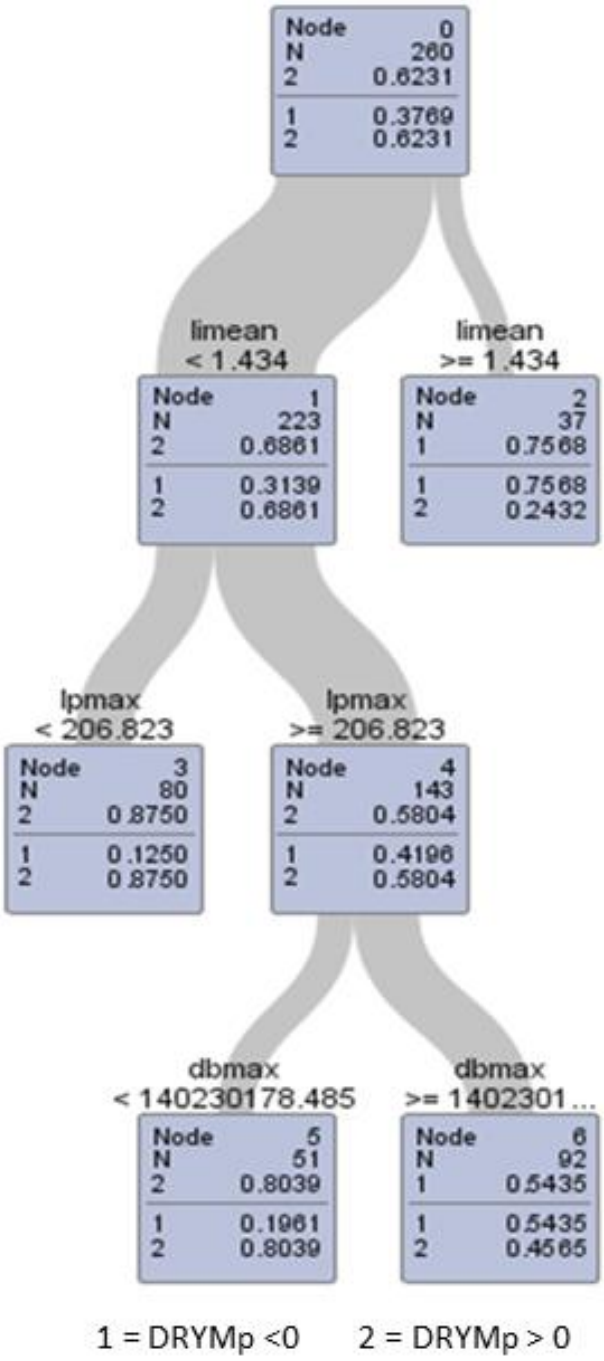


Figure 53. Decision tree model on canopy parameters from genotypes cultivated under long-term stress conditions in 2017 and 2018. Where 1 - sensitive group (DRYMp < 0), 2 - tolerant group (DRYMp > 0), limean - mean leaf inclination, lpmax - maximum light penetration depth, db - digital biomass.

3.9 Predicting tolerance from multiple parameters

In the final step, I have attempted to improve drought tolerance prediction by integrating several independent morphological and physiological variables in one model to predict drought tolerance. Since most of the morphological and a physiological variable were mostly predictive under long-term stress conditions, tolerance prediction from multiple variables focused on trait phenotypes under long-term stress conditions. For this purpose, DRYMp was predicted through multiple linear regression with stepwise variable selection (stepwise model) and with application of the Least Absolute Shrinkage and Selection Operator (LASSO model). LASSO regression is a type of linear regression that uses regularization.

3.9.1 DRYMp values in specific trial's, 2015-2018

In this case, genotype tolerance was predicted from the means of morphological and physiological values per genotype of the respective experiment and combined over years of the respective population. In population B, LI and LPD (2017) and LI (2018) were maintained by the model as predictive parameters for tolerance (Table 18). In population A (2016 experiment), LA was used as predictive parameter for tolerance. However, tolerance prediction in the 2015 experiment was not effective. DRYMp values across experiments were predicted from PH and LA in population A and from LI in population B (Table 18). The model's adj R^2 values of respective year and combined over years were higher for population B than for population A. Generally, tolerance was negatively correlated with PH and LA in population A and with LI and LPD in population B.

Table 18. Genotype tolerance prediction from multiple parameters determined under ss condition, 2015-2018. Int - intercept, para - parameter, est - estimate.

Year (s)	Para	Est	R^2	Adj R^2
2016	Int.	0.02		
	LA	-0.04	0.12	0.11
2017	Int.	4.39		
	LI	-2.77	0.33	
	LPD	-0.05	0.58	0.53
2018	Int.	4.27		
	LI	-3.01	0.50	0.47
2015-2016	Int.	-0.02		
	PH	-0.04	0.06	
	LA	-0.02	0.10	0.09
2017-2018	Int.	0.04		
	LI	-0.05	0.27	0.25

3.9.2 Prediction of mean tolerance in different environments

Finally, I tested whether the drought tolerance calculated from starch yields in different environments can be predicted from morphological and physiological parameters measured in experiments that were performed in the FGH. For this purpose, average DRYMp values were calculated from relative starch yields in drought stress experiments in the FGH and on different field sites in 2011 – 2018. Data were obtained from the projects VALDIS and TROST. Only a limited number of genotypes are represented in all experiments. The results of the tolerance prediction from average morphological and physiological values are shown in Table 19. Among the predictive parameters, PH (population A) and PH and LI (population B) were selected as main predictors for average tolerance in FGH and (FGH and Field) conditions. This result indicated that PH and LI were potential predictors for a wider range of genotypes. The model R^2 was higher in population B than population A (Table 19).

Altogether, drought tolerance prediction under long-term conditions across different test conditions (FGH, Field) and wider genotypes could be possible from LI and PH. Therefore, measuring LI and PH during drought tolerance evaluation may be helpful to predict tolerance and variety selection.

Table 19. Predicting average tolerance in FGH plants and (FGH and Field) plants, mean of morphological parameters 2011-2018. Pop - population, Int - intercept, para - parameter.

Pop	Experiment	Model	Entered	R^2	Adj r^2	Para.	Estimate
A	FGH	Stepwise	Int.	0.00			
			PH	0.11	0.09	PH	-0.05
A		LASSO	Int.	0.00			
			PH	0.11	0.09	PH	-0.05
A	FGH and Field	Stepwise	Int.	0.00		Int.	-0.01
			PH	0.10	0.08	PH	-0.03
A		LASSO	Int.	0.00			
			PH	0.09	0.07	PH	-0.02
B	FGH	Stepwise	Int.	0.00		Int.	0.03
			PH	0.46		PH	-0.07
			LI	0.62		LI	-0.05
			DB	0.68	0.55		
B		LASSO	Int.	0.00		Int.	0.01
			PH	0.26		PH	-0.04
			LI	0.46	0.36	LI	-0.02
B	FGH and Field	Stepwise	Int.	0.00		Int.	0.02
			PH	0.52		PH	-0.06
			LI	0.70	0.65	LI	-0.03
B		LASSO	Int.	0.00		Int.	0.01
			PH	0.28		PH	-0.04
			LI	0.56		LI	-0.01
			LPD	0.69	0.48		

4. Discussion

4.1 Yield and drought tolerance

In potato, water stress is most detrimental to tuber initiation, tuber bulking and thus tuber yield (Schafleitner 2009; Obidiegwu *et al.* 2015; Hirut *et al.* 2017). The yield reduction results from decreased leaf area, decreased photosynthetic rates and reduced partitioning of assimilate to tubers (Obidiegwu *et al.* 2015; Dahal *et al.* 2019). Yield reduction under water stress depends on the tolerance of genotypes to insufficient water supply. Drought tolerance variation between potato genotypes has been reported for European, Indian, USA and African potato cultivars (Stark *et al.* 1991; Sprenger *et al.* 2015; Gerhards *et al.* 2016; Mahmud *et al.* 2016; Hirut *et al.* 2017). In this study, tolerance data of 71 potato genotypes from two test populations were used; these data were based on yield and yield related traits on plants grown under screenhouse and field conditions (Table 1). The genotypes were genotyped by Prof. R. Horn at the University of Rostock as part of the collaborative project VALDIS Trost. In both populations, genotypes varied significantly in tuber yield, starch yield, tuber number, and average tuber weight under control and different water stress conditions. The stress condition included early stress (stress until flowering), late stress (stress since flowing) and long-term stress.

Higher reduction of tuber and starch yield was found after long-term water stress compared to shorter early water stress and control. Long-term stress resulted in a lower number of tubers and lower average tuber weight (Figure 10) compared to control conditions. The fact that yield reduction under drought results from decreased tuber numbers and reduced average tuber weight has been reported before (Schafleitner *et al.* 2007a; Obidiegwu *et al.* 2015; Hirut *et al.* 2017; Aliche *et al.* 2018). The effect of drought on potato depends on the timing of the drought in the developmental cycle (van Loon 1981; Obidiegwu *et al.* 2015). In this study, tuber and starch yield were more reduced by late stress during tuber bulking than by early stress during the vegetative stage. Rudack *et al.*, (2017) found that many physiological traits respond more sensitively during tuber bulking than in earlier stages. Thus, insufficient water supply during tuber bulking results in an inhibition of tuber bulking. This hypothesis is supported by our observation that average tuber weight was not affected by early stress. The more detrimental effect of long-term and late stress compared to early stress was found in both screenhouse and field (2018) experiment, suggesting that the sensitivity of the tuber bulking stage was apparent in both test environments.

Early stresses had no effect on average weight per tuber. This implies that yield reduction under early stress may have resulted from a lower number of tubers produced. (Aliche *et al.* 2018) and (Dahal *et al.* 2019) found that drought during early growth stage suppresses tuber initiation substantially. According to (Struik 2007a), tuberization occurs between 15-65 days from planting so any stress between this time range may inhibit tuber initiation and finally reduce the number of tubers. However, in our studies the effect of early stress on tuber number was too variable between years to come to a straight conclusion. Even though the effect of early stress on tuber number was not consistent in this study, early stress resulted in reduced final tuber and starch yield compared with control conditions. Different reports indicate that early stress affects plant establishment and final yield in potato (Obidiegwu *et al.* 2015; Sprenger *et al.* 2015; Dahal *et al.* 2019). The total tuber and starch yields were least reduced by early stress and most by long-term stress. Thus, our results confirm that duration and timing of drought affect tuber yield and starch yield.

4.2 Drought tolerance index

Drought indices provide a measure of drought tolerance of a genotype based on the loss of yield under drought-conditions in comparison to optimal water supply conditions (Mitra 2001; Sprenger *et al.* 2015). Different yield-based drought tolerance indices SSI, SST and GMP (Fischer and Maurer 1978; Rosielle and Hamblin 1981; Fernandez 1992) were used in different crops to quantify tolerance. In the present study, drought tolerance of genotypes were quantified by calculating the tolerance index DRYM, which is the deviation of relative yield from the overall median (DRYM) (Sprenger *et al.* 2015). Since its values centered at zero, interpretation of DRYM values is simple. DRYM value of zero indicates average tolerance, negative values indicate sensitivity and positive values indicate tolerance. In addition to the easy interpretation of the values, the DRYM is more powerful than SSI or GMP to distinguish tolerant and sensitive lines independent of the yield potential (Sprenger *et al.* 2015).

In the present study, analysis of variance on DRYMp of the genotypes indicated that there was a significant variation in drought tolerance of genotypes of both populations under screenhouse and field conditions. The analysis of variance indicated that tolerance levels of genotypes varied between years, stress timing (early, late and long-term stress) and test environments (screenhouse, field) (Table 4 and Table 5). Generally, the results suggest that more than four experiments and a range of experimental conditions are required to predict drought tolerance under variable agricultural conditions.

4.3 Laser scanner automatic phenotyping

Different automatic phenotyping platforms have been used for different crops and objectives (Chen *et al.* 2014; Li *et al.* 2014; Humplik *et al.* 2015). In this study, plants were phenotype by laser scanner, which provides repeated, non-destructive measurements of the canopy surface. Through segmentation and different algorithms of 3D point cloud extraction of canopy surface, we obtained six estimates of canopy parameters for each day. Canopy parameters generated in this study were leaf area, plant height, digital biomass, leaf area index, and leaf angle and leaf inclination.

4.3.1 Laser scanner data quality control and data deconvolution

The plants were automatically phenotyped for about five, ten, eight and seven weeks in 2015, 2016, 2017, and 2018 respectively. The data of all years were downloaded from the database of the scanner software and proc gplot and proc univariate were run in SAS to check for outlier and data uniformity. At an early stage of phenotyping, data uniformity was low as a result of differences in plant establishment. Plant establishment is affected by the type of planting material (tuber vs tissue culture plantlets) and the size or weight of seed tubers planted (Lommen and Struik 1994; Hossain *et al.* 2011). In population B, tubers seed were used for planting. As weights were not exactly equal that may have caused variation in plant establishment especially between replicated plants. In population A, tissue culture plantlets were used as plant material. Canopy structure was substantially changed in early plant establishment, which may result from the adaptation of the plantlets to the big pots. Around the end of the observation period, shoots started to log and intermingle so that data quality was reduced again at the late stage of observation. In addition to the problems above, shoots logged before attaining maturity especially in population A (Figure 19). These problems required restricting the time window for evaluation to 15 days in population A, while more than 30 days could be used for the fitting of the growth curves and the calculation of the mean values in population B.

4.3.2 Estimation of parameters from continuous data

From continuous measurements I estimated mean, maximum, diurnal range and initial growth rate (by fitting different growth curves) of canopy parameters for individual genotypes, treatments and experimental conditions. Maximum values were determined for parameters that have maximum biological limits in the phenology of the plants, like plant height. For these parameters, maximum values of genotypes were determined for each genotype and treatment as mean of maximum values

of the replicates. In contrast, leaf angle and leaf inclination were calculated as mean of all observations because values of these parameters oscillated without a clear trend in the age window, during which the measurements were performed.

Many studies indicate that canopy parameters are related to water stress tolerance and yield of different crops (Stolf-Moreira *et al.* 2010; Chen *et al.* 2014; Obidiegwu *et al.* 2015). In this study, the association of the given canopy parameters (see above) with the tolerance index calculated from tuber starch yield data of respective experiments was determined by Pearson correlation analysis and multivariate analysis after quality control and outlier removal, followed by the calculation of descriptive values (traits) (see canopy traits in Materials and Methods).

Average and Maximum values

The maximum value of leaf area, plant height, digital biomass, leaf area index and mean values of leaf angle and leaf inclination values varied significantly among genotypes. The significant genotype x treatment interaction effects on several parameters found in all experiments implied that the drought treatments affected shoot development differently in different genotypes. Different studies indicate that water stress reduces plant height (Deblonde and Ledent 2000; Anithakumari *et al.* 2012; Hirut *et al.* 2017), ground cover (Deblonde and Ledent 2000; Hirut *et al.* 2017), and leaf growth (Deblonde and Ledent 2001; Souza *et al.* 2014).

The correlation analysis in population B indicated that tolerant genotypes under stress conditions showed lower plant height, shorter light penetration depth, higher leaf area 2D, and higher leaf angle (more horizontal leaf) than sensitive genotypes. This may be because larger leaf area increases interception of solar radiation and increase the efficiency of dry matter accumulation. According to (Lommen and Struik 1994; Boyd *et al.* 2002), intercepted radiation levels are determined by leaf area. Previous studies (Deblonde and Ledent 2000; Schafleitner *et al.* 2007a; Hirut *et al.* 2017) show that groundcover, which is strongly related to leaf area index and biomass, is correlated with tuber yield under both drought and well-watered conditions. In line with this, (Stolf-Moreira *et al.* 2010) indicate that tolerant soybean cultivars exhibit a larger leaf area than less tolerant cultivars. In addition to larger leaf area, compact canopy under stress condition may help to modify micro-climate of the canopy and reduce evapotranspiration. According to (Huang 1985), a compact canopy reduces evapotranspiration from the soil and lower leaf surface.

The leaf angle affects the light interception by the leaf and greatly modifies the leaf energy balance and canopy microclimate (Ehleringer and Forseth 1990). In this study, a higher leaf angle (more

horizontal leaves) was related to high tolerance in population B. Leaves that are more horizontal may improve solar light interception and consequently photosynthesis of a plant and thus improve dry matter accumulation. However, different studies indicate that erect leaf posture is associated with reduced susceptibility to photoinhibition and reduced risk of overheating (Pastenes *et al.* 2005; Burgess *et al.* 2017). Among all the traits (descriptive parameters) considered in population B, mean leaf angle under long-term stress showed the most consistent correlation with the tolerance index calculated from data of the same experiment. Thus, leaf angle may be a promising predictor for drought tolerance under long-term stress.

In population A, plants lodged prematurely before they reached the maximum canopy development during 2015 and 2016. This may have contributed to the low reproducibility of parameters between years. As a result, tolerance prediction from maximum and mean canopy parameters in population A may not be effective.

Diurnal leaf movements

Diurnal leaf movements occur in a variety of plants in response to the sun's movement across the sky (Zhu *et al.* 2015; Feng *et al.* 2016), growth stage (Luo *et al.* 2013) and environmental stress (Haile 2000; Pastenes *et al.* 2005; Zhu *et al.* 2015). Under soil water stress, the leaves or leaflets increase their leaf angle until they are almost vertical at midday (Ehleringer and Forseth 1990). Changes in leaf orientation in response to moisture stress result in architectural modification of the canopy (Haile 2000). In this study, leaf movements caused diurnal oscillation of morphological parameters under all treatment conditions. However, the calculated daily range of the morphological parameters of genotypes did not show any significant correlation with the tolerance level. This may be because different genotypes use different adaptation mechanisms to drought. Some genotypes may have responded by changing the leaf orientation and consequently reduce heat load to the leaf (Haile 2000) , and others may have responded to drought stress with epinasty or with the leaves curving downwards (Romero *et al.* 2017). Others may have responded physiologically (Obidiegwu *et al.* 2015; Rudack *et al.* 2017). One or a combination of the above mechanism may affect the correlation between tolerance and leaf movement. Therefore, drought tolerance prediction from diurnal leaf movement may not be successful.

4.3.3 Daily canopy structure and tolerance

Canopy structure refers to the volume and distribution of above-ground plant parts. Different studies indicate that canopy structure is affected by growth stage (Luo *et al.* 2013; Feng *et al.*

2016). In this study, the association between daily mean canopy parameters and respective experimental drought tolerance index (DRYMp) was calculated by Pearson correlation.

In population A under long-term stress conditions, mean leaf area (2D and 3D) and mean leaf angle were significantly correlated with DRYMp especially during the reproductive stage (Figure 25). According to (Obidiegwu *et al.* 2015) the reproductive stage includes tuber initiation, tuber filling and tuber maturing. However, the correlation values were relatively weak. In population B, under long-term stress conditions, mean leaf area index and mean leaf angle were positively correlated with DRYMp (Figure 26 and Figure 27). On the other hand, mean light penetration depth was negatively correlated with DRYMp values. Most of the above correlations were found in late stress conditions in the 2017 experiment. Maintaining leaf area (Boyd *et al.* 2002; Stolf-Moreira *et al.* 2010) and developing a compact canopy (Huang 1985) help plants to increase photosynthetic efficiency and consequently increase assimilate production. Therefore, in this study, higher leaf area and lower light penetration may help tolerant plants to produce more assimilates to bulk the tubers than susceptible plants, especially in the reproductive stage.

In both populations the association between canopy parameters and DRYMp was relatively higher in the reproductive stage than vegetative stage of the plant. This could be because of genotype difference between the populations and/or environmental differences. Another potential source of variation for low correlation coefficients in population A could be because of lodging of the plants before they attained the biological maximum value of canopy parameters.

In both populations especially under long-term stress conditions, the association between tolerance and canopy parameters was improved in the reproductive stage than vegetative stage. Off course, this stage was identified by different studies (van Loon 1981; Obidiegwu *et al.* 2015; Rudack *et al.* 2017; Dahal *et al.* 2019) as one of the most important stages in drought tolerance. Therefore, water stress tolerance prediction from canopy parameters looks promising, especially during the reproductive stage in long-term stress conditions.

4.3.4 Initial growth rate and/or average morphological values

Plant growth follows a sigmoidal growth curve (Damgaard and Weiner 2008; Chen *et al.* 2014). The advances in plant growth modelling have allowed a deeper understanding of relationships between plants and their abiotic environment (Paine *et al.* 2012). In this study, we used time-lapse phenotypic data to model and predict plant growth under control and different water stress conditions. We used linear models for approximately linearly increased part of the data and

polynomial model for the whole part of the data set. Confounding effect of climate data for model prediction were not considered because of technical limitations.

Linear initial growth

The rate of change of morphological parameters over time was determined by linear regression.

For Population B in 2017, lower growth rates of plant height under control and late stress conditions were associated with drought tolerance. This agrees with results found for barley (Chen *et al.* 2014) which suggest that breeding for higher drought tolerance could simultaneously select lower plant height. Plant size is inversely related with growth reduction and directly related with osmotic adjustment during osmotic stress (Blum *et al.* 1997). (Boyd *et al.* 2002) show that genotypes that exhibit less reduction in growth and carbon assimilation rate under stress exhibit less tuber yield reduction. This pattern fits with results found for population A (experiment performed in 2015), where tolerant lines had larger growth rate of leaf areas as compared to sensitive lines. Therefore, the inconsistency of results in two populations may result from different genetic backgrounds of the populations and/or from environmental effects like higher VPD in the 2018 experiment. The genetic background of the two populations differ as population B contained commercial varieties that were not present in population A.

Average leaf inclination affects light interception by the leaf and greatly modifies the leaf energy balance and canopy microclimate (Ehleringer and Forseth 1990). In this study, leaf inclination changes with plant age under control (2017 and 2018) and long-term stress (2017) conditions and was positively correlated with tolerance level (DRYMp) of each trial under long-term stress conditions. The parameter leaf inclination considers leaf orientation plus leaf area (see material and methods). This could have improved the association of tolerance with leaf inclination compared to leaf angle. A more vertical leaf angle (low value of leaf angle) results in a higher value for leaf inclination. The average leaf angle changes with plant age under control condition (2018) was negatively correlated with DRYMp. According to (Falster and Westoby 2003), steeper leaf angels potentially lead to an improvement in whole day carbon gain by enhancing light absorption at low solar angles. In line with this, tolerant genotypes presented more upright leaves under control condition than sensitive genotypes. Since average leaf inclination per plant age under control conditions showed a consistent relationship with drought tolerance, it may be a useful parameter for tolerance prediction. Measurements under control conditions are preferable to those

under stress conditions as optimal soil water content is easier to maintain in managed trials than drought stress conditions (Stark *et al.* 1991).

Polynomial regression

In contrast to the linear model that was fitted for the early growth period, the polynomial model was estimated for the entire growth period until plants attained maximum canopy parameters. Generally, tolerance levels showed significantly negative correlations with linear coefficients, and positive correlations with the quadratic coefficients of the model. A lower growth rate seems to be related with tolerance. This fits with the observation that tolerant genotypes showed lower average plant heights, digital biomass and leaf area index in population B and lower growth rates of leaf area in 2016 in population A. The result was not consistent across populations and seasons, which implies that tolerance prediction from fitted polynomial growth functions could be difficult to generalize.

4.3.5 Tolerance prediction from single morphological parameters

Different reports indicate that tolerance index could be linearly predicted from single or multiple plant parameters. In wheat, (Mason and Singh 2014) predict yield from canopy temperature and covariate of plant height and days to heading. (Frels *et al.* 2018) also predict agronomic and nitrogen use (NU) traits from vegetation indices. In potato, (Hirut *et al.* 2017) predict drought tolerance from tuber yield and number of days from planting to 50 % flower bud formation. (Stark *et al.* 1991) predicted drought tolerance from canopy temperature depression. In this part of the study, DRYMp in a particular experiment was linearly predicted from average or maximum values per genotype of each single independent morphological trait. In population A, tolerance prediction was only possible in the 2016 experiment from mean LA under long-term water stress. In population B, tolerance under long-term stress conditions were predicted from either mean LA or mean LI. In 2017, tolerance was also predicted from PH and LPD. Additionally, tolerance predictions under cc (LPD or LI) and cs (LPD or PH) conditions were possible in 2017. This indicated that tolerance in the FGH could be predicted from different single independent predictors especially under long-term stress conditions. Therefore, considering these parameters during tolerance evaluation may be helpful. However, the model adj R^2 values of most traits were small especially in population A (Table 14). This indicated that tolerance prediction from single traits may not be sufficient. According to (Ekanayake 1989; Luo 2010), tolerance results from a

combination of different mechanisms, therefore tolerance prediction from a single parameter is rarely successful. Therefore, in the next step, tolerance was predicted from different morphological and physiological traits in a single model. Multivariate predictive models are discussed in detail in section 4.8 below. However, tolerance prediction from single morphological and physiological traits was only possible (e.g. CTD) or improved (e.g. LPD) for long-term stress data, therefore multiple regression, visualization and clustering analysis focused on long-term stress data sets.

4.4 Canopy temperature (CT) and canopy temperature depression (CTD)

4.4.1 Quality control and data convolution

In 2017 and 2018, CT was measured once every 30 min between 27 to 73 DFP (2017) and 24 to 74 DFP (2018) by IRT. The CT of a particular plant was selected from the continuous measurements of IRT based on the timestamps of the plantEye data. Therefore, any problems with the timestamps of the laser scanner data will affect the data quality of IRT data. For example, in the 2017 experiment the speed of the laser scanner varied throughout the measurement periods, which made timestamp calculation for particular plant positions difficult. This resulting data integration was time consuming and prone to error. As a solution, timestamps were calculated based on daily scanning speed and plant position. In addition, they were crosschecked with the start time of the continuous scanning data (Figure 28). The start time of each scan was clearly visible from continuous scanning data.

Finally, all data are available in database. A few data points are missing as a result of technical problems. The data set outliers were trimmed at plus or minus two standard deviations, but Shapiro-Wilk test indicated that the data set was not normally distributed (supplementary Table S15). This could be explained by the high CT difference betweeen genotypes (tolerant and sensitive group) (Figure 30). Therefore, the final data analysis was done after outlier removal without data transformation. This may have reduced the power of the analysis.

4.4.2 CT as a drought tolerance marker

There is a close inverse relationship between canopy temperature and transpiration cooling, which makes leaf canopy temperature a reliable indicator of plant water stress (Stark *et al.* 1991; Blum 2009; Ahmed *et al.* 2011). Different studies in alfalfa and turfgrass (Blonquist *et al.* 2009), wheat (Blum *et al.* 1982; Thapa *et al.* 2018), potato (Mahmud *et al.* 2016; Hirut *et al.* 2017), cotton

(Hatfield *et al.* 1987), durum wheat (Guendouz *et al.* 2012), and sorghum (Olufay *et al.* 1993) found that plants show higher canopy temperature under stress conditions. This relationship results from stomata closure of plants that experienced decreased water uptake due to soil water depletion. This reduces transpiration and increases canopy temperature. Likewise, in our experiments the mean CT was higher for plants under stress conditions than for plants under optimal water conditions (Figure 29).

In our study, mean VPD ((Figure 8 and Figure 9), CT and CTD (absolute values) were higher in 2018 than in 2017, indicating that plants were potentially under more stress in 2018 than in 2017. This matches the larger reduction in tuber and starch yield in 2018 compared to 2017 (Figure 11 and Figure 12). The yield reduction was observed in all treatments, which implies that another environmental stress enhanced drought effects in 2018. One of the potential environmental stresses in the 2018 experiment was high air temperature (supplementary Figure S5). As potato grows optimally at 14 - 22^{o} C (Struik 2007b), the relatively higher temperature values in the 2018 experiment may have led to heat stress and/or enhanced the effects of drought stress. Heat stress effects were reported for potatoes that grow at a temperature of 29 ^{o}C and above (Lafta and Lorenzen 2015; Krystyna 2017). In 2018, air temperature in the afternoon (12:00 -19:00) were frequently above 30 ^{o}C especially in the late stage (after 67 DFP) of the plant (supplementary Figure S5). According to (Krystyna 2017), heat stress during the growing season has a negative effect on the final yield. The effect is strongest when heat affects plants during flowering.

In both years, genotypic variance was significant for CT and CTD under all treatment conditions. CTD variation in potato genotypes has been reported for European, Indian and African potato populations (Stark *et al.* 1991; Gerhards *et al.* 2016; Mahmud *et al.* 2016; Hirut *et al.* 2017). In the present study, higher mean canopy temperatures under long-term drought stress conditions were associated with yield reduction (supplementary Table S12). High mean canopy temperatures indicate that sensitive genotypes were not able to maintain adequate transpiration rates and therefore transpiration cooling was reduced. Lower transpiration is linked to a reduction in photosynthetic rates and therefore reduction in yields. Similar results have been reported for potato (Gerhards *et al.* 2016; Mahmud *et al.* 2016), wheat (Blum *et al.* 1982) and sorghum (Mutava 2012).

Different reports suggest canopy temperature depression (CTD) as a potential predictor for drought tolerance. However, there are different opinions on the condition of CT measurements and the

determination of CTD. (Blum *et al.* 1982; Gardner *et al.* 1986) propose to measure CTD under drought stress to identify cooler canopies, because higher transpiration rates associated with high CTD indicate more growth and yield. In contrast, others have suggested measuring CTD under well-watered conditions to identify warmer canopies because smaller transpiration rates associated with warm canopies indicate greater water conservation and therefore more water for growth and reproduction later in the season (Pinter *et al.* 1990; Stark *et al.* 1991; Hirut *et al.* 2017). In the present study, DRYMp calculated from individual experiments correlated with CTD measured under long-term stress condition in the reproductive stage of plant, but not under control conditions.

4.4.3 Effect of developmental stage on CT and CTD

Potato developmental stage is classified into five distinct growth phases (Obidiegwu *et al.* 2015). The exact timing of these growth phases depends on many environmental factors, among them water supply. In this study, the effect of water stress on the relation between CT and developmental stage was assessed from CT measured throughout different plant growth stages.

In line with other reports (Blum *et al.* 1982; Reynolds *et al.* 2001; Mainassara *et al.* 2011; Talebi 2011), our tolerant potato genotypes showed a cooler (1.5 ^{o}C in 2017 and 2 ^{o}C in 2018) canopy under long-term stress conditions than the sensitive genotypes. This may be because tolerant groups may keep the stomata open for longer times and keep the canopy cooler than susceptible group. This was especially the case during tuber bulking. During this stage, tolerant genotypes maintain photosynthesis and transpiration to produce assimilates that bulk the tubers.

Different studies indicate that growth stage affects the association between CTD, yield and tolerance index in different crops. In wheat (Reynolds *et al.* 2001; Bilge *et al.* 2008; Abdipur *et al.* 2013), the association between CTD, yield and tolerance index is strongest during anthesis and the milky stage of grain development. In contrast, (Epure *et al.* 2017) suggest that the effect of growth stage on the association between CTD and yield of wheat is location dependent. In chickpea (Purushothaman *et al.* 2015), selection for grain yield through CTD is most efficient two weeks after the mean flowering time. Our result confirms that the growth stage has to be considered when measuring CTD as tolerance prediction parameter.

4.4.4 Effect of diurnal cycle on CT and CTD

CT varies through the 24-hour cycle. CT follows the diurnal cycle of the air temperature, solar radiation and vapor pressure deficit (Olufay *et al.* 1993; Balota *et al.* 2007; Thapa *et al.* 2018). CTD depends on the light-depend change in photosynthesis and thus stomatal transpiration. Photosynthesis is furthermore influenced by the internal circadian rhythm of the plant. In this study, the highest correlations between CTD and tolerance index were observed at and after noon. This may be because tolerant genotypes may continue photosynthesis and thus transpiration cooling longer in the afternoon than susceptible genotypes. Numerous reports indicate that self-cooling is often mostly effective at or after midday. In *Artemisia ordosica,* self-cooling is most effective between 9:00-16:00 (Yu *et al.* 2017). (Mason and Singh 2014) found highest CTD between 1:00 pm and 7:00 pm for sorghum. In wheat, (Amani *et al.* 1996; Abdipur *et al.* 2013) found remarkably high correlations between grain yield and CTD at or after midday. For potato, best correlations between CTD and tolerance index were found at 13:00 (Mahmud *et al.* 2016) and between 11:00 and 15:30 (Gerhards *et al.* 2016).

Furthermore, significant correlations between CTD and tolerance index were also observed during nighttime, especially in the 2018 experiment. The preliminary result of (Ramirez *et al.* 2017) indicates the presence of nighttime transpiration in potato. Nighttime transpiration has also been described in wheat (Richards *et al.* 2002; Balota *et al.* 2007) and other C3 and C4 species (Snyder *et al.* 2003; Caird *et al.* 2007) in low-humidity environments. In potato, nocturnal transpiration is linearly correlated with tuber yield (Ramirez *et al.* 2017). To our knowledge, there are no other reports in the literature of consistent genotypic differences for CTD during the night in potato.

4.4.5 Effect of micro-climate on CT and CTD

CTD is affected by many physiological factors, which makes it a powerful integrative trait. However, CTD is sensitive to many environmental factors like soil water status, air temperature, relative humidity, and incident radiation (Reynolds *et al.* 2001). In line with this, high correlation values between CT and average midday (10-14) VPD, daily mean RH, and air temperature were observed in this study (supplementary Figure S6). According to (Blum *et al.* 1982) and (Amani *et al.* 1996), CTD is best determined at high vapor pressure deficit conditions associated with low relative humidity and warm air temperature conditions. In this study, the correlations between CTD and tolerance index were higher in the 2018 than the 2017 experiment. This may result from

the higher average VPD in 2018 (Figure 9). Therefore, it is important to consider the micro-climate when deciding when to measure CT in order to estimate CTD as a predictor for drought tolerance.

4.4.6 Conclusion on CTD measurements for marker development

Under long-term stress conditions, tolerance of genotypes can be linearly predicted from CTD. The variation in DRYMp explained by our linear regression model was about 0.27 (2017) and 0.40 (2018). Different studies in wheat (Epure *et al.* 2017), chickpea (Purushothaman *et al.* 2015), potato (Gerhards *et al.* 2016), and sorghum (Mutava 2012) suggest that CTD can be used to predict yield or tolerance in different crops. This study thus confirms that the best prediction for drought tolerance in potato is to perform CT measurements around noon during tuber-bulking stage in drought-stressed plants.

4.5 Principal component analysis

Principal component analysis (PCA) is a technique used to visualize variation and strong patterns in a dataset. PCA is often used as an explorative tool to generate hypotheses. In this study, PCA was performed on morphological and physiological parameters measured under long-term stress conditions. The total variance explained by the first two components was higher than 75% and together with the third component they explained more than 97% (in population A) and 86% (in population B). This indicted that two or three components provided a good summary of the data. The first component contained the variance of leaf area and LAI. The second component depicted the variance of PH, LA, CTD and LI. Highly correlated phenotypic components pointed in roughly the same direction. Nearby points in the biplot represented samples with similar patterns (Han *et al.* 2019). In this study, LAI and leaf area (A3D and A2D) were close to each other. The other group showing a similar pattern was observed between CTD, LPD and LI. There may be high multicollinearity between these parameters, which has to be considered during the fitting of multiple regression models.

4.6 Clustering

Genotypes were clustered based on morphological and physiological parameters for each experiment and combined across experiments for each population. In population B, tolerant and susceptible genotypes were allocated to different clusters. This indicted tolerant genotypes of population B were morphologically and physiologically more similar to each other than they were

to the sensitive genotypes. In 2017, tolerant genotypes were allocated to two different clusters suggesting that the group of tolerant genotypes is not uniform under all environmental conditions. In population A, there was no clear relationship between the allocation to clusters and the drought tolerance. This implies that tolerance prediction from morphological and physiological parameters may be less effective in population A than in population B.

4.7 Decision tree

Decision trees are the most sophisticated methods for partitioning sets of items into classes (Nisbet *et al.* 2009). In this study, the tolerance classes sensitive (DRYMp <= 0) and tolerant (DRYMp > 0) were predicted from canopy parameters in a decision tree model.

In population A, no decision tree could be generated for the separate datasets in 2015 and 2016. When a decision tree was fitted on the data from both years, PH and leaf area were selected as discriminating parameter for tolerance classes. In population B, the tree model selected different discriminating parameters in each experiment and for the combined dataset of both years. In respective experiment dataset LPD, LI and PH (2017) and LI (2018) were selected as discriminating parameters. In combined 2017 plus 2018 data set, LI, LPD and DB were selected as discriminating parameters. Decision trees for each experiment as well as for the combined data set indicated that LI was consistently used as discriminating parameter between tolerant and sensitive groups.

Generally, discriminating tolerance and sensitive groups by decision tree was more efficient in population B than population A. This difference could be due to the additional canopy parameters measured in population B, the longer observation period or the higher genotypic variability in population B. In both populations, the tolerant class showed larger leaf area, shorter plant height, more compact canopies and more horizontal leaves under long-term stress conditions. Therefore, considering these parameters may be helpful to identify tolerant genotypes under long-term stress conditions.

4.8 Prediction of DRYMp from different parameters in a model

In the final step, I have tried to improve drought tolerance prediction by integrating several independent morphological and physiological variables in one model to predict drought tolerance by multiple linear regressions with either stepwise selection or Least Absolute Shrinkage and Selection Operator (LASSO) to determine the optimal number of independent variables.

Prediction of DRYM values under long-term stress conditions (FGH, 2015-2018)

The DRYMp value of a specific experiment or calculated for data of several experiments was predicted from maximum (PH, A2D, A3D, DB, LPD and LAI) and mean (LA and LI, CTD) values per genotype. In population A, the stepwise model identified LA (2016) as tolerance predictor. In data combined over the years in FGH experiments, tolerance was predicted from maximum PH and mean LA.

For population B, the stepwise model selected mean LI and maximum LPD (2017) or mean LI (2018). For the dataset that combines both years, the stepwise model selected mean LI as predictor. Relatively, the model efficiency was higher in population B than population A. This may result from differences in genotypic variation and environmental conditions between the two populations as well as from the use of more predictive parameters in population B.

In population B, the final multiple regression model maintained very few parameters; however, different canopy parameters were identified as tolerance predictors by simple linear regression models (Table 14). This may be because multiple linear regression models omitted some predictive parameters from the final model because of multicollinearity. For example, in the 2018 experiment, the final model maintained or removed CTD depending on the presence or absence of LI in the input model. If LI was in the input in the model, the final model maintained only LI. If LI was not in the input model, the final model maintained CTD and LA. The variation explained by CTD and LA was almost equivalent with that of a model containing only LI. This indicated that CTD and LA are both together could be an alternative predictor for tolerance. As handheld infrared thermometers are low cost and easily available, CTD can be measured more easily than other potential predictor like LI, LPD and LA. Therefore, for practical simplicity and applicability at any level (manual to automatic), CTD is one of the promising predictive traits for tolerance breeding in potato.

Prediction of DRYM values for different test environments

This study suggests that tolerance prediction from yield data of single, small experiments may not be effective. To get a more robust estimate, tolerance estimates from the projects TROST and VALDIS, which were based on multiple pot and field trials in 2011 - 2018 were used to develop prediction models based on morphological and physiological parameters. The average tolerance

(FGH or all test conditions) was predicted from canopy parameters by stepwise regression and LASSO model. The number of parameters maintained by the final model in the stepwise selection and LASSO models were more or less the same. This indicated that parameter shrinking in the LASSO model was not sufficient to reduce the parameters. However, the coefficients of the LASSO output were lowers compared to those the stepwise selection method indicating that shrinkage parameters were different from zero.

In both populations, the average tolerance in FGH condition and average tolerance in both test conditions (FGH and Field) were predicted by similar traits. However, the regression coefficients were different. In population A, maximum PH was selected as predictive trait. In population B, maximum PH and mean LI were selected by stepwise selection as predictive parameters for average tolerance. This indicates that similar parameters were important in both test conditions (FGH and Field), suggesting that tolerance can be predicted for both test environments from canopy parameters measured under screenhouse conditions, but the model has to be trained for each environment.

5. Conclusion on laser scanner and IR phenotyping as a source for drought tolerance markers

In both populations, water stress tolerance was affected by genotype, treatment, environmental conditions and their interaction. This suggests that several experiments in different years and under different environmental conditions are required to obtain a general assessment of a genotype's drought tolerance. Water stress reduced leaf area, DB, PH, LAI and LPD. However, water stress increased LI, CTD and LA (more vertical leaf).

The association between tolerance and canopy parameters was improved in the reproductive plant age especially in long-term stress conditions. Of course, this stage is identified by different studies (van Loon 1981; Rudack *et al.* 2017) as one of the most important stages in drought tolerance. Because of this, drought tolerance marker identification was focused on canopy parameters measured in the reproductive stage and long-term stress. Marker identification was done through liner regression and a decision tree.

In population A, the stepwise regression model selected PH and LA as tolerance predictive parameters in pooled data of both experiments. However, tolerance prediction in specific experiments was only possible from LA in 2016. The decision tree model was effective only in combined data over the years. The nodes in the tree model were determined by leaf A3D and PH. Both regression and decision tree identified PH as potential tolerance predictor in population A. Furthermore, the stepwise regression model chosen PH as average tolerance predictor over the wider dataset (2011 to 2018) in FGH experiments and in joint FGH and field dataset. This indicated that PH is one of the potential markers to predict tolerance in wider data sets. However, the model R^2 was smaller than in population B.

In population B, the stepwise regression model selected mean LI as a predictive parameter in the combined data set over the year. However, the decision tree model selected LI, LPD and DB to discriminate between the tolerant and susceptible groups. In a specific experimental year, LPD and LI (2017) and LI (2018) were maintained by the final model. Similarly, LPD and LI (2017) and LI (2018) determined the nodes in the decision tree model. As stepwise regressions as well as the decision tree model identified mean LI as predictors, LI was one of the potential tolerance predictor markers in the population B dataset. Additionally, the stepwise regression model selected LI and PH as average tolerance predictor across the wider dataset (2011 to 2018) in the FGH dataset and

in the joint FGH and field dataset. Together, mean LI could be a potential tolerance marker across different backgrounds.

As indicated by the simple linear regression model, CTD was one of the potential physiological parameters used to predicted tolerance under long-term stress, but it was not maintained in the final multiple linear regression model outputs. This was mainly because of the presence of multicollinearity problems, especially in the 2018 experiment. Therefore, from practical simplicity using CTD as an alternative tolerance predictor to LI may be important. CTD measured during the late morning and afternoon was the best predictor. Generally, this study confirms that the best prediction for drought tolerance in potato is made from the measurement of PH (population A and population B) and LI or CTD (population B) during the tuber-bulking stage in drought-stressed plants.

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7. Appendix

Supplementary Table S1. Genotypes and tolerance level from projects TROST (Sprenger *et al.* 2015) and VALDIS TROST (unpublished data from Manuela Haas).

Pedigree or Genotype	**Sample ID**	**Varity and group**[a]	**Tolerance group**	**TROST project**[b]	**Population**	
					A	B
Albatros (A)	866296	Parent	t	x	x	x
Euroresa (E)	869004	Parent	s	x	x	x
Ramses (R)	858641	Parent	s	x	x	x
AxR	899484	SP1	t		x	
AxR	899491	SP1	t		x	
AxR	899522	SP1	t		x	x
AxR	899596	SP1	t		x	
AxR	899664	SP1	t		x	
A xR	899665	SP1	t		x	x
AxR	899710	SP1	t		x	
AxR	899719	SP1	t		x	
ExA	899445	SP1	t		x	
ExA	899822	SP1	t		x	x
ExA	899905	SP1	t		x	
ExA	899922	SP1	t		x	x
ExA	899960	SP1	t		x	
ExA	899968	SP1	t		x	
ExA	900024	SP1	t		x	x
AxR	899486	SP1SP2	t		x	x
AxR	899584	SP1SP2	t		x	
AxR	899648	SP1SP2	t		x	
AxR	899659	SP1SP2	t		x	
AxR	899748	SP1SP2	t		x	x
AxR	899756	SP1SP2	t		x	
ExA	899446	SP1SP3	t		x	
ExA	899815	SP1SP3	t		x	
ExA	899891	SP1SP3	t		x	
AxR	899464	SP2	t		x	
AxR	899519	SP2	t		x	x
AxR	899530	SP2	t		x	
AxR	899569	SP2	t		x	
AxR	899620	SP2	t		x	
AxR	899626	SP2	t		x	
AxR	899646	SP2	t		x	
AxR	899660	SP2	t		x	

Pedigree	Sample ID	Varity	Tolerance	project	A	B
AxR	899663	SP2	t		x	
AxR	899704	SP2	t		x	
AxR	899708	SP2	t		x	
AxR	899717	SP2	t		x	
AxR	899732	SP2	t		x	
AxR	899745	SP2	t		x	
ExA	899788	SP2	t		x	
ExA	899834	SP2	t		x	x
ExA	899847	SP2	t		x	
AxR	899518	SP3	s		x	
ExA	899440	SP3	s		x	
ExA	899457	SP3	s		x	
ExA	899460	SP3	s		x	
ExA	899814	SP3	s		x	
ExA	899831	SP3	s		x	x
ExA	899852	SP3	s		x	
ExA	899871	SP3	s		x	
ExA	899872	SP3	s		x	
ExA	899914	SP3	s		x	
ExA	899925	SP3	s		x	
ExA	899932	SP3	s		x	
ExA	899933	SP3	s		x	
ExA	899934	SP3	s		x	
ExA	900012	SP3	s		x	
ExA	900029	SP3	s		x	
ExA	900033	SP3	s		x	
ExA	900039	SP3	s		x	
ExA	900040	SP3	s		x	
Desiree	22497	Varity	t	x	x	X
Milva	850136	>>	s	x		x
Eldena	872474	>>	s	x		x
Eurostarch	872477	>>	s	x		x
Maxi	866306	>>	s	x		x
Karlena	866309	>>	t	x		x
Pirol	866303	>>	t	x		x
Priamos	858638	>>	s	x		x

Nb: [a]- VALDIS project (in 2014), SP1- tolerant genotype based on stress index, SP2- tolerant by MAS (GC-MS and QRT-PCR) and SP3-sensitive by MAS (unpublished manuscript Haas et al.) and [b]-TROST project (2011-2013), t - tolerant and s - sensitive (Sprenger *et al.* 2015).

Supplementary Table S2. Mean starch yield (g per plant) of genotypes in control and long-term stress in FGH and Field, 2015-2016. Means comparison was done by *regwq test.* Treatments assigned the same letter are not significantly different at a p-value of 0.05. Id – Genotype Id

Id	Genotype	FGH				Field			
		2015		2016		2015		2016	
		cc	ss	cc	ss	cc	ss	cc	ss
1	22497	200^{H-J}	77^{M-R}	167^{C-K}	96^{B-J}	94^{K-M}	34^{C-F}	135^{G-J}	71^{A-E}
2	858641	243^{B-J}	69^{P-R}	149^{F-K}	75^{F-T}	291^{A-D}	73^{A-E}	236^{A-F}	78^{A-E}
3	866296	253^{B-J}	102^{C-R}	194^{A-I}	95^{B-K}	230^{A-I}	66^{A-E}	250^{A-F}	76^{A-E}
4	869004	288^{A-G}	92^{F-R}	183^{A-J}	77^{E-T}	285^{A-E}	69^{A-E}	231^{A-H}	92^{A-D}
5	899440	261^{A-J}	117^{A-O}	173^{A-K}	57^{O-U}	88^{LM}	39^{B-F}	131^{H-J}	45^{DE}
6	899445	285^{A-H}	79^{K-R}	174^{A-K}	74^{G-T}	266^{A-H}	75^{A-E}	301^{A}	89^{A-E}
7	899446	262^{A-J}	91^{G-R}	168^{C-K}	99^{A-I}	101^{J-M}	34^{C-F}	198^{B-I}	52^{C-E}
8	899457	280^{A-I}	137^{A-G}	220^{A-C}	98^{A-J}	193^{C-L}	57^{A-E}	198^{B-I}	71^{A-E}
9	899460	267^{A-J}	74^{N-R}	158^{D-K}	70^{I-U}	350^{A}	83^{AB}	259^{A-F}	82^{A-E}
10	899464	258^{B-J}	64^{QR}	157^{D-K}	52^{Q-U}	184^{C-L}	43^{A-F}	210^{A-I}	54^{C-E}
11	899484	188^{J}	91^{G-R}	137^{I-L}	54^{P-U}	51^{M}	10^{F}	97^{J}	28^{E}
12	899486	268^{A-J}	125^{A-L}	176^{A-K}	88^{C-O}	203^{C-L}	51^{A-F}	224^{A-I}	78^{A-E}
13	899491	262^{A-J}	78^{L-R}	196^{A-H}	76^{E-T}	2218^{B-J}	47^{A-F}	210^{A-I}	82^{A-E}
14	899518	280^{A-I}	130^{A-I}	158^{D-K}	67^{I-U}	277^{A-F}	80^{A-D}	272^{A-E}	65^{B-E}
15	899519	275^{A-I}	124^{A-M}	182^{A-J}	99^{A-I}	273^{A-G}	66^{A-E}	225^{A-I}	79^{A-E}
16	899522	297^{A-G}	147^{A-C}	229^{AB}	128^{A}	235^{A-I}	65^{A-E}	212^{A-I}	63^{B-E}
17	899530	238^{D-J}	103^{B-Q}	169^{C-K}	72^{H-T}	209^{B-L}	47^{A-F}	207^{A-I}	55^{B-E}
18	899569	$193I^{J}$	88^{H-R}	149^{F-K}	67^{I-U}	167^{D-M}	37^{B-F}	167^{F-J}	55^{B-E}
19	899584	270^{A-J}	142^{A-E}	188^{A-J}	98^{A-J}	158^{E-M}	32^{D-F}	167^{F-J}	64^{B-E}
20	899596	247^{B-J}	82^{J-R}	151^{F-K}	38^{U}	203^{C-L}	54^{A-F}	211^{A-I}	70^{A-E}
21	899620	231^{F-J}	84^{I-R}	184^{A-J}	49^{R-U}	180^{C-L}	64^{A-E}	213^{A-I}	93^{A-D}
22	899626	267^{A-J}	99^{D-R}	171^{B-K}	74^{F-T}	220^{B-J}	77^{A-E}	193^{B-I}	74^{A-E}
23	899646	243^{B-J}	139^{A-F}	204^{A-G}	117^{A-D}	221^{B-J}	65^{A-E}	216^{A-I}	78^{A-E}
24	899648	229^{F-J}	146^{A-D}	192^{A-I}	107^{A-F}	221^{B-J}	55^{A-F}	203^{A-I}	93^{A-D}
25	899659	263^{A-J}	131^{A-I}	171^{B-K}	86^{D-P}	154^{F-M}	47^{A-F}	196^{B-I}	72^{A-E}
26	899660	321^{A-D}	107^{B-Q}	179^{A-K}	93^{B-L}	219^{B-J}	58^{A-E}	224^{A-I}	97^{A-D}
27	899663	327^{A-C}	101^{C-R}	179^{A-K}	92^{B-M}	263^{A-H}	43^{A-F}	206^{A-I}	62^{B-E}
28	899664	277^{A-I}	139^{A-F}	180^{A-K}	108^{A-E}	166^{D-M}	40^{A-F}	184^{C-J}	67^{A-E}
29	899665	290^{A-G}	126^{A-K}	187^{A-J}	92^{B-M}	225^{A-J}	60^{A-E}	234^{A-G}	108^{A-C}
30	899704	276^{A-I}	95^{E-R}	179^{A-K}	83^{E-Q}	273^{A-G}	82^{A-C}	248^{A-F}	94^{A-D}
31	899708	252^{B-J}	73^{O-R}	190^{A-J}	72^{H-T}	289^{A-D}	76^{A-E}	178^{D-J}	79^{A-E}
32	899710	293^{A-G}	89^{H-R}	170^{C-K}	77^{E-T}	268^{A-H}	71^{A-E}	258^{A-F}	62^{B-E}
33	899717	274^{A-J}	92^{F-R}	159^{D-K}	59^{N-U}	289^{A-D}	74^{A-E}	294^{AB}	90^{A-D}
34	899719	277^{A-I}	120^{A-O}	195^{A-I}	90^{C-N}	222^{B-J}	68^{A-E}	247^{A-F}	95^{A-D}
35	899732	243^{B-J}	157^{A}	195^{A-I}	93^{B-M}	222^{B-J}	68^{A-E}	222^{A-I}	77^{A-E}
36	899745	269^{A-J}	112^{A-P}	176^{A-K}	103^{A-H}	273^{A-G}	74^{A-E}	257^{A-F}	94^{A-D}
37	899748	300^{A-G}	92^{F-R}	207^{A-F}	84^{E-Q}	298^{A-C}	80^{A-D}	211^{A-I}	90^{A-D}
38	899756	329^{AB}	157^{A}	211^{A-E}	97^{A-J}	223^{B-J}	61^{A-E}	236^{A-F}	82^{A-E}
39	899788	277^{A-I}	109^{B-Q}	155^{D-K}	60^{M-U}	242^{A-I}	57^{A-E}	253^{A-F}	78^{A-E}

Id.	Genotype	**cc**	**ss**	**cc**	**ss**	**cc**	**ss**	**cc**	**ss**
40	899814	264^{A-J}	119^{A-O}	182^{A-J}	79^{E-S}	200^{C-L}	52^{A-F}	167^{F-J}	74 $^{A-E}$
41	899815	259^{A-J}	91^{G-R}	168^{C-K}	84^{E-Q}	147^{G-M}	48^{A-F}	180^{D-J}	72^{A-E}
42	899822	297^{A-G}	128^{A-J}	196^{A-H}	86^{C-P}	236^{A-I}	62 $^{A-E}$	248^{A-F}	89^{A-E}
43	899831	261^{A-J}	129^{A-J}	202^{A-G}	94^{B-K}	183^{C-L}	68^{A-E}	231^{A-H}	89^{A-E}
44	899834	291^{A-G}	132^{A-H}	222^{A-C}	122^{AB}	217^{B-K}	70 $^{A-E}$	198^{B-I}	68^{A-E}
45	899847	303^{A-F}	98^{E-R}	191^{A-I}	66^{I-U}	301^{A-C}	82^{A-C}	285^{A-C}	95^{A-D}
46	899852	280^{A-I}	96^{E-R}	166^{C-K}	61^{L-U}	151^{F-M}	60^{A-E}	185^{C-J}	60^{B-E}
47	899871	327^{A-C}	142^{A-E}	215^{A-D}	82^{E-R}	141^{H-M}	69^{A-E}	190^{C-J}	73^{A-E}
48	899872	266^{A-J}	88^{H-R}	174^{A-K}	82^{E-Q}	222^{B-J}	54^{A-F}	246^{A-F}	89^{A-D}
49	899891	290^{A-G}	132^{A-H}	231^{A}	106^{A-G}	221^{B-J}	69^{A-E}	216^{A-I}	62^{B-E}
50	899905	296^{A-G}	134^{A-H}	166^{C-K}	81^{E-S}	230^{A-I}	65^{A-E}	211^{A-I}	75^{A-E}
51	899914	242^{B-J}	106^{B-Q}	166^{C-K}	72^{H-T}	221^{B-J}	64^{A-E}	190^{C-J}	72^{A-E}
52	899922	300^{A-G}	131^{A-I}	206^{A-F}	118^{A-C}	208^{C-L}	52^{A-F}	191^{C-J}	54^{C-E}
53	899925	249^{B-J}	93^{F-R}	146^{G-K}	66^{J-U}	277^{A-G}	80^{A-D}	226^{A-I}	95^{A-D}
54	899932	219^{F-J}	69^{P-R}	167^{C-K}	63^{K-U}	235^{A-I}	67^{A-E}	262^{A-F}	126^{A}
55	899933	235^{D-J}	142^{A-E}	90^{L}	86^{C-P}	188^{C-L}	33^{D-F}	197^{B-I}	57^{B-E}
56	899934	302^{A-F}	125^{A-L}	205^{A-G}	104^{A-H}	134^{I-M}	40^{A-F}	199^{B-I}	54^{C-E}
57	899960	262^{A-J}	105^{B-Q}	185^{A-J}	66^{J-U}	181^{C-L}	64^{A-E}	172^{E-J}	82^{A-E}
58	899968	240^{C-J}	79^{K-R}	142^{H-L}	54^{P-U}	251^{A-I}	70^{A-E}	224^{A-I}	73^{A-E}
59	900012	257^{B-J}	95^{E-R}	203^{A-G}	48^{S-U}	299^{A-C}	69^{A-E}	255^{A-F}	93^{A-D}
60	900024	345^{A}	150^{AB}	200^{A-H}	77^{E-T}	203^{C-L}	65^{A-E}	254^{A-F}	75^{A-E}
61	900029	319^{A-E}	106^{B-Q}	203^{A-G}	84^{E-Q}	269^{A-H}	88^{A}	222^{A-I}	72^{A-E}
62	900033	232^{E-J}	105^{B-Q}	151^{E-K}	72^{H-T}	303^{A-C}	85^{AB}	276^{A-D}	116^{AB}
63	900039	282^{A-H}	55^{R}	123^{KL}	49^{S-U}	337^{AB}	88^{A}	236^{A-F}	92^{A-D}
64	900040	213^{G-J}	121^{A-N}	131^{J-L}	45^{TU}	84^{LM}	31^{EF}	126^{IJ}	44DE
	Mean	268	109	178	81	219	61	217	76

Supplementary Table S3. Mean starch yield (g per plant) of genotypes in control and different water stress condition in FGH, 2017 and 2018. Means comparison was done by *regwq test.* Treatments assigned the same letter are not significantly different at a p-value of 0.05. Id – genotype ID.

ID	Genotype	2017				2018			
		cc	**cs**	**sc**	**ss**	**cc**	**cs**	**sc**	**ss**
1	22497	129^{DE}	81^{C-E}	108^{E-G}	69^{BC}	58^{EF}	25^{E-G}	37^{E-G}	12^{F-H}
2	850136	125^{DE}	55^{G-I}	97^{GH}	27^{FG}				
3	858638	121^{DE}	70^{D-G}	114^{D-G}	67^{BC}	113^{A-C}	50^{B}	62^{B-D}	39^{A-D}
4	858641	128^{DE}	77^{D-F}	97^{GH}	56^{C-E}	80^{DE}	28^{D-G}	52^{B-F}	16^{E-H}
5	866296	119^{DE}	67^{E-H}	103^{FG}	54^{C-E}	98^{B-D}	42^{B-D}	67^{B-D}	31^{B-E}
6	866303	154^{A-D}	81^{C-E}	119^{C-G}	55^{C-E}	99^{B-D}	40^{B-E}	63^{B-D}	22^{D-G}
7	866306	78^{F}	27^{J}	58^{IJ}	20^{FG}	49^{F}	16^{G}	37^{E-G}	15^{E-H}
8	866309	167^{A-C}	90^{A-D}	143^{A-D}	82^{AB}	58^{EF}	28^{D-G}	32^{FG}	7^{GH}
9	869004	96^{EF}	38^{IJ}	72^{HI}	34^{E-G}	61^{EF}	23^{FG}	50^{C-G}	4^{H}
10	872474	128^{DE}	49^{HI}	106^{FG}	42^{D-F}	84^{C-E}	27^{D-G}	46^{D-G}	21^{E-H}
11	872477	70^{F}	20^{J}	41^{J}	19^{G}	37^{F}	14^{G}	28^{G}	
12	899486	177^{AB}	103^{AB}	149^{AB}	93^{A}	109^{A-D}	45^{BC}	74^{AB}	31^{B-E}
13	899519	141^{B-D}	74^{D-G}	120^{B-G}	63^{B-D}	93^{B-D}	37^{B-F}	69^{B-D}	41^{A-C}
14	899522	171^{A-C}	108^{A}	170^{A}	96^{A}	135^{A}	66^{A}	92^{A}	50^{A}
15	899665	120^{DE}	74^{D-G}	105^{FG}	67^{BC}	100^{B-D}	33^{C-F}	60^{B-E}	25^{C-F}
16	899748	156^{A-D}	87^{B-E}	132^{B-F}	74^{A-C}	111^{A-D}	34^{C-F}	63^{B-D}	29^{C-F}
17	899822	121^{DE}	58^{F-I}	106^{FG}	52^{C-E}	93^{B-D}	23^{FG}	48^{C-G}	17^{E-H}
18	899831	136^{CD}	73^{D-G}	108^{-G}	53^{C-E}	92^{B-D}	32^{C-F}	62^{B-D}	22^{D-G}
19	899834	185^{A}	89^{A-E}	148^{A-C}	82^{AB}	123^{AB}	41^{B-D}	73^{A-C}	47^{AB}
20	899922	153^{A-D}	98^{A-C}	137^{B-E}	80^{AB}	93^{B-D}	45^{BC}	63^{B-D}	29^{C-F}
21	900024	129^{DE}	75^{D-G}	113^{E-G}	64^{BC}	101^{B-D}	38^{B-F}	60^{B-E}	13^{F-H}
	Mean	132	70	109	60	89	34	57	26

Supplementary Table S4. Mean starch yield (g per plant) of genotypes in control and different water stress condition in Field, in 2017 and 2018**.** Means comparison was done by *regwq test.* Treatments assigned the same letter are not significantly different at a p-value of 0.05.

Genotype	**2017**				**2018**			
	cc	**cs**	**sc**	**ss**	**cc**	**cs**	**sc**	**ss**
22497	108^{C}	96^{C}	86^{C}	69^{A-D}	129^{BC}	60^{B}	122^{E}	54^{F}
850136	169^{A-C}	175^{A-C}	149^{A-C}	27^{FG}	135^{A-C}	92^{AB}	120^{E}	68^{C-F}
858638	175^{A-C}	175^{A-C}	148^{A-C}	67^{A-D}	135^{A-C}	77^{AB}	125^{DE}	67^{D-F}
858641	189^{A-C}	195^{AB}	155^{AB}	56^{C-F}	161^{A-C}	81^{AB}	147^{B-E}	81^{B-E}
866296	172^{A-C}	205^{AB}	147^{A-C}	54^{C-F}	167^{A-C}	92^{AB}	174^{A-C}	83^{B-D}
866303	199^{A-C}	189^{A-C}	153^{A-C}	55^{C-F}	153^{A-C}	80^{AB}	154^{A-E}	67^{C-F}
866306	254^{A}	230^{AB}	129^{BC}	20^{G}	173^{A-C}	97^{AB}	164^{A-E}	81^{B-E}
866309	166^{A-C}	165^{A-C}	106^{BC}	82^{A-C}	141^{A-C}	75^{AB}	134^{C-E}	73^{B-F}
869004	197^{A-C}	222^{AB}	165^{AB}	34^{E-G}	166^{A-C}	96^{AB}	189^{AB}	94^{A-C}
872474	195^{A-C}	167^{A-C}	143^{A-C}	42^{D-G}	105^{C}	69^{AB}	129^{C-E}	55^{EF}
872477	242^{AB}	261^{A}	202^{A}	19^{G}	182^{A-C}	103^{AB}	159^{A-E}	112^{A}
899486	220^{AB}	199^{AB}	158^{AB}	93^{AB}	168^{A-C}	87^{AB}	154^{A-E}	77^{B-F}
899519	177^{A-C}	176^{A-C}	112^{BC}	63^{B-E}	179^{A-C}	99^{AB}	165^{A-E}	85^{B-D}
899522	156^{A-C}	160^{BC}	154^{AB}	96^{A}	215^{A}	104^{AB}	189^{AB}	90^{A-D}
899665	163^{A-C}	186^{A-C}	114^{BC}	67^{A-D}	164^{A-C}	103^{AB}	134^{C-E}	91^{A-D}
899748	185^{A-C}	204^{AB}	140^{A-C}	74^{A-C}	183^{A-C}	79^{AB}	136^{C-E}	79^{B-F}
899822	205^{A-C}	248^{AB}	171^{AB}	52^{C-F}	172^{A-C}	113^{A}	184^{AB}	96^{AB}
899831	220^{AB}	219^{AB}	164^{AB}	53^{C-F}	161^{A-C}	73^{AB}	185^{AB}	88^{A-D}
899834	165^{A-C}	183^{A-C}	111^{BC}	82^{A-C}	150^{A-C}	77^{AB}	146^{B-E}	67^{C-F}
899922	144^{BC}	161^{BC}	130^{BC}	80^{A-C}	150^{A-C}	91^{AB}	170^{A-D}	80^{B-F}
900024	159^{A-C}	183^{A-C}	166^{AB}	64^{B-E}	209^{AB}	114^{A}	197^{A}	90^{A-D}
Mean	180	186	140	60	160	87	156	79

Supplementary Table S5. Pearson correlation of SY and DRYMp between FGH and Field in 2015-2018. Where * significant at p values of 0.05. T - treatment.

Year	**2015**		**2016**		**2017**				**2018**			
T	**cc**	**ss**	**cc**	**ss**	**cc**	**cs**	**sc**	**ss**	**cc**	**cs**	**sc**	**Ss**
SY	0.2	-0.2	0.1	0.1	-0.48^{*}	-0.54^{*}	-0.4	-0.4	0.2	0.1	0.3	-0.0
DRYMp		0.1		-0.1		-0.0	0.4	-0.0		-0.1	0.1	-0.2

Supplementary Table S6. Combined ANOVA summary over trials (FGH and field) in population A and B. Where * and ** significant at p values of 0.05 and 0.01, para - parameter.

P	Para	Model	G	T	Y	E	G*Y	G*T	G*E	G*Y*E	G*T*Y*E
A	DF	260	63		1	1	63		63	64	
	DRYMp	6^{**}	5^{**}		99^{**}	10^{**}	3^{**}		6^{**}	3^{**}	
B	DF	254	20	2	1	1	20	40	20	20	81
	DRYMp	5^{**}	2^{**}	49^{**}	8^{**}	35^{**}	3^{**}	1^{ns}	2^{**}	2.77^{*}	3^{**}

Late stress (2017) in field experiment was excluded from combined analysis. Where, P – population, G - Genotype, T - Treatment, Y - Year and E - Experiment (FGH or Field).

Supplementary Table S7. Summary of the maximum plant height (PH), leaf area (A2D, A3D), digital biomass (DB), leaf area index (LAI), light penetration depth (LPD) and mean leaf angle (LA) and leaf inclination (LI) of population A and population B in different treatments (T) under in FGH experiments 2015 to 2018. Means with the same letter were not significantly different at p value of 0.05. Pop - population, T - treatment, com - combined over experiments of respective population.

P	year	T	PH (mm)	A3D (mm^2)	A2D (mm^2)	LA (º)	DB (mm^3)	LAI	LPD (mm)	LI
A	2015	cc	401^{A}	252061^{A}	2077^{A}	20^{A}				
		ss	365^{B}	248812^{B}	2040^{B}	20^{A}				
A	2016	cc	409^{A}	237252^{A}	1968^{A}	18^{B}				
		ss	400^{B}	213882^{B}	1774^{b}	20^{A}				
B	2017	cc	625^{A}	329820^{A}	240373^{A}	71^{AB}	179^{A}	1.01^{A}	184^{C}	1.37^{C}
		cs	494^{C}	322598^{B}	236541^{A}	70^{B}	144^{D}	0.99^{A}	175^{D}	1.39^{B}
		sc	625^{A}	309352^{C}	221852^{B}	71^{AB}	170^{B}	0.95^{B}	203^{A}	1.41^{A}
		ss	529^{B}	311942^{C}	222145^{B}	72^{A}	150^{C}	0.96^{B}	191^{B}	1.41^{A}
B	2018	cc	673^{A}	301409^{A}	216157^{A}	47^{A}	176^{A}	0.96^{A}	468^{B}	1.37^{D}
		cs	611^{B}	300831^{A}	214447^{A}	46^{C}	167^{B}	0.96^{A}	430^{C}	1.42^{A}
		sc	693^{A}	275531^{B}	196588^{B}	46^{B}	156^{C}	0.88^{B}	520^{A}	1.39^{C}
		ss	617^{B}	277347^{B}	196555^{B}	46^{C}	142^{D}	0.88^{B}	447^{BC}	1.41^{B}
A	com	cc	631^{A}	309548^{A}	2570^{A}	19^{B}				
		ss	486^{B}	291789^{B}	2445^{B}	20^{A}				
B	com	cc	651^{A}	316715^{A}	229168^{A}	59^{A}	178^{A}	0.99^{A}	323^{B}	1.37^{D}
		cs	551^{C}	311801^{B}	225333^{B}	58^{B}	156^{c}	0.98^{B}	302^{C}	1.40^{B}
		sc	659^{A}	293559^{C}	209761^{C}	59^{A}	164^{b}	0.92^{C}	360^{A}	1.39^{C}
		ss	573^{B}	294433^{C}	209163^{C}	59^{A}	147^{d}	0.92^{C}	319^{B}	1.41^{A}

DB (in ten thousand), LAI (mm^2/mm^2) and LI (mm^2/mm^2)

Supplementary Table S8. Eigenvalues of the correlation matrix of respective trial, 2015-2018. P- principal value. Pop - population.

Pop A		**2015**				**2016**			
	P	**Eigen value**	**Differ ence**	**Propo rtion**	**Cumul ative**	**Eigenv alue**	**Differ ence**	**Propo rtion**	**Cumul ative**
	1	2.30	1.37	0.58	0.58	2.39	1.52	0.60	0.60
	2	0.94	0.24	0.23	0.81	0.88	0.20	0.22	0.82
	3	0.70	0.63	0.17	0.98	0.68	0.63	0.17	0.99
	4	0.06		0.02	1.00	0.05		0.01	1.00
Pop B		**2017**				**2018**			
	1	4.87	2.91	0.54	0.54	3.20	0.61	0.36	0.36
	2	1.95	0.86	0.22	0.76	2.59	0.61	0.29	0.64
	3	1.09	0.43	0.12	0.88	1.98	1.04	0.22	0.86
	4	0.66	0.36	0.07	0.95	0.95	0.76	0.11	0.97
	5	0.29	0.22	0.03	0.98	0.18	0.11	0.02	0.99
	6	0.07	0.01	0.01	0.99	0.07	0.06	0.01	1.00
	7	0.06	0.05	0.01	1.00	0.02	0.01	0.00	1.00
	8	0.01	0.01	0.00	1.00	0.01	0.01	0.00	1.00
	9	0.00		0.00	1.00	0.00		0.00	1.00

Supplementary Table S9. Eigenvectors of PC of respective trial, 2015-2018.

Year	**para**	**Prin1**	**Prin2**	**Prin3**	**Prin4**	**Prin5**	**Prin6**	**Prin7**	**Prin8**	**Prin9**
2015	PH	-0.24	0.94	0.22	-0.04					
	A3D	0.63	0.12	0.25	0.73					
	A2D	0.61	0.03	0.42	-0.68					
	LA	-0.43	-0.3	0.84	0.12					
2016	PH	-0.30	0.94	-0.12	0.10					
	A3D	0.60	0.30	0.25	-0.70					
	A2D	0.60	0.16	0.34	0.70					
	LA	-0.43	-0.01	0.90	-0.05					
2017	PH	-0.24	0.54	-0.29	-0.12	0.22	-0.55	0.29	0.33	0.00
	A2D	0.43	0.14	0.04	0.23	-0.21	-0.39	0.36	-0.64	-0.09
	A3D	0.43	0.17	0.06	0.13	-0.25	-0.07	-0.14	0.32	0.76
	DB	0.07	0.69	-0.13	0.01	0.20	0.33	-0.48	-0.35	-0.02
	LPD	-0.37	0.32	0.05	0.25	-0.49	0.47	0.48	0.05	0.04
	LA	0.40	-0.05	-0.18	0.22	0.60	0.40	0.46	0.14	0.02
	LAI	0.43	0.17	0.07	0.16	-0.28	-0.01	-0.16	0.49	-0.64
	CTD	-0.26	0.04	0.53	0.70	0.31	-0.19	-0.15	0.05	0.01
	LI	0.13	0.23	0.75	-0.54	0.14	0.07	0.22	0.02	-0.01
2018	PH	-0.25	0.26	0.53	-0.07	0.35	0.67	-0.07	-0.04	-0.03
	A2D	0.52	0.19	0.01	0.09	-0.20	0.20	0.23	-0.74	0.03
	A3D	0.50	0.28	-0.03	0.04	0.08	0.04	-0.07	0.36	-0.73

para	Prin1	Prin2	Prin3	Prin4	Prin5	Prin6	Prin7	Prin8	Prin9
DB	0.04	0.26	0.62	-0.10	0.19	-0.69	0.07	-0.12	0.02
LPD	-0.26	0.40	0.19	0.46	-0.70	0.01	-0.06	0.15	0.01
LA	0.18	-0.44	0.30	0.48	0.08	0.08	0.61	0.27	0.05
LAI	0.49	0.28	-0.03	0.03	0.10	0.08	-0.20	0.39	0.68
CTD	-0.19	0.27	-0.34	0.66	0.53	-0.14	-0.07	-0.18	0.00
LI	-0.20	0.48	-0.29	-0.32	0.03	0.01	0.72	0.16	0.06

Supplementary Table S10. Model-based fit statistics for selected tree of population A and population B. com A - combined over years of population A, com B - combined over years of population B dataset.

Year	Model-Based Fit Statistics for Selected Tree of population A and Pop B								
	N	ASE	Mis-class	Sensitivity	Specificity	Entropy	Gini	RSS	AUC
2017	4	0.14	0.17	0.71	0.89	0.63	0.27	35.54	0.81
2018	2	0.21	0.32	0.32	0.97	0.87	0.42	53.69	0.64
Com B	6	0.16	0.22	0.54	0.92	0.71	0.32	82.54	0.82
Com A	3	0.23	0.37	0.72	0.55	0.94	0.46	327.5	0.64

Mis-class - Misclassification, AUC - Area under the Curve for Binary Classification Trees

Supplementary Table S11. Variable importance for selected tree of population A and B. com A - combined over years of population A, com B - combined over years of population B dataset.

Year	2017			2018	Com B			Com A	
Variable	LP	LI	PH	LI	LI	LP	DB	A3D	PH
Importance	4.05	2.10	1.96	3.15	4.05	2.98	2.81	4.40	2.56

Supplementary Table S12. Genotype's mean starch yield (SY) (g/plant), CT (ºC) and CTD (ºC) under ss condition in 2017 and 2018. Means comparison was done by *regwq test.*Means followed by the same letter are not significantly different at p value of 0.01.

Genotype	2017			2018		
	SY	CT	CTD	SY	CT	CTD
22497	67^{B-C}	22.0^{M}	-0.9^{L}	12^{G-I}	23.3^{GH}	-0.07^{HI}
850136	25^{FG}	22.3^{H-K}	-0.6^{HI}			
858638	66^{B-D}	22.2^{KL}	-0.7^{K}	37^{A-D}	23.3^{GH}	-0.05^{G-I}
858641	52^{DE}	22.5^{C-G}	-0.4^{C-E}	11^{G-I}	23.5^{D-F}	0.12^{C-E}
866296	55^{DE}	22.3^{H-L}	-0.6^{IJ}	35^{B-E}	23.4^{G}	-0.01^{F-H}
866303	61^{D}	22.4^{D-H}	-0.5^{GF}	25^{C-H}	23.6^{B-D}	0.22^{C}
866306	13^{G}	22.7^{AB}	-0.3^{B}	16^{E-I}	23.5^{D-F}	0.12^{C-E}
866309	85^{AB}	22.7^{AB}	-0.3^{B}	4^{I}	24.0^{A}	0.61^{A}

	SY	CT	CTD	SY	CT	CTD
869004	23^{FG}	22.5^{CD}	-0.4^{CD}	7^{HI}	23.7^{B}	0.38^{B}
872474	35^{EF}	22.7^{A}	-0.2^{A}	22^{DI}	23.5^{DE}	0.16^{CD}
872477	14^{G}	22.4^{D-H}	-0.5^{EF}		22.9^{J}	-0.45^{K}
899486	96^{A}	22.2^{L}	-0.7^{K}	40^{A-D}	23.3^{G}	-0.03^{F-I}
899519	58^{D}	22.5^{C-E}	-0.4^{DE}	44^{A-C}	22.9^{J}	-0.47^{K}
899522	99^{A}	22.2^{I-L}	-0.7^{KJ}	56^{A}	23.4^{G}	-0.03^{F-I}
899665	62^{CD}	22.6^{BC}	-0.3^{CB}	30^{C-G}	23.1^{I}	-0.30^{J}
899748	72^{CD}	22.5^{C-F}	-0.4^{C-E}	34^{B-F}	23.6^{CD}	0.20^{C}
899822	54^{DE}	22.3^{G-J}	-0.6^{GH}	18^{E-I}	23.7^{BC}	0.33^{B}
899831	52^{DE}	22.3^{E-H}	-0.6^{GH}	18^{E-I}	23.4^{E-G}	0.07^{D-F}
899834	82^{AB}	22.3^{F-I}	-0.6^{GH}	51^{AB}	23.4^{FG}	0.00^{F-H}
899922	81^{A-C}	22.2^{L-K}	-0.7^{KJ}	28^{C-G}	23.4^{FG}	0.05^{E-G}
900024	61^{D}	22.1^{M}	-0.9^{L}	14^{F-I}	23.2^{H}	-0.13^{I}

Supplementary Table S13. Summary statistics of lasso model in 2017 and 2018.

Summary statistics	Year	
	2017	2018
Root MSE	0.10	0.09
Mean	0.02	0.02
R-Square	0.15	0.12
Adj R-Sq	0.13	0.10
AIC	-375.68	-356.20
AICC	-375.07	-355.76
SBC	-471.10	-446.50
ASE (Train)	0.01	0.01
ASE (Test)	0.01	0.01

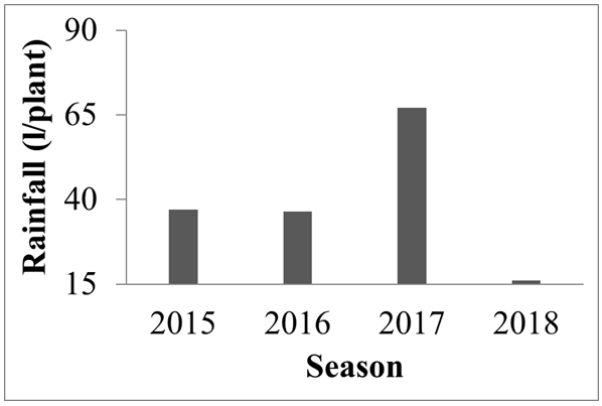
AIC - Akaike's information criterion, AICC - Corrected Akaike's information criterion, SBC - Schwarz Bayesian information criterion, ASE - average square error

Supplementary Table S14. Selection of the diurnal time interval by LASSO model, 2017 and 2018.

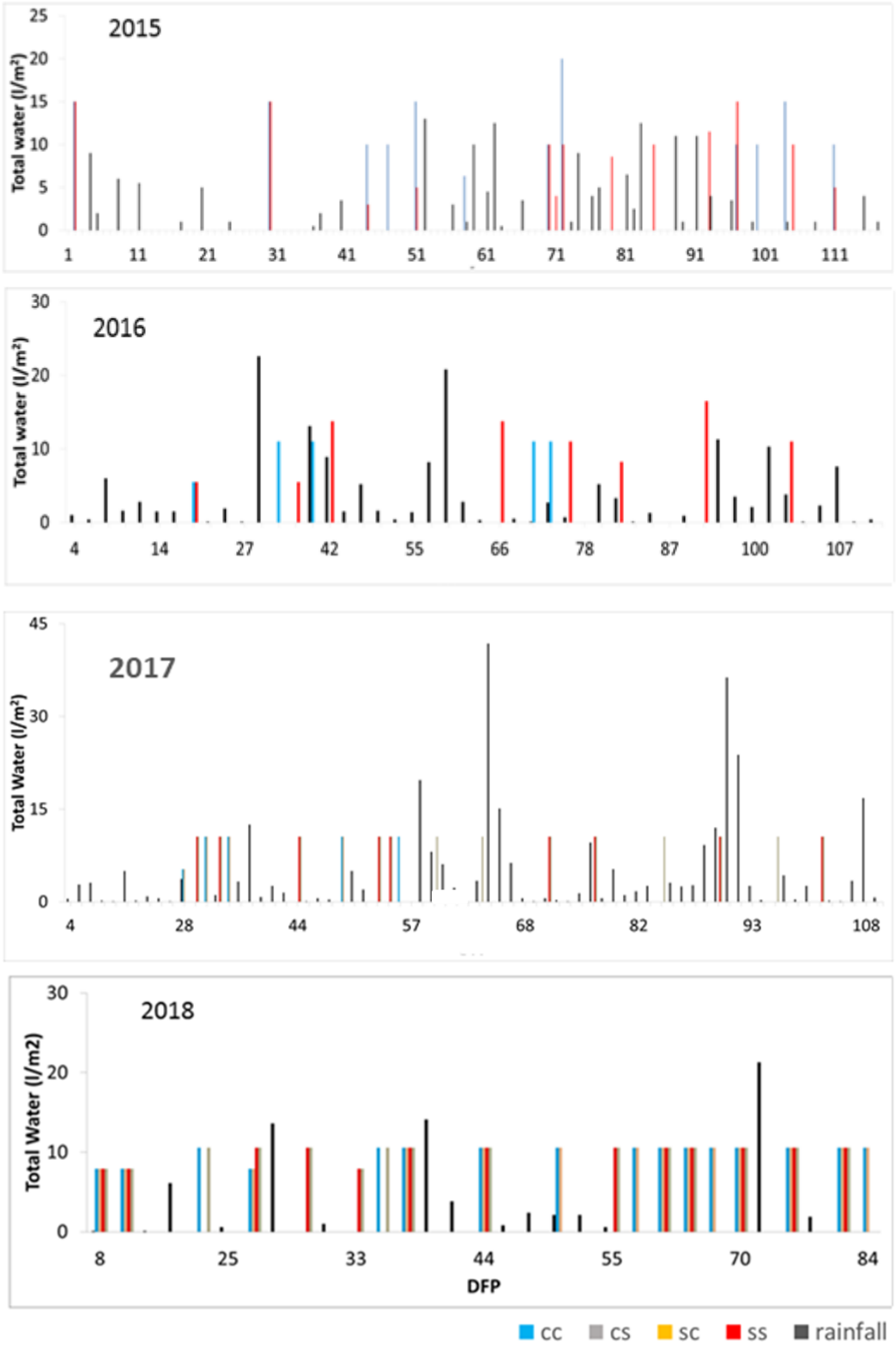
Parameter	Year	
	2017	2018
Intercept	0.010	0.050
8:00		-0.016
10:00		-0.015
11:00	-0.028	
14:00	-0.017	
16:00	-0.045	

Supplementary Table S15. Normality Test by Shapiro-Wilk for maximum/mean canopy parameters in 2015-2018.

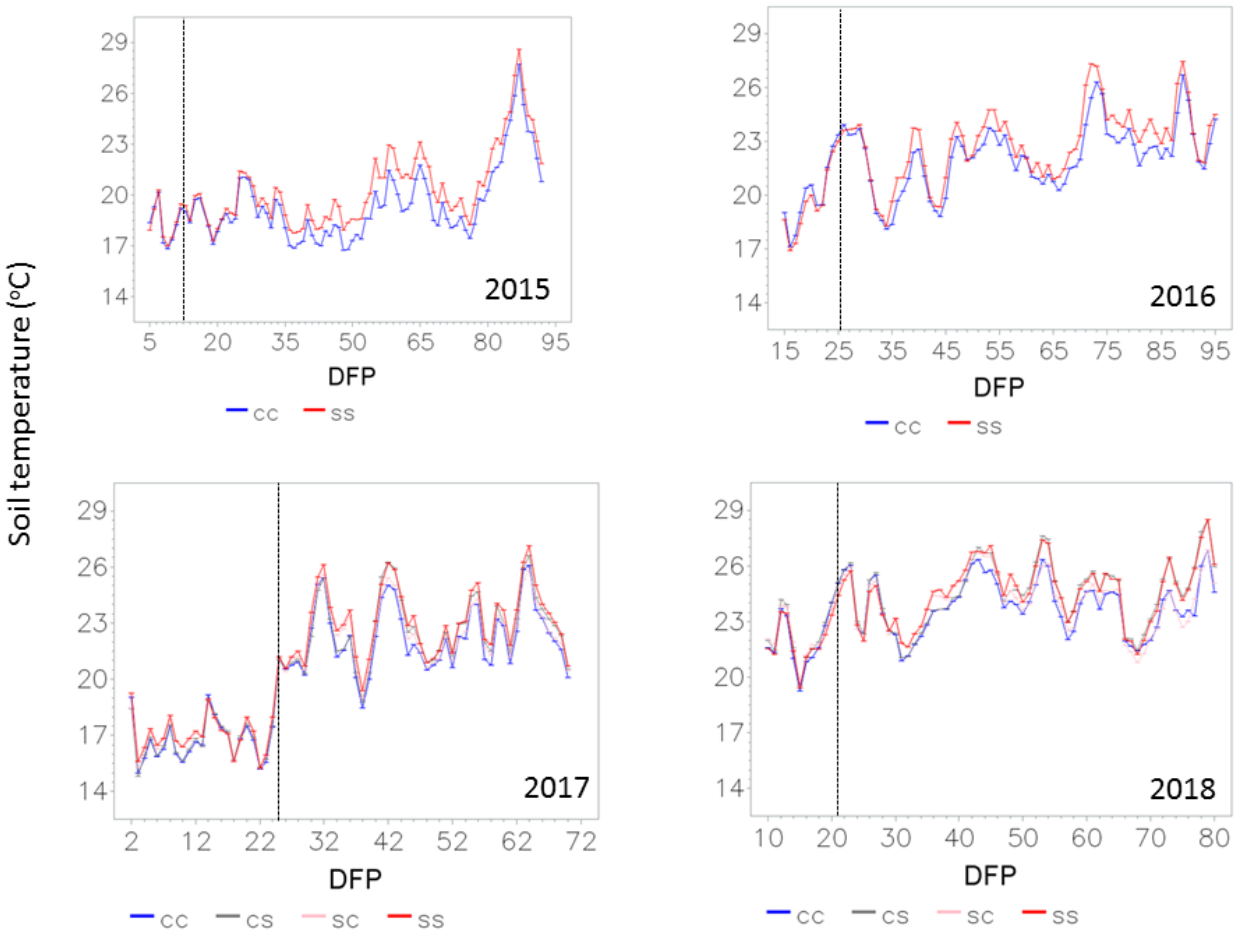
Year	Treatment	A2d		A3D		LA		PH	
		Test	p Value	Test	p Value	Test	p Value	Test	p Value
2015	cc	0.99	0.18	0.98	0	0.99	0.31	0.99	0.16
2016	cc	0.99	0.01	0.99	0.27	0.99	0.24	0.99	0.03
2017	cc	0.95	<0.0001	0.93	<0.0001	0.54	<0.0001	0.98	0.03
2018	cc	0.99	0.29	0.99	0.18	0.99	0.92	0.93	<0.0001
2017	cs	0.96	0	0.96	<0.0001	0.63	<0.0001	0.98	0.06
2018	cs	0.99	0.91	0.99	0.65	0.99	0.59	0.99	0.28
2017	sc	0.98	0.01	0.98	0.01	0.99	0.73	0.99	0.14
2018	sc	0.99	0.43	0.99	0.46	0.99	0.81	0.85	<0.0001
2015	ss	0.98	<0.0001	0.99	0.01	0.99	0.05	1	0.66
2016	ss	0.98	0	0.99	0	0.99	0.01	1	0.72
2017	ss	0.98	0.03	0.98	0.1	0.99	0.29	0.99	0.36
2018	ss	0.98	0.03	0.98	0.03	0.99	0.61	0.97	0.01
		DB		LAI		LI		LP	
2017	cc	0.99	0.47	0.93	<0.0001	0.96	0	0.96	0
2018	cc	0.99	0.61	0.98	0.02	0.99	0.43	0.98	0.14
2017	cs	0.99	0.51	0.96	<0.0001	0.99	0.25	0.99	0.08
2018	cs	0.99	0.45	0.99	0.78	0.98	0.02	0.98	0.07
2017	sc	0.99	0.43	0.97	0	0.98	0.04	0.99	0.40
2018	sc	0.99	0.37	0.99	0.40	0.99	0.63	0.99	0.42
2017	ss	0.98	0.08	0.98	0.10	0.97	0	0.99	0.19
2018	ss	0.99	0.87	0.98	0.03	0.99	0.65	0.99	0.29
		CTD							
2017	cc	0.95	<0.0001						
2018	cc	0.98	0.02						
2017	cs	0.97	0						
2018	cs	0.97	0						
2017	sc	0.97	0						
2018	sc	0.98	0.05						
2017	ss	0.96	0						
2018	ss	0.98	0.06						



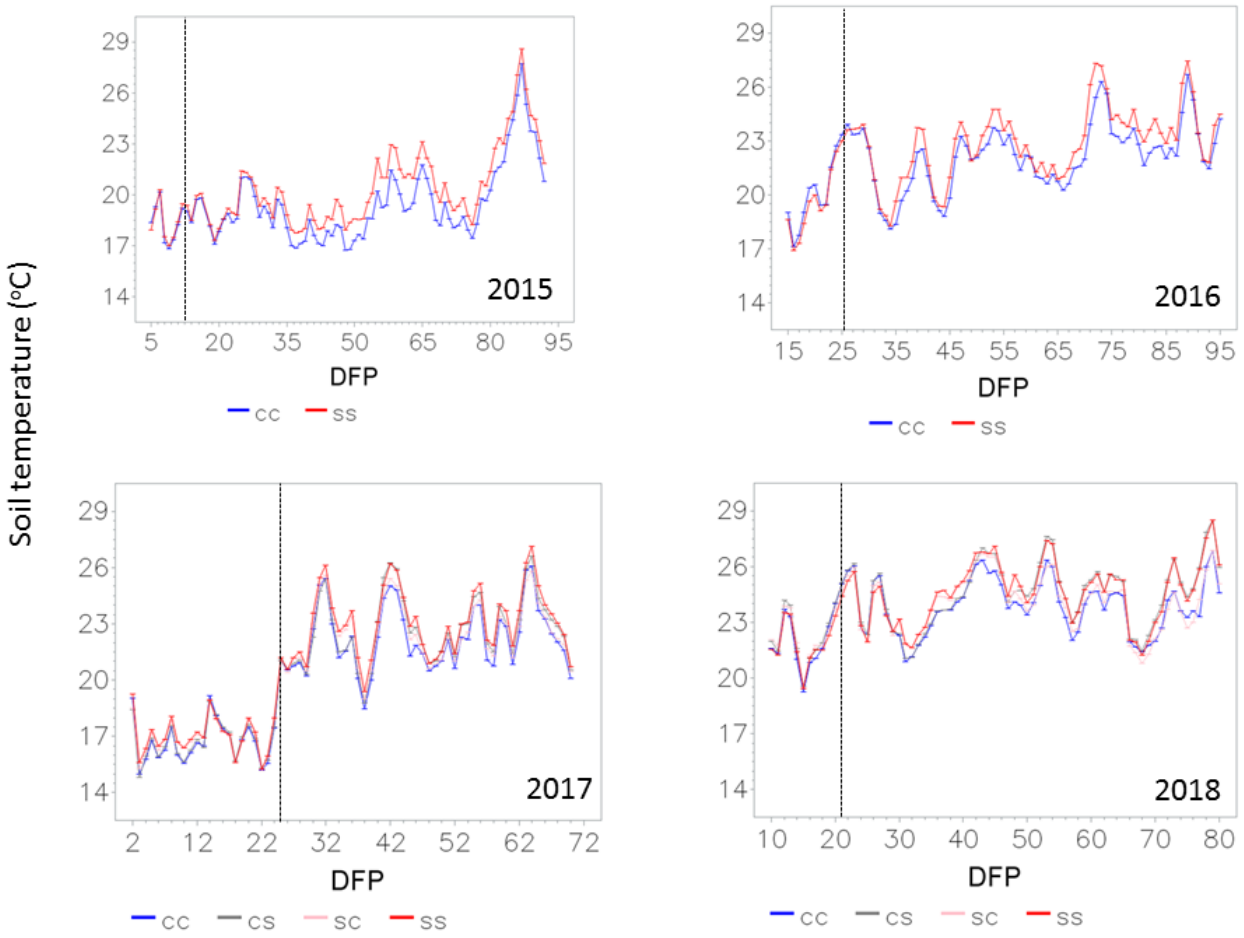
Supplementary Figure S1. Amount of rainfall water received by plants in cc (population A and B), cs (2017) and sc (2018) treatment.



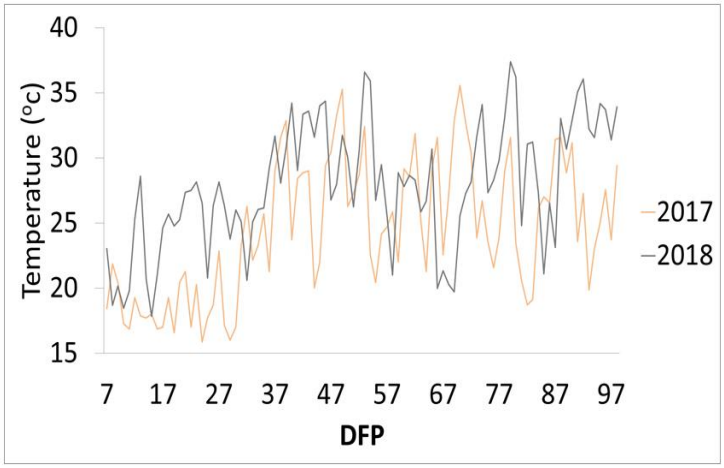
Supplementary Figure S2. Daily amount irrigation water applied to different treatments (cc, sc, ss and cs) and rainfall (see supplementary Figure 1) received by plants per days from planting (DFP) in the field experiments 2015-2018.



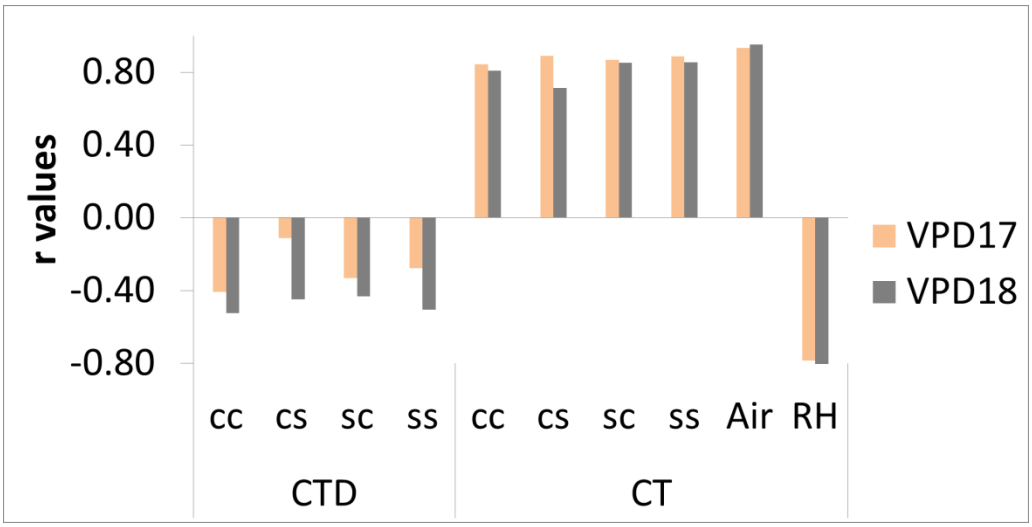
Supplementary Figure S3. Daily mean soil temperature of treatments in FGH experiments 2015 - 2018. Vertical broken line indicated stress initiation date.



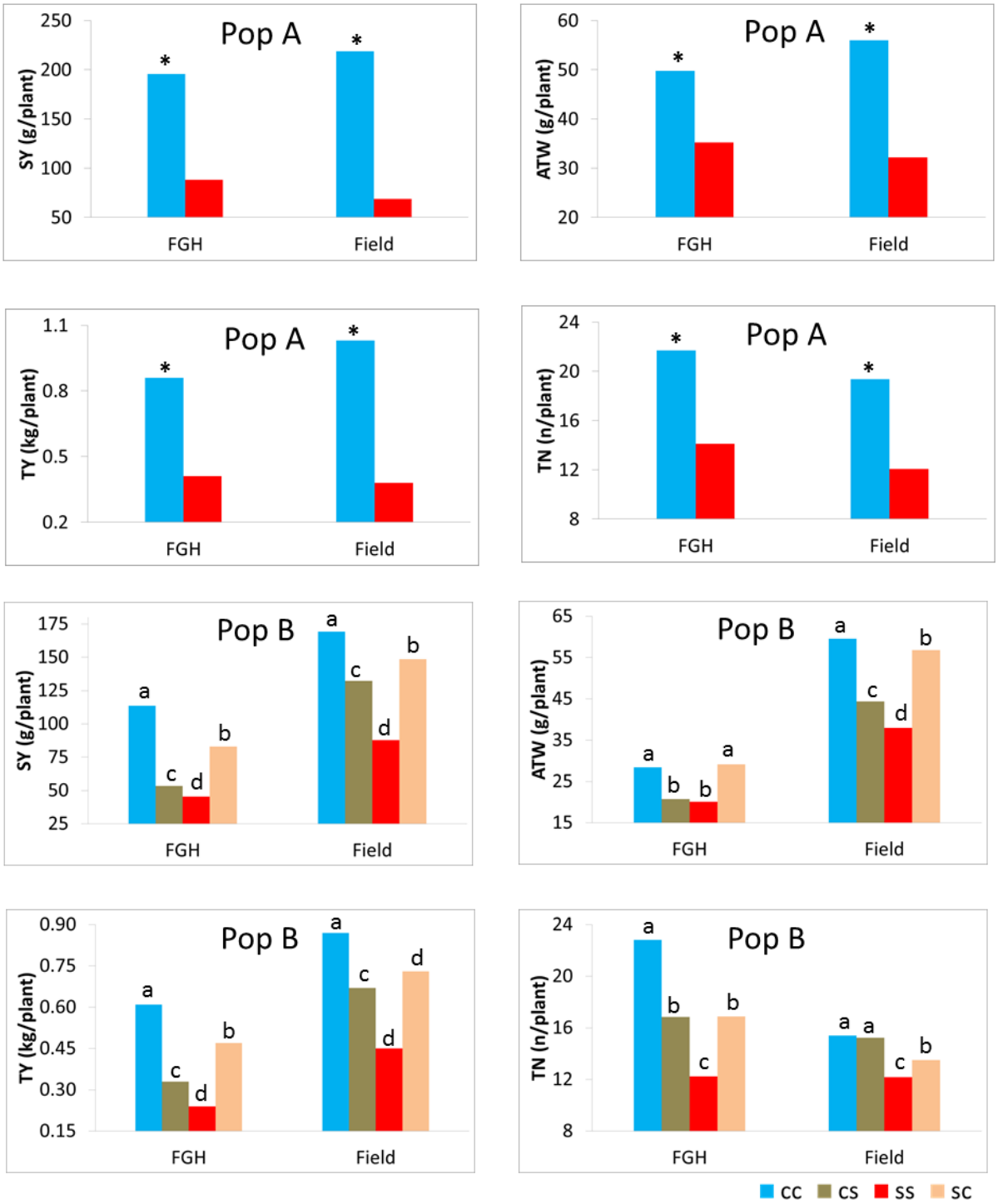
Supplementary Figure S4. Daily mean soil temperature of treatments under field condition in 2015 - 2018. Vertical broken line indicated stress initiation date.



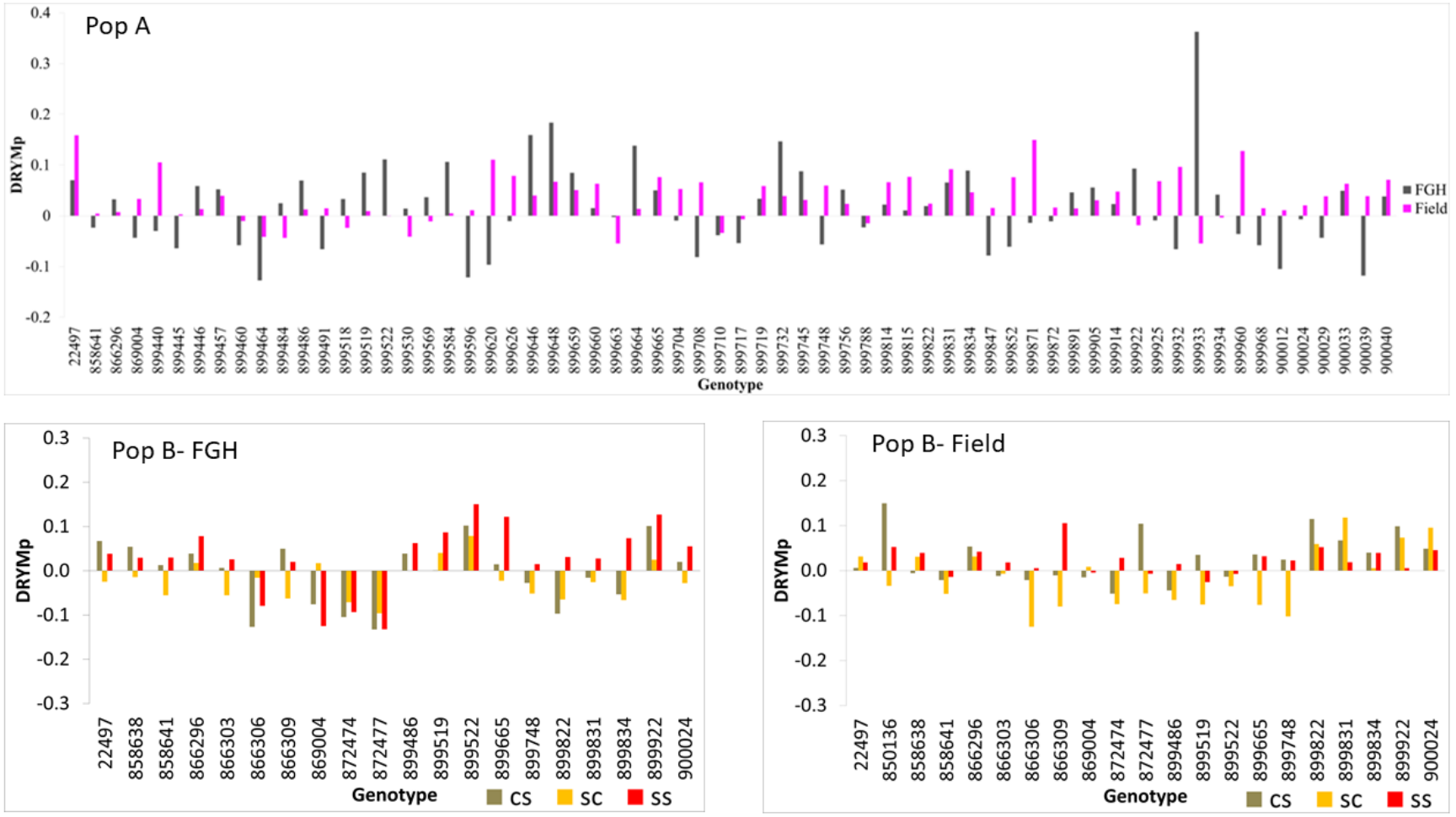
Supplementary Figure S5. Daily mean air temperature (12:00-19:00) in FGH experiment 2017 – 2018



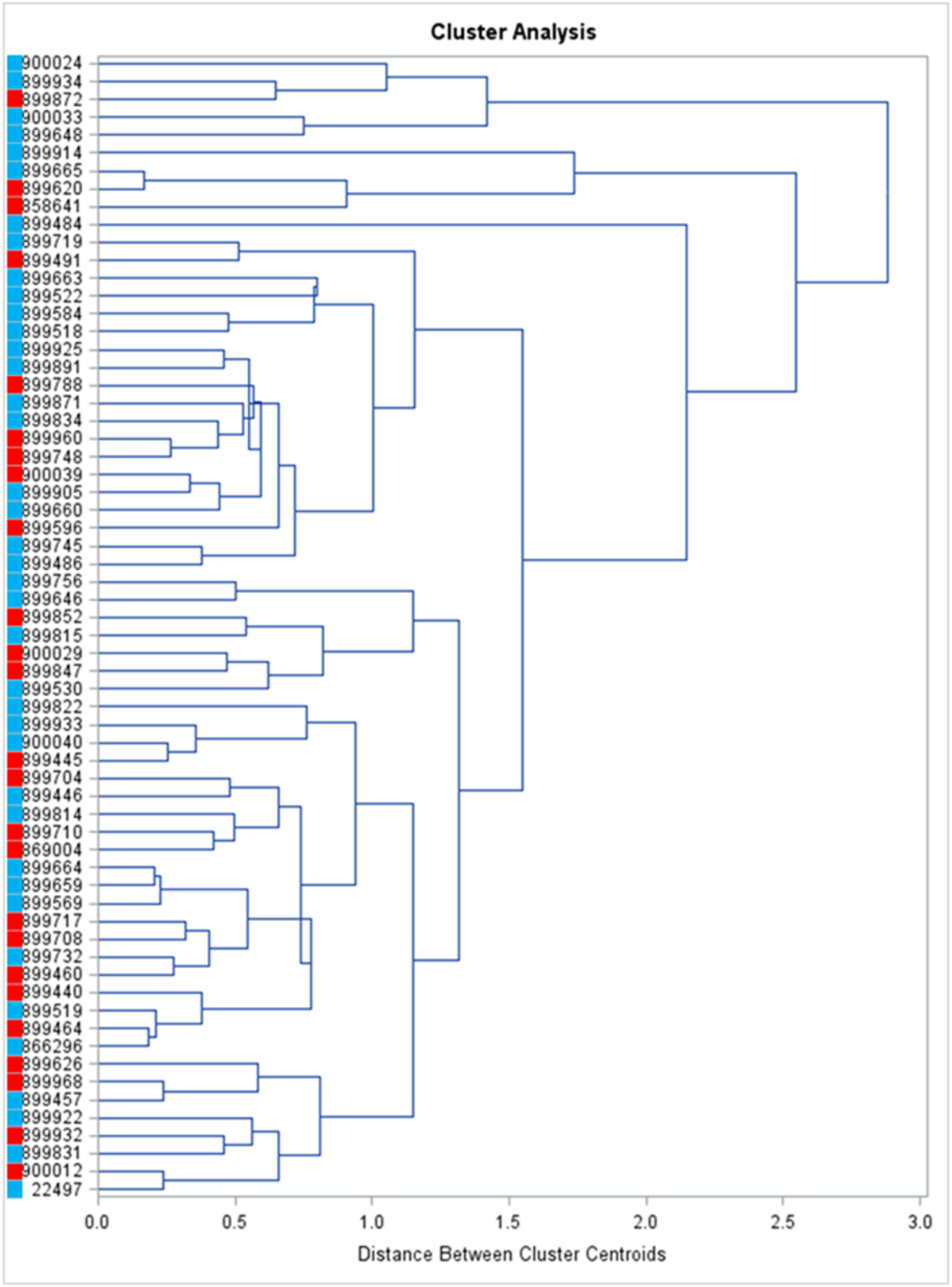
Supplementary Figure S6. Pearson correlation values of mean VPD (10-14hr) with CTD, CT, Air temperature, and RH in FGH experiments 2017 - 2018.



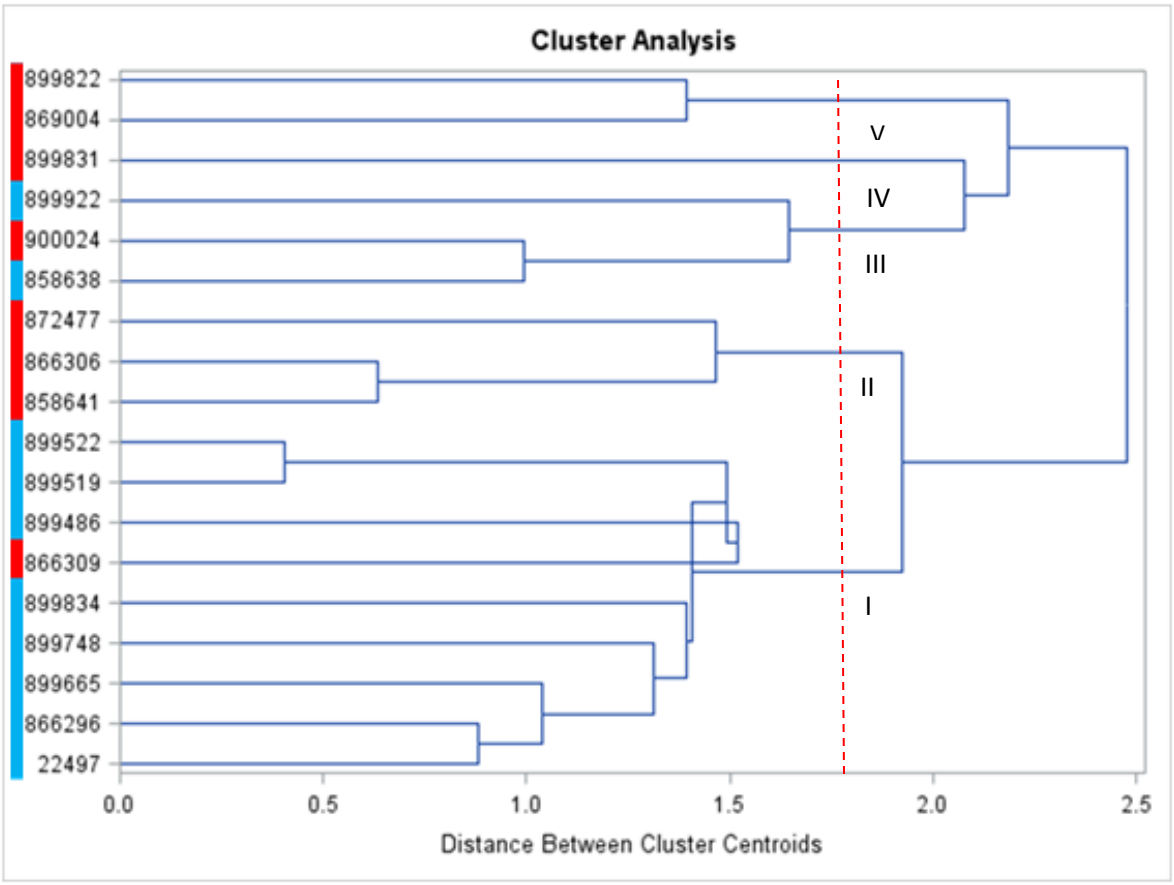
Supplementary Figure S7. Mean tuber yield (TY), starch yield (SY), average tuber weight (ATW) and tuber number (TN) per plant of population A (top) and B (bottom) in screenhouse (FGH) and field experiments. Mean separation was done by *regwq*. Treatments assigned the same letter were not significantly different at P values of 0.01. Pop A - Population A and Pop B - Population B.



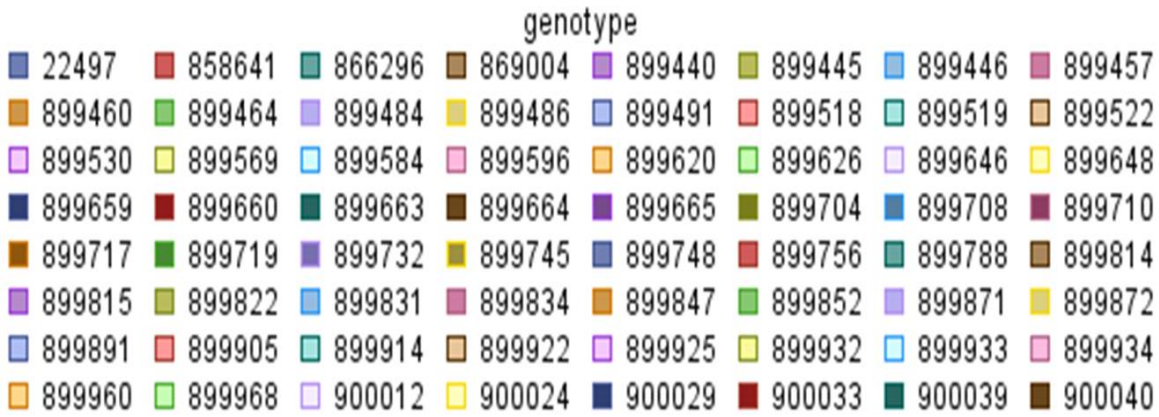
Supplementary Figure S8. Median DRYMp values of population A (top) and population B genotypes in FGH (bottom left) and field (bottom right) experiments. Pop A - Population A and Pop B - population B.



Supplementary Figure S9. Cluster analysis for canopy parameters combined over season under ss treatment 2017 - 2018. Blue and red bars in front of the genotypes code represents the DRYMp values greater than one (blue) and less than one (red).



Supplementary Figure S10. Cluster analysis for canopy parameters combined over season under ss treatment 2017 - 2018. Blue and red bars in front of the genotypes code represents the DRYMp values greater than one (blue) and less than one (red). Vertical broken line indicates a Centroid distance of 1.75.



Supplementary Figure S11. Genotype color code

Syntax

```
proc univariate normal plot data=example; var canopy parameter;
histogram canopy parameter /normal (color=red w=5);
run;

proc sql;
create table outliers as
select *, std(canopy parameter) as std, mean(canopy parameter) as avg,
   case when ((canopy parameter - calculated avg)/(calculated std) < -2.0) or ((canopy parameter
- calculated avg)/(calculated std) > 2.0) then 'Outlier'
   else 'Normal'
   end as outlier_status
from data
group by Genotype*treatment*replication;
quit;

proc glm data=data;
class genotype treatment;
model yield/canopy parameters=genotype treatment genotype*treatment;
random genotype genotype*treatment;
means genotype /regwq;
means treatment/regwq;
run;

proc means mean std max min range  data=data noprint;
  by genotype treatment replication;
  var canopy parameter / yield;
  output out=meansout  mean=mean min=minimum max=maximum STD=STD range=range
stderr=stderr;
run;

proc corr data=data pearson spearman sscp cov;  by class ;
  var canopy parameters and yield;
run;


proc reg outest=test2 rsquare;
    by genotype treatment plant_position;
model 1 canopy parameters=dfp;
model 2 canopy parameters =dfp dfp*dfp;
run;

ods graphics on;

proc factor data=data
```

```
priors =smc msa residual
rotate =promax reorder
outstat =fact_all
plots =(scree initloadings preloadings loadings);
run;
ods graphics off;


ods graphics on;

proc hpsplit data=data cvmethod=random(10) seed=123 intervalbins=500;
  class Tolerance;
  grow gini;
  model Tolerance = canopy parameters;
  prune costcomplexity;
run;


proc cluster noeigen method=centroid rsquare nonorm out=tree data=o;
id genotype;
var canopy parameters;
run;
quit;

proc surveyselect data=data out=traintest seed=130
samprate=0.7 method=srs outall;
run;
proc glmselect data=traintest plots=all seed=130;
partition role=selected(train='1' test='0');
model DRYMp=canopy traits /selection=stepwise slentry=.05;
run;
proc glmselect data=traintest plots=all seed=130 ;
partition role=selected(train='1' test='0');
model DRYMp = canopy traits  / selection=lasso(stop=none choose=sbc);
run;
```

r scripts

```
> library(ggplot2)
> library(ggfortify)
> df <- data[c(ci---cn)]
> autoplot(prcomp(df))
> theme <- theme(panel.background =
element_blank(),panel.border=element_rect(fill=NA),panel.grid.major =
element_blank(),panel.grid.minor =
element_blank(),strip.background=element_blank(),axis.text.x=element_text(colour="black"),axi
```

```
s.text.y=element_text(colour="black"),axis.ticks=element_line(colour="black"),plot.margin=unit
(c(1,1,1,1),"line"))

> p <-  autoplot(prcomp(df), data = data, colour = 'Tolerance', shape = FALSE, label.size = 6,
        loadings = TRUE, loadings.colour = 'blue',
        loadings.label = TRUE, loadings.label.size = 6)
> p <- p+theme
> p
```

About the Author

Gedif Mulugeta Aneley was born on December 12, 1981 in Finoteselam, Ethiopia. He obtained his BSc in Horticulture in 2005 from Jimma University College of Agriculture. From November 2005, Gedif worked as an instructor in Gambella ATVET Collage than in February 2017 he joined Amhara Region Agricultural Research Institute as junior potato breeder. He then joined Bahir Dar University for his Masters study in plant breeding and graduated in 2012. His master's thesis was on genotype by environment interaction and stability analysis for tuber yield and quality traits of Potato. From June 2014**,** Gedif worked as a research associate in International Potato Center (CIP), Ethiopia. In February 2016, he started his PhD study in Biology, Potsdam University. His PhD work on drought tolerance prediction of potato by automatic phenotyping of morphological and physiological traits is presented in this thesis.