Evaluation of drought adaptation of different tomato genotypes

Master thesis

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submitted by

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„Water has no taste, no color, no odor;

it cannot be defined, art relished while ever mysterious.

Not necessary to life, but rather life itself.

It fills us with a gratification that exceeds the delight of the senses. "

ANTOINE DE SAINT-EXUPERY, 1939

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List of Abbreviations

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ABA .... abscisic acid
BBCH.. .... scale for phenological development stages of a plant
Chl*a* and *b* .... chlorophyll *a* and *b*
CI........ .... confidence interval
DAR.... .... Days after repotting
DW .... dry weight
F´m .... maximal fluorescence in the light
F_t........ .... fluorescence immediately after the flash
FW .... fresh weight
GMO... .... genetically modified organism
LBR .... LBR 11
NC NC-37
NPQ.... .... non-photochemical quenching
OOTP . ... Organic Outdoor Tomato Programme
PAM.... .... pulse-amplitude modulated
PEG.... .... polyethylene glycol
Phantasia......... Phantasia F1
Philovita .... Philovita F1
PS I..... .... photosystem I
PS II.... .... photosystem II
qP .... photochemical quenching
ROS.... .... reactive oxygen species
RSR.... .... root/shoot ratio
RuBisCo .... ribulose-1,5-bisphosphate carboxylase/oxygenase
RuBP ribulose-1,5-bisphospate
RWC... .... relative water content
SPAC.. .... soil-plant-atmosphere continuum
TW .... turgidity weight
WC .... water content
WSD ... . water saturation deficit

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1. INTRODUCTION

1.1 Taxonomy, evolution and origin of tomato (*Lycopersicon esculentum*)

The center of origin of the *Lycopersicon* genus is in South America (Peru, Mexico). The center of greatest varietal diversity is in Mexico. It is assumed that the cherry tomato (*L. esculentum* var. *cerasiforme*) was the immediate ancestor of the cultivated types. After discovery of America it was brought to Europe and other parts of the world. Primarily tomato was used as an ornamental plant, since world war I it attained more and more importance as vegetable (ROEMER, 1962, p. 351).

The cultivated tomato *Lycopersicon esculentum* Mill. belongs to the Solanaceae family of plants. All nine species of the genus Lycopersicon have 2n = 24 chromosomes. *L. pimpinellifolium*, *L. hirsutum*, *L. cheesmanii*, *L. chmielewskii*, *L. parviflorum* and *L. pennellii* can be hybridized with *L. esculentum*. Crosses with *L. peruvianum* and *L. chilense* are only successful with embryo rescue (KALLOO, 1991).

Tomato is highly self-pollinating although some cultivated varieties and *L. esculentum* var. *cerasiforme* types may have higher levels of outcrossing, depending on temperature conditions and occurrence of carpenter bees (*Xylocopa s*pp.). The enclosure of stigma and style by the anther cone enhances self-fertilization and reduces chances of cross pollination. Higher outcrossing rates are sometimes associated with a style and stigma that extends beyond the anther cone. To maintain varietal purity, isolation and roguing of off-types is required for such genotypes (HORNEBURG and MYERS, 2012).

Tomato chromosomes can be identified easily; with development of trisomics, monosomics and translocation through chromosome engineering, the research on tomato cytogenetics has become one of the most advanced in agricultural research (PASSAM et al., 2007).

1.2 Distribution and production

Tomato is one of the most popular and important vegetables all over the world, with a production of 159 million tons in 2011 (FAO, 2011). It plays a vital role in providing

vitamin C, carotenoids, flavonoids and phenolics for human diet (HORNEBURG and MYERS, 2012).

There are some problems in outdoor production, but tomatoes have a high potential especially for organic production systems. Compared to glasshouse production they cause less amount of work and energy consumption. Irrigation could be reduced or even neglected, moreover the extent of fruits for rotation is extended (VOGEL, 1996). However, tomato fruit production is generally restricted by fungal infestations in the field. The most serious disease is late blight caused by *Phytophthora infestans* (class Oomycota, family Pythiaceae). It appears in most of the tomato-producing areas and can cause complete loss (FOOLAD et al., 2008, BEDLAN, 1999).

Outdoor production takes place in Spain, Italy, and the Balkans, protected production in the Netherlands, Germany and Austria. Outdoor production in large parts of Europe is mainly restricted to home gardeners, small scale organic, or traditional market garden businesses mainly due to economic pressure. In some regions, both field and protected tomato production systems are used. The processing market is supplied exclusively from field production, while protected production is used mainly for the fresh markets (LAMMERTS VAN BUEREN et al., 2011).

The main differences between protected organic and conventional production systems are that in organic production, plants are grown in soil and fertilized with certified organic fertilizers, while conventional production is based on mineral fertilizers and is increasingly reliant on hydroponic systems. As a result, steam soil treatments, disease suppressive composts, and grafting onto soil borne diseases resistant rootstocks are often used in conventional as well as in organic production (LAMMERTS VAN BUEREN et al., 2011).

1.3 Organic Tomato Breeding

Plant breeding is based on genetic variability, selection and recombination. Compared to conventional plant breeding, there are some limitations on the choice of the method in organic plant breeding. Methods which are forbidden are interspecific crossing, protoplast fusion, genetic modification, and induced mutations; conditionally permitted are the use of hybrid cultivars, somatic embryogenesis, meristem culture, and in vitro micropropagation anther culture. Hence intraspecific crossing,

backcrossing, mass selection and individual selection remain as permitted methods (ZDRAVKOVIC et al., 2010).

Special attention in breeding for organic systems has to be paid to specific adaptation for certain traits. Yield and yield stability, tolerance and resistance to biotic and abiotic stressors, and nutrient use efficiency are more important for varietal performance in organic systems as in conventional systems. Organic tomato also has to meet high expectations of consumers, when it comes to quality and flavor of fruits (HORNEBURG and MYERS, 2012).

Most contemporary tomato cultivars are bred with resistance to fusarium (*Fusarium osysporum f.s. lycopersici*), verticillium (*Verticillium dahliae*) leaf mold (*Cladosporium fulvum*), and nematodes. Independently of production system is the occurrence of tomato mosaic virus (ToMV), tomato spotted wilt virus (TSWV), early blight (*Alternaria solani*), and late blight (*Phythophthora infestans*). When it comes to seed-borne diseases (tomato mosaic virus (ToMV), tobacco mosaic virus (TMV), bacterial speck and bacterial spot (caused by *Pseudomonas syringae* pv. *tomato* and *Xanthomonas campestris* pv. *vesicatoria* and *Clavibacter michiganense*) organic agriculture has to rely on genetic resistance as the most important strategy (HORNEBURG and MYERS, 2012).

HORNEBURG and MYERS (2012) stated that the main challenges for organic (tomato) breeding are the effects of the selection environment on breeding success. Of importance are also the determination of genotype and production system interaction, the nature of induced resistances and its induction by organic matter or soil microflora, and the plant genetics of this response and the pyramiding of resistances. Moreover focus has to be put on a greater international networking within the organic breeding community and of course the below-ground components of growth, including nutrient acquisition, uptake and soil microbial interactions.

1.4 Organic Outdoor Tomato Project

The "Organic Outdoor Tomato Project" was launched in 2003 by the University of Göttingen. A participatory screening and breeding method on a national scale was developed. 3500 accessions were screened and genotypes with potential for improvement were identified. The best parent genotypes were used as source material for the breeding program, based on their yield, early maturity, and harvest

period and fruit quality. The correlation of field resistance against late blight with low fruit weight remains a restricting factor. Small fruited "wild" tomato genotypes show a higher field resistance against *Phytophthora infestans* in comparison to genotypes with larger fruit size (HORNEBURG, 2010).

Market gardeners, seed savers, advisors, and scientists work together to ensure that the best use of genetic resources is made in a well targeted program to meet the demand in horticulture. The first pure line varieties resulting from the Project, the cocktail tomatoes "Clou", "Dorada", and "Primavera", were released in 2010 (HORNEBURG, 2010, EUROPEAN COMISSION, 2013).

1.5 Water Relations of Plants

1.5.1 Water in Plant Cells

Water in plant cells occurs in several forms. It is a chemically bound constituent of the protoplasm. As water of hydration it is associated with ions, dissolved organic substances and dispersed macromolecules filling the gaps between the fine structures of the protoplasm and the cell wall. Therefore it is stored in vesicles and vacuoles as a reserve. And finally, as interstitial water, it serves as a transport medium in the compartments between cells and in the conducting elements of the xylem and phloem systems (vascular water) (LARCHER, 2003, p.233).

Water is stored mainly in cell compartments which are reservoirs for solutions. This water is osmotically bound to dissolved substances such as sugars, organic acids, secondary plant compounds and ions. The osmotic pressure, π, of the solution increases when absolute temperature rises and when the number of dissolved particles increases. Macromolecular substances can be present in considerable amounts in terms of mass. Through the polymerization of small molecules to macromolecules (e.g. sugar to starch) the cell can rapidly alter its osmotic pressure and the net balance of water can be regulated that way (LARCHER, 2003, p.234).

The thermodynamic state of the water influences the biochemical activity of the protoplasm. The water potential, ψ, is understood as the work necessary to raise bound water to the potential level of free water. Osmotically bound water in solutions only becomes available if energy is added. The osmotic potential, ψ_π, is lower than that of pure water and therefore it is expressed as negative pressure. The matric potential ψ_τ is the water bound to colloids and hydrophilic surfaces and it is also

negative. The availability of water is expressed as the water potential of the aqueous system (cell, cell compartment, external solution) with respect to the potential of pure water. It implies that the availability of water is lower, the more negative the potential of the system under consideration is (LARCHER, 2003, p.234).

Between sites of different water potentials there is a potential difference $\Delta\psi$ which drives mechanisms to reduce this difference. In the cell water transport always happens in the direction of lower (more negative) water potential. As long as there is no obstacle to diffusion, a thermodynamic steady state is immediately attained within the cell, and between the cells and their surroundings. A high water vapour deficit in the air or a hypertonic medium can also cause water to leave the cell which results in a lower water potential. And water flows from the surroundings into cells with a more negative water potential. At a given state of hydration, the water potential of the whole cell, ψ_{cell}, results from the difference between the osmotic potential ψ_π and the pressure potential ψ_ρ. The protoplast attains its greatest volume and exerts the greatest pressure on the cell wall when water-saturated. Due to the turgor pressure (internal pressure) the cell wall is maximally distended. Then net water uptake into the cell is stopped because the wall pressure compensates for the osmotic effect of the cell sap. At this point $\psi_{cell} = 0$ and $\psi_{\pi =} \psi_\rho$ (LARCHER, 2003, p.235).

1.5.2 Water Uptake

Plants can absorb water over their entire surfaces; however, most of the water is supplied through the root-soil-interface. In higher plants, roots are the specialized organs for water and nutrient absorption. The transport of water is governed by rules analogous to those for the flow of electricity (Ohm´s law). The potential gradient in the soil-plant-atmosphere continuum (SPAC) is the driving force for water transport through the plant (LARCHER, 2003, p.244).

After precipitation water infiltrates the soil and steadily percolates to the ground water table. Parts of the infiltrating water, the capillary water, is held back and stored in the pores of the soil. It depends on the nature of the soil and the distribution of pore sizes within it when it comes to the amount of water that retains as capillary water, and how much penetrates as gravitational water. The water-holding capacity of soils is described by the soil water content of natural soils that remains in the soil after the gravitational water has percolated and it is called field capacity (LARCHER, 2003, p.240).

As a result of extraction of soil water from the immediate surroundings of roots, water is drawn from moister zones in the soil. This phenomenon is called mass flow of water and takes place by capillarity. Therefore it is very slow and occurs only over short distances (a few mm to cm). Water in the soil can also move in the form of water vapour and as dew in the upper layers of the soil (LARCHER, 2003, p.242).

A plant can take up water from the soil only as long as the water potential of its fine roots is more negative than that of the soil solution in its immediate surroundings. The amount of water that roots can absorb is proportional to the exchange area in the rooting zone of the soil and to the water potential difference between root and soil, and it is inversely proportional to the transfer resistance of water movement within the soil and in the passage from soil to the plant. The older parts of the root system suberize, due to continuous growth at the root tip the active root surface area increases (LARCHER, 2003, p.241).

1.6 Drought Stress and Response

The term "stress" is commonly used but its meaning needs to be defined more precisely for this work. According to LEVITT (1973, p.5) stress in physics and mechanics refers to a force applied to a body. Hence, strain is used to describe the deformation of the body resulting from the stress. There are two main differences when it comes to biological stress and strain. Firstly, biological stress has to be measured in units of energy instead of units of force. And secondly, biological stress is connoted by possible injury (e.g. irreversible or plastic strain). In accordance with the definition above, stress is an external factor acting on an organism (e.g. water stress), and strain is any physical or chemical change produced by a stress (e.g. changes in osmotic potential).

LARCHER (2003, p.401) defined drought as a situation where too little water is available in a suitable thermodynamic state. This situation can occur under soil dryness, high evaporation, osmotic binding of water in saline soils, or in frozen soils, and as a result of inadequate water uptake by plants growing in soils too shallow for the development of an adequate root system. BLUM (1988) stated that drought occurs if a plant is not able to meet its evapotranspirational demand.

Additionally the terms "water stress" and "drought stress" are to be defined further. Drought is a meteorological term, commonly defined as a period without significant

rainfall. Drought stress therefore expresses a lack of water whereas water stress can either describe a lack of water or an excess of water (LEVITT, 1973, p.25). CHAVES et al (2003) suggested considering time scale of stress. Slowly developing water shortage (within days to weeks or month) or short-term water deficits (hours to days) can show completely different results in terms of physiological response or adaptation.

In this thesis the term "drought stress" is used because it is equivalent to what is going to be discussed.

Abiotic stresses (heat, cold, drought, salinity, wounding, heavy metals toxicity, excess light, excess water (flooding), high speed wind, nutrient loss, anaerobic conditions and radiation) and biotic stresses (pathogens [bacteria, fungi, and viruses], herbivores, weeds, insects, nematodes, and mycoplasma) cause plant responses either on individual cells or on the whole organism entirely. Firstly the stress signal is perceived by receptors of the plant cells, and then this signal information is transduced, resulting in the activation of various stress-responsive genes. The products of these stress genes lead to a stress tolerance response or plant adaptation, and help the plant to survive and surpass unfavorable conditions (JONES, 1989, MAHAJAN and TUTEJA, 2005).

The dynamic concept of stress denotes that the organism under stress passes through a sequence of characteristic phases. The impact of stress factors destabilizes vital structures inducing an alarm phase, in which functional declines occur (stress reaction). Stress reactions are offset by counter-reactions (restitution), which may lead to over-compensation (hardening). Under ongoing exposure to constant stress, a higher degree of resistance is developed and this may result in restabilization (adjustment). If the organism is overstrained by stress (exhaustion), irreversible damage occurs. If the impairment was temporary, damage may be repaired in a phase of regeneration (LARCHER, 2003, p.347).

The survival strategy of plants in stress-dominated habitats is thus not directed at maximizing productivity, but rather at achieving a compromise between yield and survival (LARCHER, 2003, p.353). Generally plants show three mechanisms which are used to resist drought stress. Some plants complete their life cycle within a seasonal rain (escape drought), other plants develop deep roots or reduce

transpiration area or increase wax layers on leaves (avoid drought) and finally there are plants that continue to grow with lower tissue water content (tolerate dehydration). These classifications are not mutually exclusive; plants express their response to drought stress differently (KRAMER and BOYER, 1995, p.187, LEVITT, 1973).

The first and most sensitive response to water deficiency within cells is a decrease in turgor and therefore a decrease of growth (cell elongation) is the result. A much lower water potential is required for stomatal closure, which is followed by effects on photosynthesis (repression) and respiration (activation) since these processes depend on the flow of O_2 and CO_2. Furthermore intercellular space is decreasing (LARCHER, 2003, p.34, LEVITT, 1973) and permeability of membranes is changing because of displacement of membrane proteins, which contributes to a loss of membrane integrity, selectivity, disruption of cellular compartments, and loss of membrane-based enzyme activity. The high concentration of cellular electrolytes due to the dehydration of the protoplasm may also cause disruption of the cellular metabolism (SHINOZAKI and YAMAGUCHI-SHINOZAKI, 2007).

Moderate water stress induces synthesis of abscisic acid (ABA) from carotinoids in the roots. As a "root signal" it is transmitted to different parts of the plant where it determines a variety of effects. Under the influence of synthesized hormones in the leaves and roots in response to drought, a number of changes occur: change in allocation of assimilates, altered ratio of root to shoot growth, development of characteristic morphogenetic features and enhancement (seed maturation) and impairment of reproductive processes (LARCHER, 2003, p.406, SHINOZAKI and YAMAGUCHI-SHINOZAKI, 2007).

Another negative impact of drought is disruption of the ionic and osmotic equilibrium of the cell. Ca^{2+} concentration has been found to increase in cytoplasm, chloroplasts and nucleus under drought stress (MA et al., 2009). This signal is first perceived at the membrane level by the receptors and then transduced in the cell to switch on various stress-responsive genes for mediating tolerance. The products of stress-inducible genes function in the initial stress response as well as in establishing plant stress tolerance. Some genes have been reported to be upregulated in response to more than one stress indicating the presence of cross-talk between the different stress signaling pathways. In this case evolution of reactive oxygen species (ROS)

represents a universal mechanism in cellular responses to environmental stresses (TUTEJA, 2009). FUJITA et al (2006) visualized stress signaling networks in the following figure.

Figure 1: Convergence points in abiotic and biotic stress signaling networks (FUJITA et al., 2006)
(ROS= reactive oxygen species, ABA= abiscisic acid, SA= salicylid acid, JA= jasmonic acid, ET= ethylene, MAP= mitogen-activated protein)

After closure of stomata CO_2 deficiency occurs and ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) activity is decreasing, photosystem II starts declining as well as CO_2 levels cause reduction of components of the electron transport chain. Therefore electrons get transferred to oxygen at photosystem I hence ROS are generated (MAHAJAN and TUTEJA, 2005, TUTEJA, 2009). Plants also accumulate a variety of osmoprotectants that improve their ability to combat abiotic stresses (TUTEJA, 2009). GIMENEZ et al (1992) for example examined the effects of water stress of two sunflower hybrids on photosynthesis. Concerning the amount of chlorophyll, soluble protein, RuBisCO protein and initial activity of RuBisCO and its activation state no differences were found between the two hybrids. After four days of water stress, assimilation of both decreased due to a decrease of photosynthesis. This is explained by a decrease of RuBP (ribulose-1,5-bisphospate) amount.

The avoidance of dehydration is defined as the ability of the plant to retain a relatively higher level of "hydration" during the period of water stress (BLUM, 1988). The plant protects its various growth related physiological, biochemical, and metabolic processes from the external water stress. Retaining a higher turgor at a given tissue water potential is called osmotic adjustment and it is an example for dehydration avoidance. It is obtained by production and accumulation of compatible organic solutes (e.g. amino acids, glycine, betaine, sugars, proline and ectoine) in the cytoplasm (FOOLAD, 2007). The process of osmotic adjustment also protects cells from extreme desiccation and allows gas exchange to continue (BLUM, 1988).

Relative root growth may be enhanced, which facilitates the capacity of the root system to extract more water from deeper soil layers. The components of drought and salt stress cross-talk as both these stresses ultimately result in dehydration of the cell and an osmotic imbalance. Overall, drought stress signaling encompasses three important parameters. Firstly plants try to reinstate the osmotic as well as the ionic equilibrium of the cell to maintain cellular homeostasis. Secondly, control as well as repair of stress damage by detoxification. And thirdly, signalling to coordinate cell division to meet the requirements of the plant under stress (LIU and ZHU, 1998).

1.7 Drought tolerance

By now screening for drought tolerance is very difficult, firstly because of the complexity of this trait and secondly because of a lack of screening methods. Recently, conventional breeding methods were enhanced with bio-molecular methods. Thus it is possible, not only to select and cross high yield cultivars with robust, drought tolerant cultivars, but also to transfer genes that are responsible for forming stress tolerance into plants that are grown in arid environments (MISHRA et al., 2012, VALLIYODAN and NGUYEN, 2006). However, this genetic modification (GMO) is strictly not allowed for organic production systems.

Most commercial cultivars of *L. esculentum* are sensitive to abiotic stresses during all stages of plant development. The cultivated species of tomato has a very narrow germplasm due to several genetic bottlenecks during its domestication and evolution (FOOLAD, 2007). The best genetic sources of drought resistance for cultivated tomato are coming from other species in the genus, *L. pennellii* and *L. chilense*. They are indigenous to arid and semi-arid environments in South America. *L. pennellii* regulates stomatal aperture efficiently during drought stress while *L. chilense* invests

in deep root growth (O´CONNELL et al., 2007). MARTÍNEZ-ANDÚJAR et al (2011) found out that an early osmotic treatment ("osmopriming" just after germination) of tomato seedlings improves biomass production in adult plants under abiotic stresses (drought or salinity). Higher photosynthesis efficiency and a lower incidence of leaf senescence under stress are explained by a reduced sensitivity to ABA. Adult plants showed increased ABA contents and increased transpiration.

1.8 Climate change and importance of drought tolerance

Climate change is regarded as one of the greatest challenges for future food production. With climate change, the importance of drought (soil and/or atmospheric water deficit) in conjunction with high temperature and radiation, and the area of irrigated land with saline soils are expected to increase significantly (LAMMERTS VAN BUEREN et al., 2011). Additionally SANTOS et al. (2012) stated that climatic factors represent major forcing factors on crops as well as they may influence biotic factors, which may be triggering pests and diseases. These biotic factors can therefore be modulated by abiotic factors like water quality, salinization, inorganic and organic pollutants and soil acidity. Crop´s sensitivity is mainly dependent on species, for instance, plants with C4 or CAM mechanisms (e.g. maize, sorghum, cassava, pineapple) tend to be more tolerant to climate change than C3 plants (e.g. tomato, rice, wheat, oat, barley, potato, apple, banana). Better water resource and soil management measures, development of more tolerant species through direct breeding, improved stomatal behavior, suitable selection of specific rootstocks, and genetic engineering and the increase of photosynthetic efficiency (e.g. C4 systems in C3 crops) might be recommended for mitigating negative impacts of drought. Secondary effects of climate change e.g. modified behavior of insects, negative effects on beneficial insects, modified host ranges of plant bacterial and fungal pathogens, effects on competition with weeds and new weed species should also be mentioned.

It is broadly accepted that breeding for drought and salinity tolerance has proven to be difficult due to very complex and till date sometimes poorly understood tolerance mechanisms. When it comes to drought stress, organic farmers may give higher priority to these traits as they want to be less dependent on inputs (LAMMERTS VAN BUEREN et al., 2011).

2. RESEARCH QUESTION

The research question was defined in collaboration with Bernd Horneburg from University Göttingen. He is the leader of the Organic Outdoor Tomato Breeding Programme. Selection criteria for this breeding project are mainly early fruit ripening, low susceptibility to *Phytophthora* and generally vital plants which includes tolerance to drought stress (HORNEBURG and BECKER, 2011).

Which of the 13 genotypes show reactions to limited availability of soil water and to which extent? Which physiological parameters provide relevant information for evaluation of drought stress?

The current study aims to investigate tomato plants in pots under water stress by measuring different parameters like biomass production (weight, root/shoot ratio, water content) and physiological parameters (chlorophyll concentration, chlorophyll fluorescence and cellular osmotic potential). It has to be analysed if there are differences between non-stressed and stressed plants (in general as well as between genotypes).

3. MATERIAL AND METHODS

3.1 Plant material

Thirteen genotypes, which were chosen by Horneburg, were analyzed. The following table summarizes information about them.

Table 1: Differentiation of tomato genotypes regarding their breeding environment

genotype	origin	breeding environment
Philovita F1	breeder De Ruiter, recently registered cultivar for conventional production	modern cultivars conventionally bred (also used as parents)
Phantasia F1	breeder De Ruiter, recently registered cultivar for conventional production	
Hildares F1	breeder Hild, old registered cultivar	old cultivars conventionally bred
Matina	Dreschflegel, old registered cultivar	
LBR 11	from Thailand, originally probably from AVRDC (The World Vegetable Center)	"exotic" genotypes
NC-37	from Israel (Bar Ilan University), pure line	
226-11-4	selected from Philovita F1	bred in the OOTP (and their parents)
Primavera	Cerise gelb x Zuckertraube, cultivar released from the OOTP	
Clou	Golden Currant x Matina, cultivar released from the OOTP	
Resi	selected from screenings of Uni Göttingen, Dreschflegel	selected from screenings in the OOTP
Golden Currant	selected from screenings of Uni Göttingen, *L. Pimpinellifolium*	
Cerise gelb	selected from screenings of Uni Göttingen	
Zuckertraube	breeder Reinsaat, registered cultivar	parent used in the OOTP

3.2 Experimental Setup

The pot experiment was carried out in the greenhouse of University of Vienna (Biozentrum, Althanstraße) from April to August 2012. Tomato plants (20 plants/cultivar) were grown in pots (14 cm diameter, volume of 1.1 liter), containing a mixture of coco peat and quartz sand at a ratio of 3:1. The main advantage was that roots can be cleaned from the substrate easily. The pots were set up in rows. The plants developed at 26/21°C (day/night) and under a relative humidity of about 60% for the entire growth period. They were fertilized twice, in July after transplanting and in August, using liquid fertilizer. The experiment was built up of 260 plants, 20 plants per cultivar, 10 without water stress (watered regularly) and 10 under water stress (watered only when they visibly started wilting). Water stress treatment started the day after repotting. As soon as the plants started wilting all stressed plants were watered.

Table 2: Records of the pot experiment conducted in the greenhouse

Records	DAR	
11th May 2012		tomato seeds were sown
21st May 2012		pricking
14st June 2012	0	repotting (in 14 cm pots)
11th July – 7th August 2012	28– 55	measuring of fresh, dry and saturation weight of leaves
18th - 20th July 2012	35– 37	measuring of chlorophyll concentration and chlorophyll fluorescence
8th - 10th August 2012	56– 58	measuring of fresh and dry weight of whole plants

DAR...Days after repotting

3.3 Data Collection

3.3.1 Biomass of leaves, roots and shoots (dry matter, fresh matter)

From 5 plants of each genotype samples were obtained by taking the terminal leaflet of one leaf of the last fully developed ones (5 to 10) for ascertaining dry weight and the terminal leaflet of another leaf for ascertaining full turgid fresh weight. Leaves were weighed and afterwards put either in water saturated foam block ("Oasis") for 24 hours or put into aluminum bowls for drying in a drying oven at a temperature of 85°C until constant weight was achieved. After saturation and drying respectively leaf weights were recorded again.

Figure 2: Terminal leaflets in "Oasis" for saturation and in aluminum bowl for drying

The roots were cleaned; afterwards root and shoot were separated by cutting. The substrate was removed only manually, hence some error is expected in root weight. Fresh weights of shoot and root were taken, they were wrapped in aluminum foil, dried at 85°C in a drying oven until constant weight was achieved, and then they were weighed again.

Data obtained through this step were used to calculate water content of tissue, root/shoot ratio, and percentage of stressed plant´s weight on non-stressed plant´s weight.

TURNER (1981) suggests expressing water content (WC) on a dry weight (DW) or fresh weight (FW) basis:

$$WC_{(DW\ basis)} = \frac{FW - DW}{DW} \times 100$$

$$WC_{(FW\ basis)} = \frac{FW - DW}{FW} \times 100$$

Root/shoot ratio was calculated on dry weight and on fresh weight basis:

$$RSR_{DW} = \frac{DW_{root}}{DW_{shoot}}$$

$$RSR_{FW} = \frac{FW_{root}}{FW_{shoot}}$$

3.3.2 Leaf chlorophyll concentration

Chlorophyll concentration was measured non-destructively on the terminal leaflets of the last three fully developed leaves, which were big enough (from 6th to 10th) with the Chlorophyll Content Meter (CCM-200 plus, Opti-Sciences, Inc., USA). Six plants per genotype were investigated, three of non-stressed plants and three of stressed plants. By using absorbance of light by the intact leaf the CCM-200 plus estimates the chlorophyll concentration in leaf tissue. The absorbance of two wavelengths (660nm - red, 940nm – near infrared) is used to calculate a Chlorophyll Concentration index (CCI, dimensionless) value that is proportional to the amount of chlorophyll in the sample and has to be related to the water content of the tissue. The red light is absorbed by chlorophyll; the near-infrared light is a "reference wavelength" that is used to adjust for differences in leaf structure (RICHARDSON et al., 2002). Chlorophyll concentration is a good indicator for plant health; furthermore it is a non-destructive method and quick to implement.

Figure 3: Measuring chlorophyll concentration with CCM 200 plus

The chlorophylls (Chl *a* and Chl *b*) are the antenna pigments in leaf chloroplasts which absorb solar radiation and transfer the resulting excitation to the reaction centre pigments. A photochemical process is set in motion by electrons, which are released by excited reaction centres. Leaf chlorophyll concentration is firstly an interesting trait for ascertaining limitation of photosynthetic potential and hence primary production because absorption of solar radiation by leaves is largely dependent on foliar concentration of photosynthetic pigments (CURRAN et al., 1990). Secondly, nitrogen is one of the main elements of chlorophyll molecule composition (chlorophyll a), and therefore quantifying chlorophyll concentration gives indirect information about nutrition status, especially the N status (FILELLA et al., 1995, PENG et al., 1996). Thirdly, stress physiology can be described by pigmentation because under stress and during senescence carotenoids increase and chlorophylls decrease (PEÑUELAS and FILELLA, 1998). And fourthly, abiotic factors (e.g. light) can cause changes in the relative concentration of pigments (LARCHER, 2003).

3.3.3 Chlorophyll fluorescence

Chlorophyll fluorescence was analysed on terminal leaflet and the two following compound leaflets from the last three fully developed leaves, which were big enough (6th to 10th, two measures per leaf, six per plant, dimensionless) with a pulse-amplitude modulation fluorometer (Mini-PAM, Walz, Effeltrich, Germany), equipped with a leaf clip holder 2030-B. Actinic illumination was provided through an optic fibre by a halogen lamp. "Modulated" measuring systems have a light source to measure

fluorescence which switches on and off at high frequency, therefore, the relative yield of fluorescence can be measured in the presence of background illumination (BOLHÀR-NORDENKAMPF and ÖQUIST, 1993).

Figure 4: Measuring chlorophyll fluorescence with Mini-PAM

Most of the light energy absorbed by chlorophyll molecules in a leaf can be used to drive photosynthesis, so the energy level of chlorophyll is raised and electrons are displaced into higher energy orbitals. Some of the excitation energy can also be dissipated as heat or as light emission (fluorescence), as the electron moves back to the ground state (DeELL et al., 1999). These three processes occur in competition, such that any increase in the efficiency of one will result in a decrease in the yield of the other two. For fluorescence measurement leaf is exposed to light of defined wavelength and the amount of light re-emitted at longer wavelength can be recorded. Firstly actinic light is applied at appropriate intervals, further saturation flashes for measuring F'_m, the fluorescence maximum in the light. The steady-state value of fluorescence immediately prior to the flash is termed F_t. After a flash, removal of actinic light (preferably whilst simultaneously giving a far-red light) allows measurement of F'_0 (MAXWELL and JOHNSON, 2000).

When a leaf is transferred from darkness into light, PSII reaction centers are progressively closed. This gives rise (during the first second of illumination) to an increase in the yield of chlorophyll fluorescence (KAUTSKY and HIRSCH, 1931). Afterwards the fluorescence level drops again over a time-scale of a few minutes.

This phenomenon (fluorescence quenching) occurs firstly because of "photochemical quenching", i.e. an increase in the rate of electron transport away from PSII; this is due to the light-induced activation of enzymes involved in carbon metabolism and the opening of stomata. At the same time, there is an increase in the efficiency with which energy is converted to heat, i.e. "non-photochemical quenching" (NPQ).

The most useful parameter is the Genty-parameter. It measures the efficiency of Photosystem II photochemistry (φ_{PSII}) and is calculated as: $\rho_{PSII} = (F'_m - F_t)/F'_m$. Genty-parameter describes the proportion of the light absorbed by chlorophyll associated with PSII that is used in photochemistry (GENTY et al., 1990)**.**

Another fluorescence parameter, measuring photochemistry, is "photochemical quenching", qP. This is calculated as: $qP = (F'_m - F_t)/(F'_m - F'_0)$. It is very similar to φ_{PSII}, but whilst φ_{PSII} is the proportion of absorbed energy for photochemistry, qP gives information about PSII reaction centers that are open (MAXWELL and JOHNSON, 2000).

3.3.4 Leaf osmotic potential

According to KALLOO (1991, p.160) for obtaining osmotic potential leaves were used, because of their importance in osmoregulation and osmotic adjustment. The osmotic potential can be ascertained after destruction of the plant tissue. The destruction of the cell walls exposes the cell content of osmotically active compounds (EHLERS and GOSS, 2003, p.168). For that purpose tissue (saturated leaves) were wrapped in aluminium foil and deep frozen at -18°C. Afterwards samples were defrosted, cell sap was pressed out by a fine pored garlic press and then they were processed with a Vapor-Pressure Osmometer (Vapro 5520f, Wescor, Inc., USA). By measuring osmolality, the number of solute particles which are dissolved in one kilogram of solvent can be determined.

3.4 Statistical Analysis

Statistical analyses were performed using the IBM SPSS Statistics/PASW Statistics 18 (2009) software (SPSS Inc., Chicago, IL, USA). The guideline for processing analyses with SPSS was JANSSEN and LAATZ (2013), the theoretical background for statistical analyses was LANDAU and EVERITT (2004).

First of all results of measurements were presented in bar charts, two bars for each genotype, one represents the mean of all non-stressed plants the other one represents the mean of all stressed plants. Charts and the description of the results are going to be found in chapter 4 (results). More detailed information is gathered in chapter 9 (annex).

Since data were normally distributed but homogeneity of variances was not given, differences were revealed by two sample t-test (Student´s t-test) and Welch-test (which is performed automatically in SPSS when requirements for the t-test are not given) respectively ($p<0.05$) (LANDAU and EVERITT, 2004). Additionally significant difference was differentiated into "low significant difference" ($p = 0,005 – 0,05$) and "high significant difference" ($p < 0,005$).

4. RESULTS

In this chapter the results of statistical analysis of biomass production and physiological parameters are presented.

4.1 Biomass traits (weight of roots and shoots, root/shoot ratio, water content)

Obviously fresh root-biomass from non-stressed plants is higher than fresh root-biomass of stressed plants (Figure 5). Fresh weight of roots of all non-stressed plants (16.3 g) is significantly higher than of all stressed plants (8.7 g). With exception of 'LBR 11' (21.3 g/11.1 g) the differences within all cultivars between non-stressed and stressed plants are significant. 'LBR 11' shows a high mean variation. Moreover 'Phantasia' (16.1 g/8.0 g), 'Philovita' (19.5 g/8.3 g) ,'Matina' (12.9 g/6.3 g), 'NC-37' (16.3 g/7.8 g), '226-11-4' (16.7 g/7.9 g), 'Resi' (17.9 g/8.6 g), 'Golden Currant' (17.1 g/8.0 g), 'Cerise gelb' (20.1 g/10.0 g), and 'Zuckertraube' (15.9 g/9.4 g) showed highly significant differences. 'Primavera' and 'Clou', both bred in the OOTP, showed very low root weight of both non-stressed and stressed plants (11 g/9.8 g; 10.1 g/7.6 g) and therefore less significant difference between them. Additionally 'Hildares' (15.7 g/9.9 g) showed a less significant difference too.

Figure 5: Fresh weight of roots of the 13 genotypes,
non-stressed and stressed separately (average of five replications each, error
bars show the confidence interval at 95%)

Similar to the fresh root-biomass fresh shoot-biomass of non-stressed plants is higher than fresh shoot-biomass of stressed plants (Figure 6). Shoot – biomass of all non-stressed plants (40.2 g) is significantly higher than that of all stressed plants (25.0 g). Apart from 'Matina' (37.8 g/29.3 g) statistically significant differences within all cultivars between non-stressed and stressed plants were noticed. Non-stressed plants of 'Philovita' (32.1 g/17.7 g), 'LBR 11' (27.1 g/16.4 g), 'NC-37' (31.5 g/17.4 g), '226-11-4' (42.9 g/20.3 g), 'Primavera' (57.7 g/38.0 g), 'Resi' (53.0 g/22.8 g), 'Cerise gelb' (37.7 g/20.9 g), and 'Zuckertraube' (39.6 g/26.3 g) showed higher fresh weights

of shoots with high significant difference. Also significant but less than the above mentioned were differences of 'Phantasia F1' (29.5 g/18.2 g), 'Hildares' (51.6 g/35.0 g), 'Clou' (45.0 g/32.8 g), and 'Golden currant' (38.7 g/28.3 g).

Non-stressed as well as stressed plants of 'Phantasia' and 'Philovita' (both conventionally bred modern cultivars) and 'LBR 11' and 'NC-37' ("exotic" genotypes) generally showed low shoot weight.
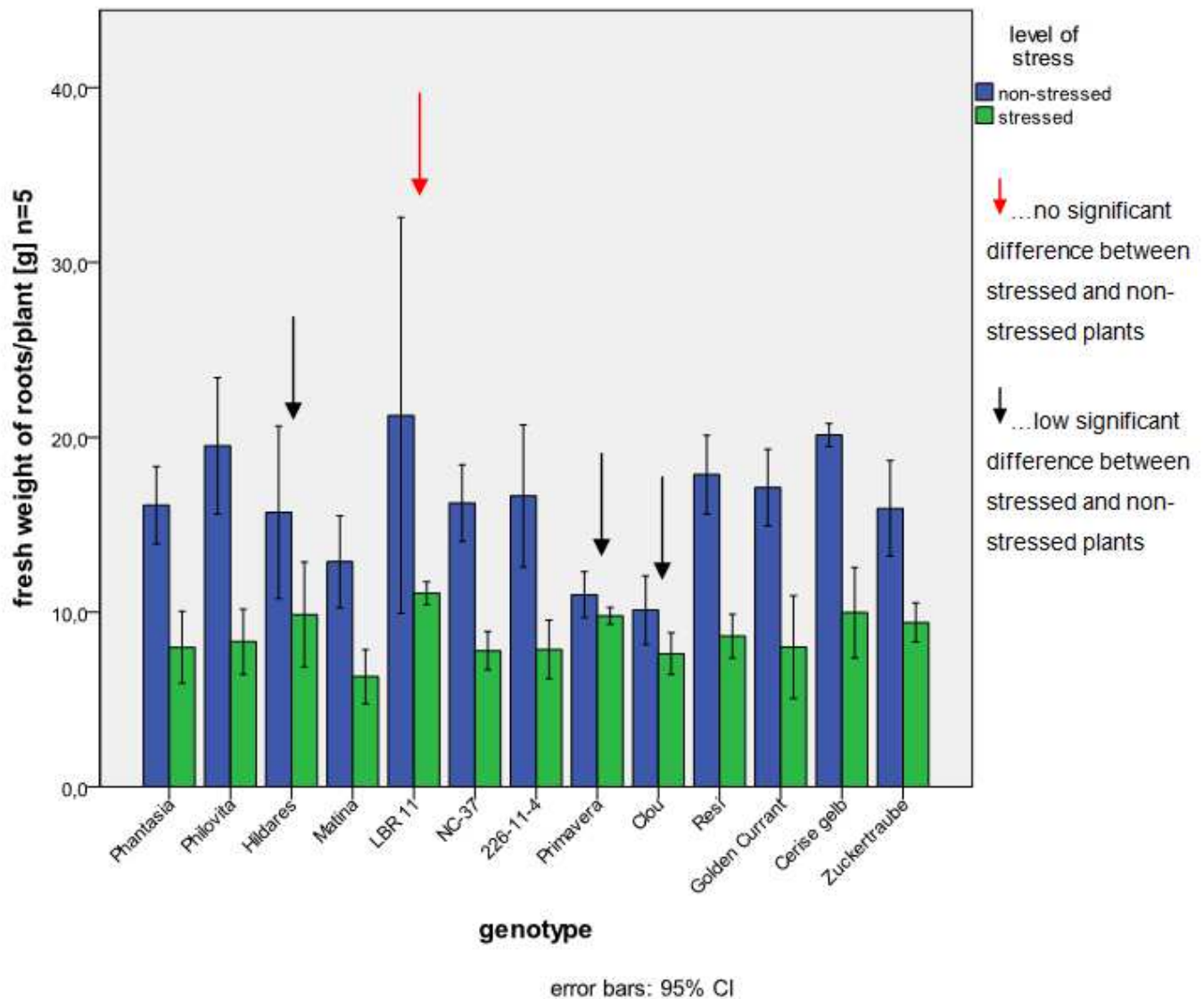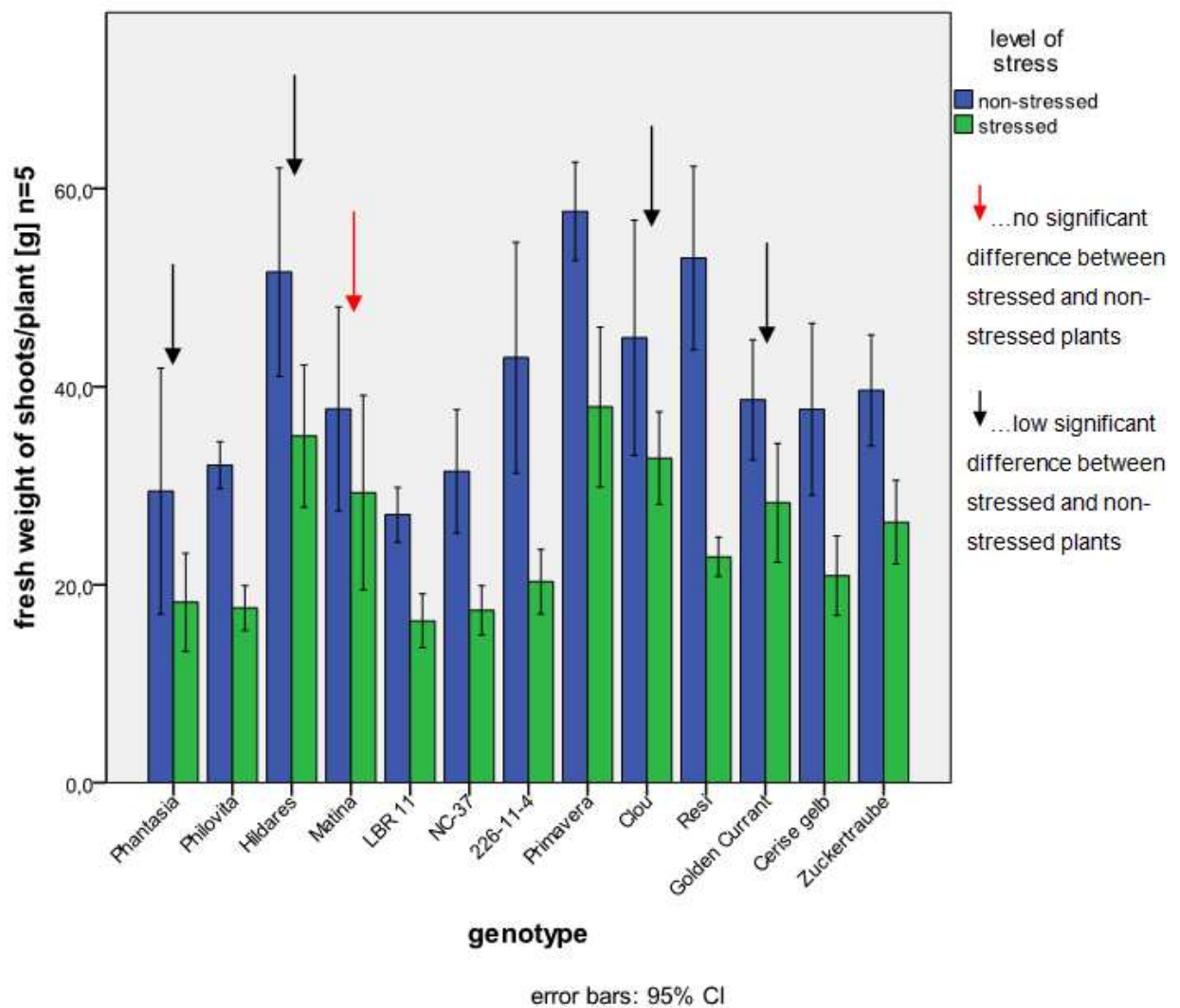

Figure 6: Fresh weight of shoots of the 13 genotypes,
non-stressed and stressed separately (average of five replications each, error bars show the confidence interval at 95%)

Root/shoot ratio of fresh weights (Figure 18 in annex) did not reveal significant differences generally (all non-stressed and all stressed plants together respectively) (0.43/0.39). 'Phantasia' (0.48/0.44), 'Hildares' (0.31/0.28), 'LBR 11' (0.74/0.64), 'NC-37' (0.53/0.45), '226-11-4' (0.41/0.43), 'Primavera' (0.24/0.29), 'Clou' (0.23/0.24), 'Resi' (0.37/0.38), and 'Zuckertraube' (0.43/0.36) did not show significant differences at all. 'Philovita' (0.61/0.47) and 'Matina' (0.35/0.25) differed significantly and 'Golden currant' (0.47/0.28) differed highly significant.

In general dry root-biomass of non-stressed plants (2.9 g) was higher than that of stressed plants (2.1 g). 'Phantasia' (2.5 g/1.8 g), 'NC-37' (2.6 g/1.7 g), '226-11-4' (2.8 g/1.9 g), and 'Cerise gelb' (4.2 g/2.6 g) showed significantly higher dry root-biomass of non-stressed plants (Figure 7). In 'Matina' (1.8 g/1.3 g) difference observed was significant. With exception of 'Primavera' and 'Clou' mean values of dry root-biomass decreased from non-stressed to stressed plants. Stressed plants of 'Primavera' weighed 2.4 g whereas non-stressed plants showed lower dry root-biomass (2.0 g). However, these differences are not statistically significant. Stressed and non-stressed plants of 'Clou' remained statistically at par with each other (1.8 g). In 'Philovita' (3.2 g/2.5 g), 'Hildares' (3.2 g/2.1 g), 'LBR-11' (3.1 g/2.6 g), 'Primavera' (2.0 g/2.4 g), 'Clou' (1.8 g/1.8 g), 'Resi' (3.7 g/2.3 g), 'Golden currant' (3.1 g/2.4 g), and 'Zuckertraube' (3.0 g/2.3 g) dry root-biomass in response to drought stress did not differ significantly.

'Matina' and 'Clou' generally (non-stressed as well as stressed plants) showed low dry root-biomass. 'Cerise gelb' and 'Resi' reached the highest dry root weight of non-stressed plants with 4.2 g and 3.7 g respectively.

**Figure 7: Dry weight of roots of the 13 genotypes,
non-stressed and stressed separately (average of five replications each, error
bars show the confidence interval at 95%)**

Dry shoot-biomass generally tended to decrease from 5.8 g (non-stressed plants) to 3.5 g (stressed plants), additionally each genotype showed higher dry shoot-biomass of non-stressed plants. 'Matina' (4.7 g/4.1 g) did not reveal significant difference between non-stressed and stressed plants. Significant differences were found in 'Clou' (5.6 g/4.1 g), 'Golden currant' (5.6 g/4.0 g), 'Cerise gelb' (5.1 g/2.9 g), and 'Zuckertraube' (5.1 g/3.7 g), whereas 'Phantasia' (5.8 g/2.8 g), 'Philovita' (5.4 g/2.8 g), 'Hildares' (6.8 g/4.4 g), 'LBR 11' (4.3 g/2.3 g), 'NC-37' (5.0 g/2.8 g), '226-11-4' (6.0

g/2.7 g), 'Primavera' (8.3 g/5.1 g), 'Resi' (8.1 g/3.1 g) revealed highly significant difference in response to water stress (Figure 8).

'Primavera', 'Resi' and 'Hildares' showed high dry weights of shoots of non-stressed plants. Low fresh weights of stressed plant roots were reached by the modern conventionally bred cultivars 'Phantasia' and 'Philovita', by the exotic genotypes 'LBR-11' and 'NC-37',by '226-11-4', a genotype selected by Horneburg, and by 'Cerise gelb' and 'Resi', derived from Horneburg´s screening trials.
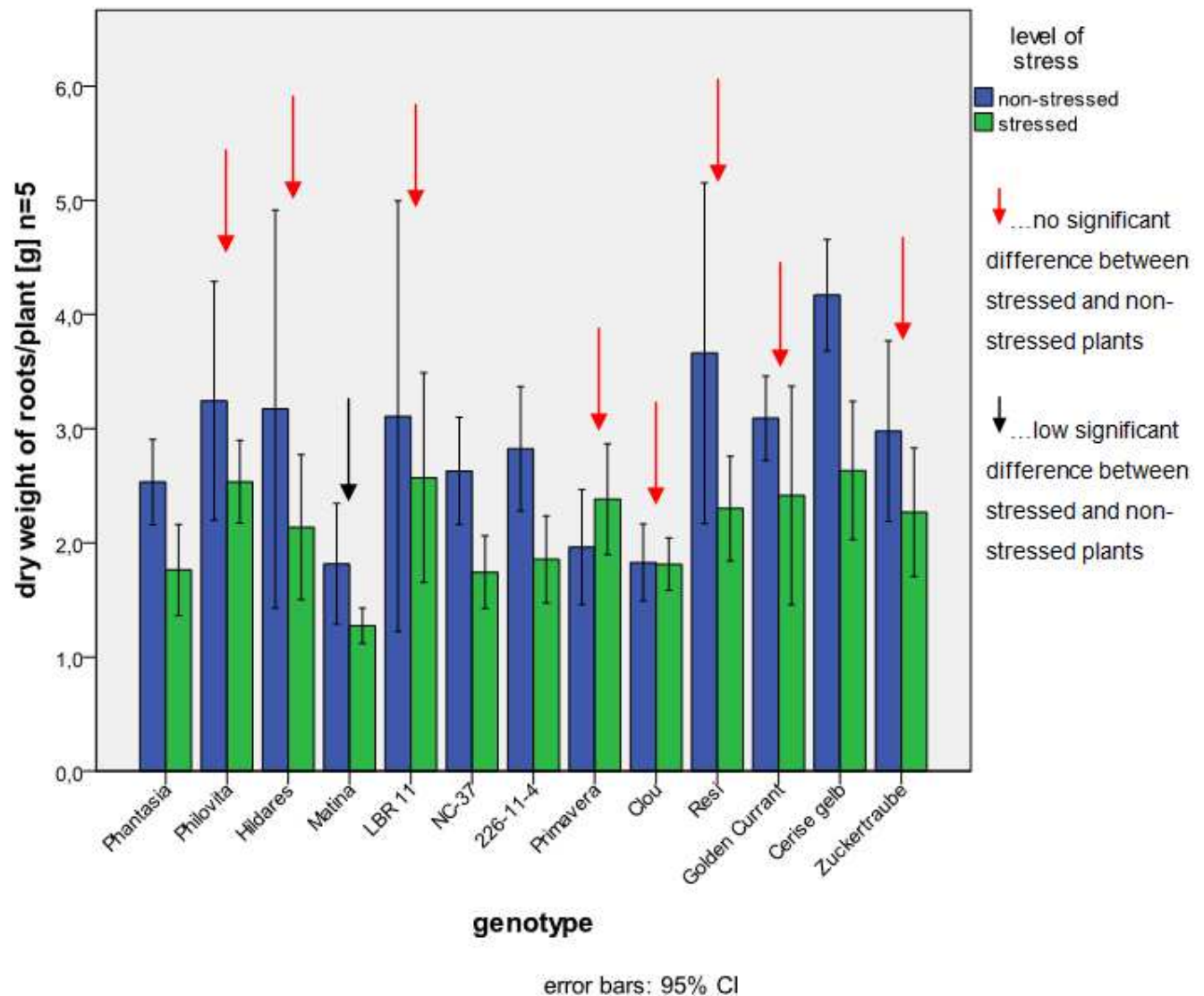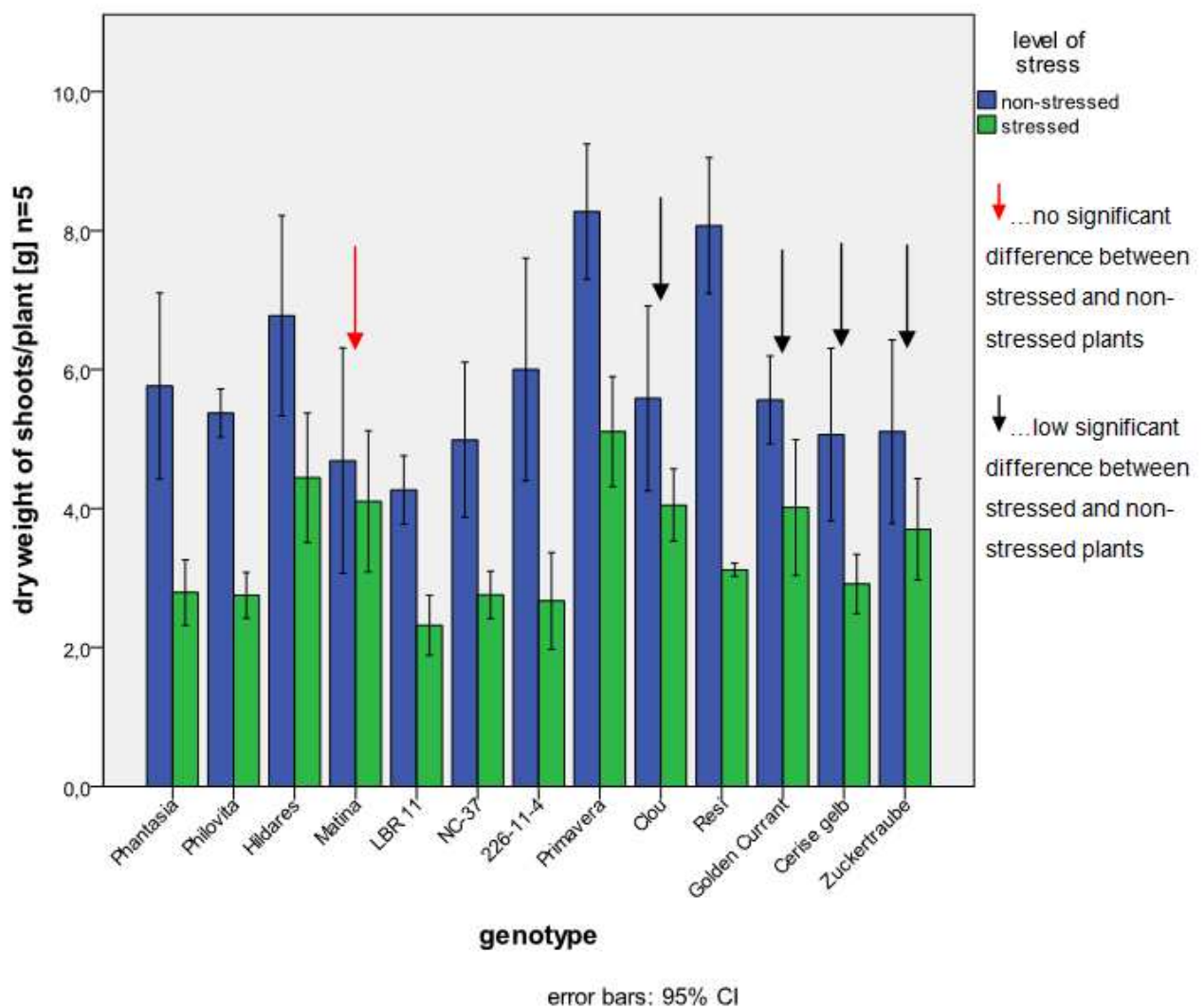

Figure 8: Dry weight of shoots of the 13 genotypes,
non-stressed and stressed separately (average of five replications each, error
bars show the confidence interval at 95%)

Root/shoot ratio on dry weight basis (Figure 9) differed significantly between non-stressed (0.55) and stressed plants (0.69). 'Hildares' (0.47/0.58), 'Matina' (0.39/0.36), 'LBR 11' (1.02/1.15), 'NC-37' (0.54/0.64), '226-11-4' (0.49/0.73), 'Primavera' (0.24/0.60), 'Clou' (0.34/0.45), 'Golden currant' (0.56/0.59), 'Cerise gelb' (0.86/0.90), and 'Zuckertraube' (0.60/0.61) did not show significant differences between non-stressed and stressed plants. 'Phantasia' (0.45/0.63) revealed significant difference, whereas 'Philovita' (0.60/0.92) and 'Resi' (0.45/0.73) revealed highly significant difference between non-stressed and stressed plants. 'LBR' and 'Cerise gelb' showed very high root/shoot ratio, whereas 'Matina' and 'Clou' showed very low root/shoot ratio.
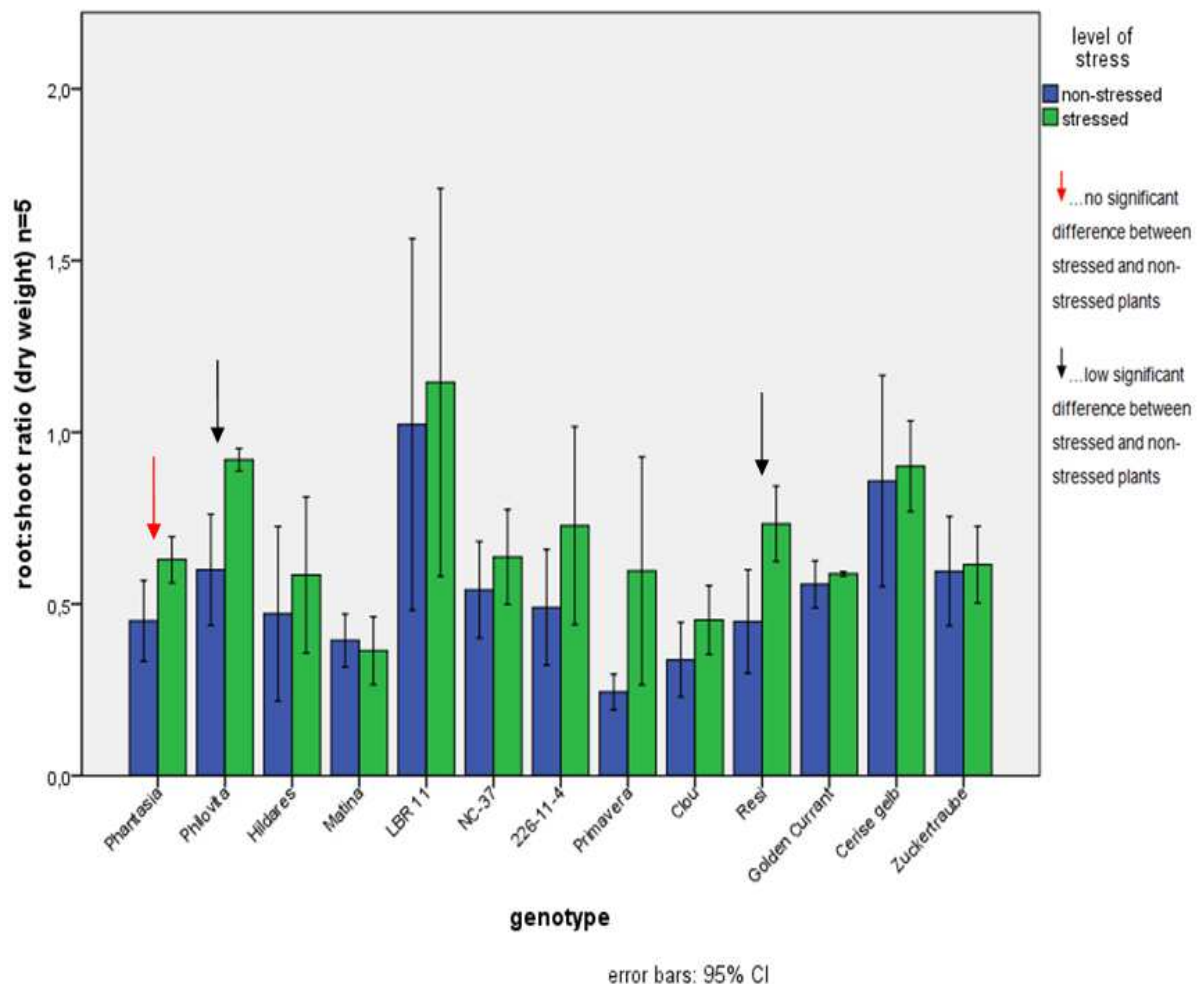

Figure 9: Root/shoot ratio (on dry weight basis) of the 13 genotypes, non-stressed and stressed separately (average of five replications each, error bars show the confidence interval at 95%)

For assessing stress induced growth reduction, percentage of stressed roots, shoots and plants (root + shoot) in relation to non-stressed plants was calculated (Table 3). As mentioned above biomass of stressed plants is lower than biomass of non-stressed plants. 'Clou' for example reached nearly equal root weight of non-stressed and stressed plants, whereas root weight of 'Primavera' in response to drought stress only reached 26 % of non-stressed root´s weight. All the other genotypes ranged from 62 – 84 %. Shoots percentages ranged from 38 – 87 %, with 'Matina' showing the highest and 'Resi' the lowest value (**Fehler! Verweisquelle konnte nicht gefunden werden.**).

Table 3: Percentage of dry weight of stressed roots, shoots and plants on dry weight of non-stressed roots, shoots and plants of the 13 genotypes (average of five replications each)

	roots	shoots	total plants
Phantasia	70	48	55
Philovita	78	51	61
Hildares	84	66	71
Matina	82	87	86
LBR 11	62	60	61
NC-37	66	55	59
226-11-4	66	44	51
Primavera	26	75	44
Clou	99	73	79
Resi	63	39	46
Golden currant	78	72	74
Cerise gelb	63	58	60
Zuckertraube	76	72	74
mean	**70**	**62**	**63**

4.2 Physiological traits

4.2.1 Leaf chlorophyll concentration

Leaf chlorophyll concentration between non-stressed and stressed plants differed significantly (15.6/19.5). No significant difference was notices in 'Phantasia' (16.6/14.6), 'Hildares' (15.9/18.4), 'Matina' (12.2/11.0), 'NC-37' (16.4/19.7), '226-11-4' (17.1/20.1), 'Primavera' (17.1/17.7), 'Resi' (23.2/25.4), whereas 'LBR 11' (8.8/13.3), 'Clou' (15.9/25.1), 'Golden currant' (16.3/24.1), 'Cerise gelb' (12.9/20.7), 'Zuckertraube' (13.3/17.9), and 'Philovita' (17.3/25.5) showed significant differences between non-stressed and stressed plants. 'Matina' and 'LBR 11' generally reached lower values; 'Resi' reached high values (Figure 10).
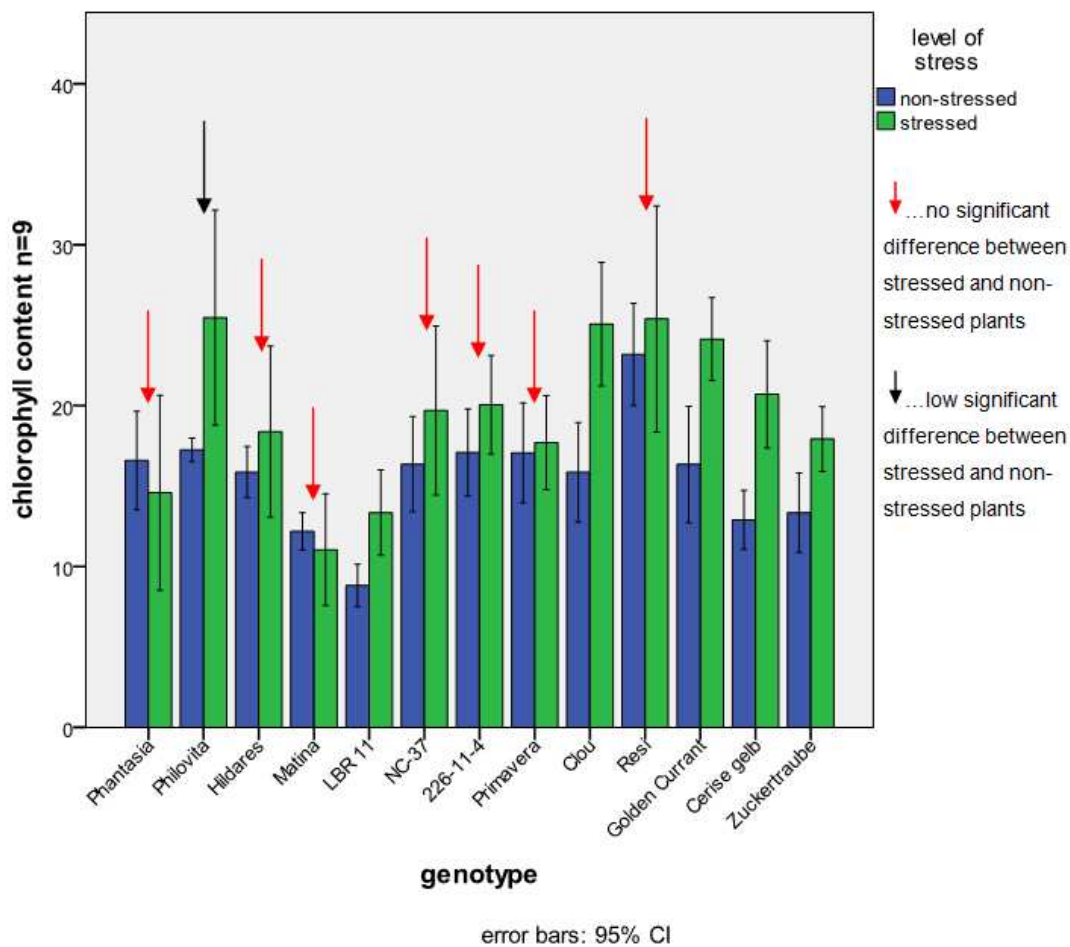

Figure 10: Leaf chlorophyll content of the 13 genotypes, non-stressed and stressed separately (average of nine replications each, error bars show the confidence interval at 95%)

4.2.2 *Chlorophyll fluorescence*

Generally chlorophyll fluorescence (F_v/F_m) showed homogenous data; although differences between all non-stressed (0.73) and all stressed (0.69) plants were significant. Within cultivars no significant difference was found in 'Philovita' (0.78/0.78), 'LBR 11' (0.48/0.56), 'NC-37' (0.75/0.72), 'Clou' (0.68/0.63), 'Golden currant' (0.73/0.73), and 'Zuckertraube' (0.78/0.78). 'Phantasia' (0.75/0.61) and 'Hildares' (0.78/0.75) showed significant difference, whereas 'Matina' (0.78/0.70), '226-11-4' (0.71/0.61), 'Primavera' (0.80/0.78), 'Resi' (0.79/0.74), and 'Cerise gelb' (0.71/0.64) showed highly significant differences. 'LBR 11' reached generally low values of chlorophyll fluorescence <0.6 (Figure 11).
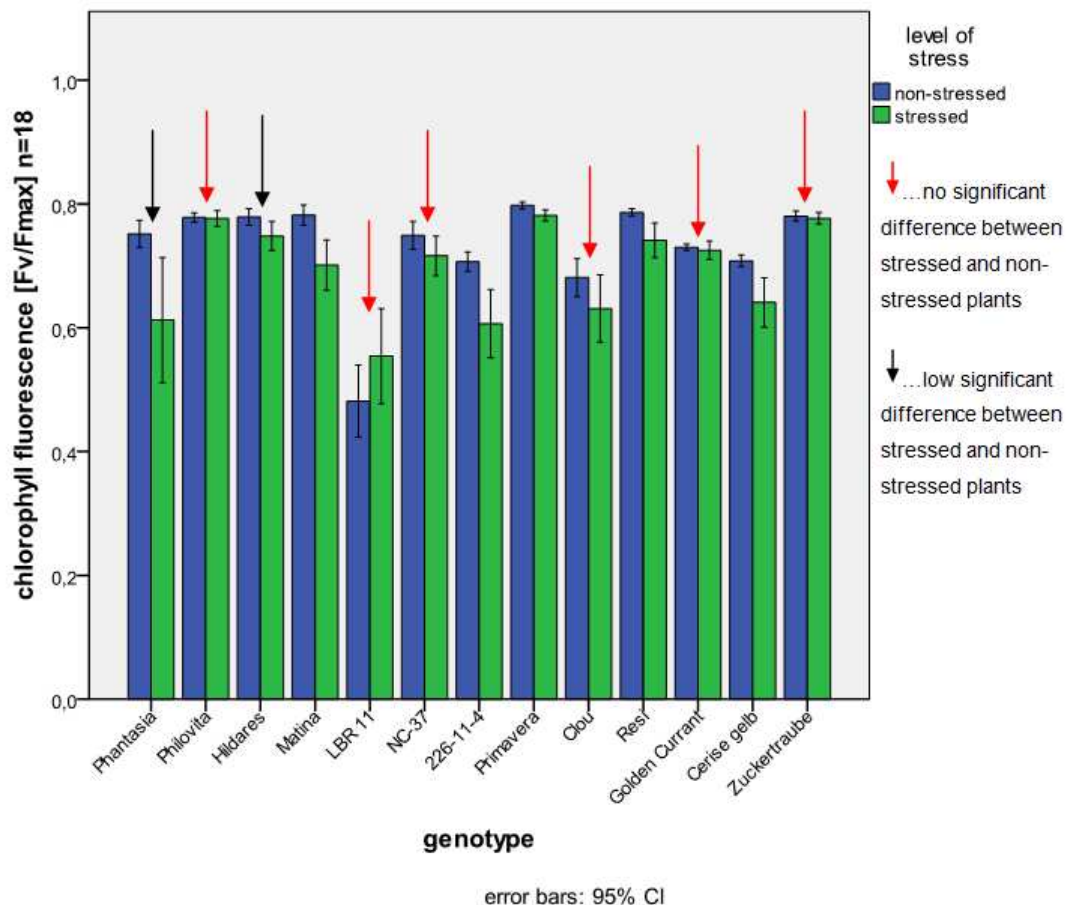

Figure 11: Chlorophyll fluorescence (F_v/F_m) of the 13 genotypes, non-stressed and stressed separately (average of eighteen replications each, error bars show the confidence interval at 95%)

4.2.3 Leaf osmotic potential

Generally non-stressed plants showed significantly less negative osmotic potential than stressed plants (-0.98 MPa/-1.06 MPa). Within genotypes osmotic potential between non-stressed and stressed plants is either equal or more negative under stress (Figure 12). 'Hildares' (-0.91 MPa /-0.89 MPa), 'LBR 11' (-0.92 MPa /-0.96 MPa), 'Primavera' (-0.93 MPa /-0.95 MPa), and 'Resi' (-0.99 MPa /-1.01 MPa) revealed no significant difference, whereas 'Zuckertraube' (-1.10 MPa /-1.05 MPa) showed significant difference and 'Phantasia' (-1.01 MPa /-1.14 MPa), 'Philovita' (-1.00 MPa /-1.20 MPa), 'Matina' (-0.94 MPa /-1.03 MPa), 'NC-37' (-0.89 MPa /-1.18 MPa), '226-11-4' (-0.97 MPa /-1.07 MPa), 'Clou' (-0.95 MPa /-0.99 MPa), 'Golden currant' (-1.11 MPa /-1.28 MPa), and 'Cerise gelb' (-0.94 MPa /-1.04 MPa) showed highly significant differences. Stressed plants of 'Phantasia', 'Philovita', 'NC-37', and 'Golden currant' reached very low values of osmotic potential.
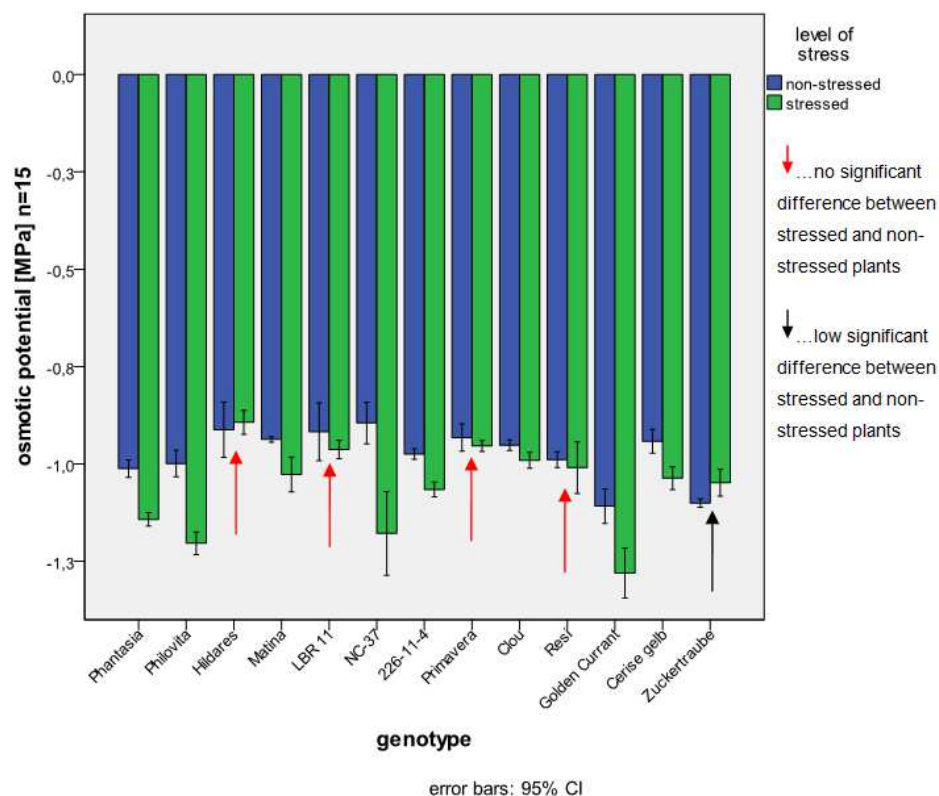

Figure 12: Leaf osmotic potential of the 13 genotypes,
non-stressed and stressed separately (average of five replications each, error
bars show the confidence interval at 95%)

5. DISCUSSION

The objective of this study was to evaluate the effect of drought stress (water deficiency in the growing substrate) on biomass production (fresh and dry weight, root/shoot ratio, water content) and physiological parameters (osmotic potential of leaves, chlorophyll concentration, and chlorophyll fluorescence).

5.1 Biomass traits

5.1.1 Biomass production

The most sensitive physiological process to water stress in plants is plant growth. Plant water status strongly influences plant growth and biomass production particularly through its effect on leaf and root extension (BEADLE et al., 1993). Inhibition of growth as well as impaired protein metabolism and synthesis of amino acids result from a decrease in osmotic potential of the cell, which is essential for preserving turgor (RUDICH and LUCHINSKY, 1987, LARCHER, 2003). Deep-root systems, biomass of roots, root length (KALLOO, 1991, p.160), and high root/shoot ratios (NAHAR and GRETZMACHER, 2011) are morphological characteristics expressing avoidance of tissue dehydration by higher uptake.

Results show in accordance to GEORGE et al. (2013) a decline in various plant attributes as response to stress, which is commonly observed and is due to tolerance level in plants. Reduced growth in tomato cultivars under PEG induced drought stress has been reported in several studies (AAZAMI et al., 2010, KULKARNI and DESHPANDE, 2007).

Growth of stressed plants was inhibited markedly. Dry weight of roots as well as dry weight of shoots decreased from non-stressed to stressed plants in general. 'Primavera' showed an increased dry weight of roots of stressed plants in comparison to non-stressed plants, though it is not significant, it is a sign for drought tolerance according to KALLOO (1991, p.160). 'Philovita', 'Hildares', 'LBR', 'Primavera', 'Clou', 'Resi', 'Golden currant' and 'Zuckertraube' showed non-significant decrease of root dry weights, therefore they did not exhibit clear reactions to drought stress. Since 'Phantasia', 'NC-37', '226-11-4' and 'Cerise gelb' showed significantly lower root dry weight of stressed plants they seem to be less drought-tolerant. 'Matina' did not show

significant effects of drought on dry weight of shoots, hence it appears to be the most drought – tolerant genotype out of the thirteen investigated. The less responsive genotype in terms of shoot dry weight are 'Phantasia', 'Philovita', 'Hildares', 'LBR-11', 'NC-37', '226-11-4', 'Primavera' and 'Resi'. All of them showed significant reduction of shoot dry weight (Figure 5, Figure 6, Figure 7 and Figure 8).

5.1.2 Root/shoot ratio

According to LARCHER (2003, p.409) and BEADLE et al. (1993) root/shoot ratio is shifted in favor of the roots the longer plants are exposed to drought. Because rapid growth of roots into deeper soil layers leads to improved water uptake due to an extensive root system with a large active surface area (LARCHER, 2003, p.409). Additionally KALLOO (1991, p.160) stated that root morphology (deep-root systems, more biomass of roots, root length) is a basic trait for drought resistance. LEVITT (1973, p.116) reported that the adaptation to drought in the rhizosphere is also improved by a high root/shoot ratio because less water needs to be absorbed per unit root area to supply the top. Increasing root/shoot ratio from non-stressed to stressed plants is noticed also in accordance to CHAVES et al. (2003), who found that an increased root/shoot ratio is a long term response to stress. Roots of field-grown tomatoes were investigated concerning enhanced root production due to soil water deficit by REID and RENQUIST (1997). Results showed clearly that tomatoes produce extra roots, especially in the subsoil, in response to an increasing soil water deficit or a decreasing soil matric potential.

Since all plants were grown in pots, root extension obviously developed less markedly than under field conditions. TRACHSEL et al. (2011) developed a phenotyping method for field conditions called "shovelomics" for investigating maize roots.

Generally 'Primavera' showed high root/shoot ratios of non-stressed and stressed plants. Also stressed plants of 'Golden currant' reached high root/shoot ratios. Significantly higher root/shoot ratio was found in non-stressed plants of 'Matina' (Figure 9); all the other genotypes did show values around 4, so root/shoot ratio as a trait for evaluating drought tolerance is not conclusive.

5.1.3 *Water content of tissue*

Water content on fresh weight basis (leaves, root, shoot) reached very homogenous values (around 80%), hence it can be assumed that adaptation to drought seemed to be similar. However, because dry weight can change diurnally and/or seasonally, comparisons of water content on a dry weight basis are unsatisfactory. When water content is expressed on a fresh weight basis, the problems of changing dry weight are still present, but, additionally, water content on a dry weight basis tends to minimize changes in water content as expressed on other bases (TURNER, 1981). The relative turgidity technique to determine RWC (relative water content) and WSD (water saturation deficit) respectively was suggested by BARRS and WEATHERLEY (1962) and reveals more valuable information about effects of drought on plants. Therefore information about water content is not conclusive.

When considering relatively constant water content of non-stressed and stressed plants (Figure 19) it is assumed that osmotic adjustment worked well.

5.2 Physiological parameters

5.2.1 *Chlorophyll content*

Further information about investigating chlorophyll concentration of tissue is offered by RICHARDSON et al. (2002), who evaluate different non-invasive methods for estimating foliar chlorophyll concentration.

VOLLMANN et al. (2011) tried to gain information about nodulation and nitrogen fixation of soybeans by applying SPAD spectrometer and a simple leaf digital analysis procedure where results correlate significantly. Another objective of that study was to compare nodulating and non-nodulating soybean lines. Chlorophyll concentration, leaf size, plant height, number of pods per plant, 1000-seed weight, seed protein and oil content were affected by nodulation type. It is suggested that the biometric parameters related to photosynthesis and nitrogen fixation could be used to determine the nitrogen status of a soybean crop and subsequently in forecasting seed quality parameters of the harvest product. After HALL (1993, p.37) chlorophyll values can be related to plant weight.

Chlorophyll content did not differ significantly in 'Phantasia', 'Hildares', 'Matina', 'NC-37', '226-11-4', 'Primavera' and 'Resi' (Figure 10), therefore it is concluded that these genotypes did not show significant reaction to drought stress regarding chlorophyll content.

5.2.2 Chlorophyll fluorescence

Chlorophyll fluorescence can give information about the state of Photosystem II (flow of electrons through PSII, PSII photochemistry), which often is one of the first indications of stress in leaves (MAXWELL and JOHNSON, 2000). As results show, chlorophyll fluorescence values are homogenous and not strongly influenced by applied drought conditions. 'LBR' showed the lowest values of chlorophyll fluorescence and is therefore the most drought tolerant genotype concerning chlorophyll fluorescence (Figure 11).

In many cases chlorophyll fluorescence measurements are used in combination with other techniques, particularly, gas exchange measurements, in order to obtain a wider image of the plants´ responses to different factors (MAXWELL and JOHNSON, 2000). Photosynthetic gas-exchange analysis can be used to describe the responses of carbon fixation to changes in the environment. However, there is no information about the partitioning of dry matter into new leaf area (which can have a considerable influence on production), or about the competing sinks which determine the fate of the carbon fixed during photosynthesis, e.g. conversion into oil or carbohydrates (HALL, 1993, p.36). HAUPT-HERTING and FOCK (2000) for example investigated tomatoes concerning the effects of drought on photosynthesis by CO_2 exchange, oxygen evolution and chlorophyll fluorescence. Our results (Figure 11) showed markedly that fluorescence measurements reached higher rates of electron flux than from CO_2 measurements in stressed leaves, which was attributed to an increased electron transfer to oxygen. VAN DER TOL et al. (2009) studied the relationship between passively measured chlorophyll fluorescence and actual photosynthesis. They found out that variations in total chlorophyll fluorescence correlate well with variations in actual photosynthesis in the late morning and afternoon, by then, photosynthesis is light saturated and limited by stomatal regulation.

When exposed to salinity, cucumbers showed reduced relative water content in leaves. Net photosynthetic rate, stomatal conductance, transpiration rate and maximum quantum efficiency of photosystem II was decreased by salt treatments. It is indicated that salinity has an effect on photosynthesis through stomata closure and non-stomatal factors (STĘPIEŃ and KŁBUS, 2006).

Results of chlorophyll fluorescence (Figure 11) showed that there is not much difference between non-stressed and stressed plants and also between the genotypes, only 'LBR' showed lower values.

5.2.3 Osmotic potential

Osmotic adjustment is described as one of the major characteristics of drought tolerance by many authors (BLUM, 1988, KRAMER and BOYER, 1995, LUDLOW and MUCHOW, 1990). LARCHER (2003, p.412), states, that a decrease in osmotic potential of the cell (especially in leaves), which is essential for preserving the turgor-pressure, takes place later during water stress, and results in an inhibition of growth. Because of osmoregulation and osmotic adjustment a higher osmotic potential is characteristic for drought-resistant genotypes (RUDICH and LUCHINSKY, 1987, KALLOO, 1991). Once water potential of the cell decreases, osmoregulatory measures are initiated. Osmotic adjustment causes an accumulation of low-molecular organic substances (water-soluble carbohydrates and organic nitrogen compounds) in the cell compartments and the cytosol via synthesis, translocation and conversion of starch. Osmotic potential decreases and water is attracted into the cell by osmotic influx. Therefore turgor pressure and cell volume stay at an adequate level. Furthermore loss of turgor in leaf mesophyll is delayed and therefore carbon assimilation of plants is benefiting (CHANDRA BABU et al., 1999, p.407, LARCHER, 2003). KIKUTA and RICHTER (1988) concluded that drought stress serves only as a driver for lowering osmotic potential, while the metabolic events are favored by high or intermediate water contents.

In this study press sap was used for determination of osmotic potential. In another study KIKUTA and RICHTER (1992) investigated two standard methods (thermocouple hygrometry of press saps and of freeze-thawed leaf discs) using tissue of different species. In leaf discs a lower (more negative) osmotic potential was

found, which is explained due to the fact that osmotically active substances are not completely extracted by pressing tissue and that the discs contain a smaller amount of apoplastic water than saps on average.

ÖGREN and ÖQUIST (1985) measured gas exchange and fluorescence light emission of attached willow leaves and found that the light-saturated photosynthetic CO_2 uptake became progressively inhibited with decreased leaf water potential both at high, and especially at low intercellular CO_2 pressure.

Our results show a more negative osmotic potential of drought stressed plants than of control plants, which is a sign for osmotic adjustment. In stressed plants of 'Phantasia', 'Philovita', 'NC-37' and 'Golden currant' more negative osmotic potentials were noticed. Additionally 'Phantasia', 'Philovita' and 'NC-37' also showed very low shoot weight (fresh and dry) (Figure 6 and Figure 8) and plant weight (root + shoot) (Figure 16).

When considering relatively constant water content of non-stressed and stressed plants (Figure 19) it is assumed that osmotic adjustment worked well.

5.3 Plant material and experimental setup

Due to the complexity of drought tolerance and drought stress responses of plants the validity of these results is limited. Additionally the experiment was carried out until BBCH 51 (1st flower open), therefore effects on later developmental stages were not observed. CHAVES et al. (2003) stated that it is important to differ between long-term and short-term drought stress. Shoot growth inhibition, osmotic adjustment, sustained root growth and increased root/shoot ratio are mentioned as some of the long-term responses. These traits might have expressed more clearly in a longer trial.

Drought stress was induced by reduced irrigation of plants which means there were just two levels of drought stress. Additionally there has not been exact measurement of drought level, i.e. plant available soil water. For more precise quantification and graduation of stress, drought can be induced for example by polyethylene glycole (PEG) (ZGALLAÏ et al., 2005, VERMEULEN et al., 2008, GEORGE et al., 2013, SHAMIM et al., 2013), calcium chloride ($CaCl_2$) (MINGCHI et al., 2010) or NaCl

(STĘPIEŃ and KŁBUS, 2006). Anyways, for breeding evaluation experiments under field conditions are the most valuable.

FOOLAD (2007) suggests that the most reliable criteria for breeding tomatoes for drought tolerance are agronomic characteristics (yield), and absolute and relative plant growth under stress and non-stress environments. Such criteria, however, may not be efficient to apply because in most breeding projects often a large number of individuals, families or populations are used. Alternative criteria based on physiological characteristics such as photosynthetic rates, stomatal resistance and leaf water potential might be more efficient. These traits generally show rather strong correlations with agronomic characteristics. Other alternatives are the identification of biochemical characters such as enzyme activities and protein contents. These methods, however, often lack a strong correlation with agronomic characteristics and are expensive. Like other abiotic stresses, the identification and utilization of molecular markers associated with different tolerance-related physiological, morphological or agronomic criteria might be an efficient way to improve drought tolerance screening in tomato.

5.4 Conclusion

Under drought stress, the new organically bred cultivars 'Primavera' and 'Clou', the old conventionally bred cultivars 'Hildares' and 'Matina' and the "wild-type" tomato 'Golden currant' produced highest shoot dry matter, whereas without stress, 'Primavera' and 'Resi', a cultivar received from a private seed saver, produced most shoot dry matter. 'Hildares' also showed above-average growth when not stressed, whereas the remaining genotypes showed average growth.

The modern, conventionally bred cultivars 'Phantasia' and 'Philovita' showed significant difference between shoot dry matter of non-stressed and stressed plants. Root dry matter only showed significant difference in 'Phantasia' and not in 'Philovita'. Therefore it may be concluded that 'Philovita' invests in root growth under limited availability of soil water. 'Phantasia' showed a low significant difference in chlorophyll fluorescence, both showed a significant difference between non-stressed and stressed plants in osmotic potential, 'Philovita' showed a low and 'Phantasia' no significant difference in chlorophyll content.

 'Hildares' and 'Matina' are old, conventionally bred cultivars. 'Matina' was the only cultivar without significant reduction of shoot dry matter when stressed; shoot dry matter of non-stressed plants was the lowest of all cultivars and in the mean of both water supply levels, also root dry matter was lowest of all cultivars. Therefore it can be concluded that 'Matina' generally has smaller plants and may have higher potential to be drought tolerant. When it comes to physiological parameters, namely chlorophyll fluorescence and osmotic potential, we found significant differences between non-stressed and stressed plants of 'Matina', whereas chlorophyll content did not show a significant difference.

'Hildares' generally showed high shoot dry weight of non-stressed plants and a significant difference to stressed plant´s dry weight. Root growth showed average values and no significant difference. Additionally there were not found highly significant differences in physiological parameters which leads to the conclusion that this genotype is generally less responsive to drought stress.

'LBR 11' and 'NC-37', the "exotic" genotypes from Thailand and Israel respectively, were expected to show a higher drought tolerance, but they did not fulfill the expectation. Only root dry weight of 'LBR 11' did not differ significantly. 'LBR 11'

showed highly significant difference in chlorophyll content, 'NC-37' showed highly significant difference in osmotic potential and no significant difference was found in chlorophyll fluorescence for both genotypes.

'226-11-4', 'Primavera' and 'Clou' were bred in the OOTP. '226-11-4' was selected from 'Philovita F1', parents of 'Primavera' (released from the OOTP) are 'Cerise gelb' and 'Zuckertraube', and parents of 'Clou' (released from the OOTP) are 'Golden currant' and 'Matina' (Table 1).

'226-11-4' showed significant difference between shoot and root dry matter of non-stressed and stressed plants. There was no significant difference in chlorophyll content but in osmotic potential and chlorophyll fluorescence. When compared with 'Philovita', '226-11-4' had similar shoot dry weights of non-stressed as well as of stressed plants. Root dry weight was generally lower in '226-11-4' and it also differed significantly between non-stressed and stressed plants.

'Primavera' was the only cultivar showing no reduction of root dry matter when stressed, whereas shoot dry matter of stressed plants showed a significant reduction. Generally 'Primavera' showed high shoot dry matter and average root dry matter. Chlorophyll fluorescence differed significantly; osmotic potential and chlorophyll content did not show significant differences. The parents of 'Primavera', 'Cerise gelb' and 'Zuckertraube' showed a reverse reaction of dry weight in general. While shoot dry weight of Primavera was relatively high, that of 'Cerise gelb' and 'Zuckertraube' was low and root dry weight of the parents was higher than of 'Primavera', although it was not that clear at root dry weight.

'Clou' showed only a low significant difference between shoot dry matter of non-stressed and stressed plants and no significant difference between dry matter of non-stressed and stressed roots. Hence it is supposed to be drought stress tolerant. Chlorophyll fluorescence did not show a significant difference between non-stressed and stressed plants but osmotic potential was significantly higher negative and chlorophyll content was significantly higher in stressed plants. Compared to 'Golden currant' and 'Matina' shoot dry weight of 'Clou' is more similar to that of 'Golden currant' whereas root dry weight of 'Clou' resembles that of 'Matina'.

'Resi', 'Golden currant' and 'Cerise gelb' were selected from screenings in the OOTP. 'Resi' showed nearly the highest shoot dry weight of non-stressed plants whereas

stressed plants reached relatively low shoot dry weight. However, root dry weight did not show a significant difference as well as chlorophyll content and osmotic potential.

'Golden currant' did not show (highly) significant differences between root and shoot dry weight of non-stressed and stressed plants. Chlorophyll fluorescence was not influenced significantly by drought stress, whereas chlorophyll content of stressed plants was significantly higher and osmotic potential of stressed plants was significantly higher negative. It is assumed that 'Golden currant' is drought stress tolerant.

'Cerise gelb' showed significantly lower root dry weight of stressed plants and a low significant difference in shoot dry weight. All physiological reactions of this genotype were highly significant. Therefore it can be concluded that 'Cerise gelb' is generally less drought tolerant.

'Zuckertraube', was also used as a parental line in the OOTP. It reached a low significant difference of shoot dry weight and no significant difference of root dry weight. Osmotic potential and chlorophyll content differed significantly, whereas chlorophyll fluorescence did not show a significant difference. In summary 'Zuckertraube' can be seen as drought stress tolerant.

6. ZUSAMMENFASSUNG

In der vorliegenden Arbeit wurden 13 Tomaten-Genotypen verschiedener Herkunft auf ihre ökophysiologische Reaktion auf Trockenstress untersucht. Die Einteilung erfolgte in alte und neue Sorten, konventionell und ökologisch gezüchtete Sorten, Genotypen aus anderen Kontinenten, Elternlinien und deren Nachkommen. Es handelt sich dabei um Genotypen, die im Rahmen eines Züchtungsprogrammes der Universität Göttingen („Ökologisches Freiland-Tomatenprojekt") geprüft werden.

Im Glashaus der Universität Wien (Althanstraße) wurde ein Topfversuch mit zwei Stufen der Wasserversorgung durchgeführt (normal, reduziert). Nach fünf Wochen wurden Chlorophyllfluoreszenz und Chlorophyllgehalt der Blätter gemessen. Der aktuelle Wassergehalt und das osmotische Potential wurden ebenso ermittelt. Nach acht Wochen wurden Frisch-, Trockengewicht und aktueller Wassergehalt der Ganzpflanze (Wurzel und oberirdischer Teil getrennt) erhoben.

Die Ergebnisse zeigten generell ein niedrigeres Frischgewicht der Wurzeln und Sprosse (Stängel und Blätter). Das Trockengewicht der Sprosse zeigte bei 'Clou', 'Golden currant', 'Cerise gelb' und 'Zuckertraube' einen lediglich gering signifikanten Unterschied. Alle anderen Genotypen wiesen keinen signifikanten Unterschied auf. Die Wurzeltrockenmasse unterschied sich signifikant bei 'Phantasia', 'NC-37', '226-11-4' und 'Cerise gelb'. Keinen bzw. einen geringen signifikanten Unterschied zeigten 'Philovita', 'Hildares', 'Matina', 'LBR-11', 'Primavera', 'Clou', 'Resi', 'Golden currant' und 'Zuckertraube'. Die physiologischen Parameter zeigten allgemein Unterschiede zwischen nicht-gestressten und gestressten Pflanzen, haben allerdings für die Beurteilung der Stresstoleranz wenig Aussagekraft.

7. ABSTRACT

The aim of this study was the evaluation of the eco-physiological responses of tomato genotypes from different breeding environments to drought stress. In total, thirteen genotypes of different origins were screened. They were categorized in old and new cultivars, conventionally and organically bred ones, and genotypes bred in different continents, as well as parental lines and progenies. These genotypes were a subset of genotypes tested within the framework of the breeding programme "Organic Outdoor Tomato Project" in field experiments at the University of Göttingen.

A pot experiment with two levels of water supply (normal, deficient) was conducted. After growing the plants in pots for five weeks, chlorophyll fluorescence and chlorophyll concentration of leaves were measured and current water content and osmotic potential of single leaves were assessed. After eight weeks in the pots, fresh weight, dry weight, and current water content of shoots and roots were determined.

Results show lower fresh weight of roots and shoots (shoot + leaves) of stressed plants. Dry weight of shoots of 'Clou', 'Golden currant', 'Cerise gelb' and 'Zuckertraube' showed just a low significant difference. All the remaining genotypes did not show a significant difference. Root dry weight differed significantly in 'Phantasia', 'NC-37', '226-11-4' and 'Cerise gelb'. Now and low significant differences respectively were found in 'Philovita', 'Hildares', 'Matina', 'LBR-11', 'Primavera', 'Clou', 'Resi', 'Golden currant' und 'Zuckertraube'. Physiological parameters did show differences between non-stressed and stressed plants but they do not provide conclusive information about evaluation of drought stress tolerance.

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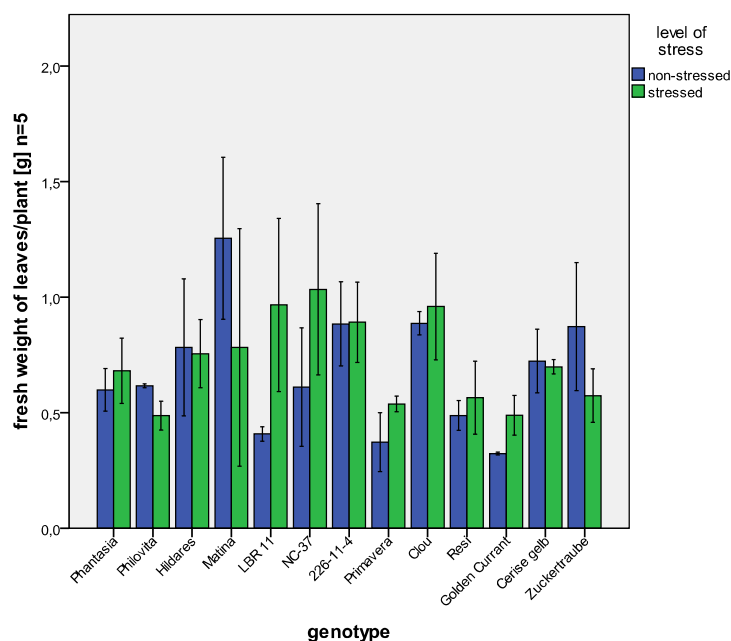
9. ANNEX

Figure 13: Fresh weight of leaves of the 13 genotypes,

non-stressed and stressed separately (average of five replications each, error
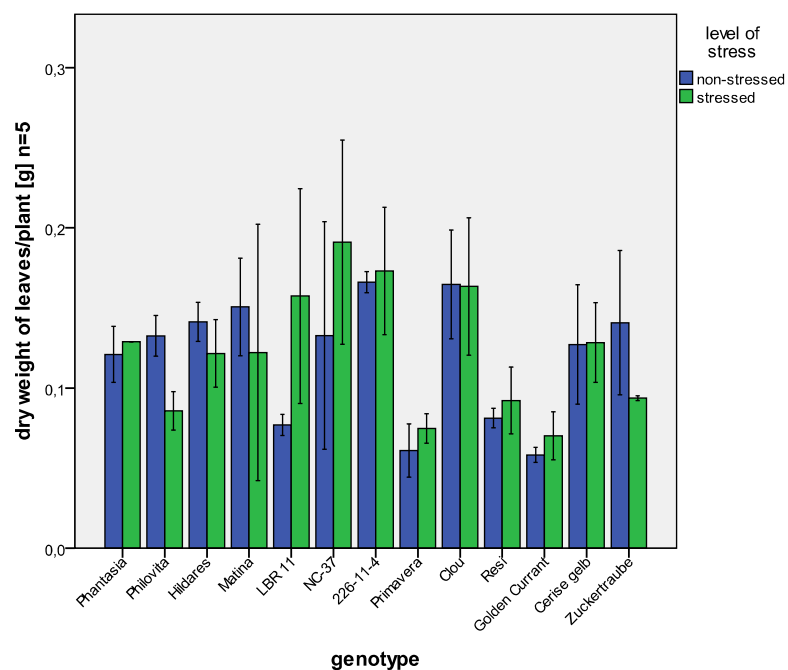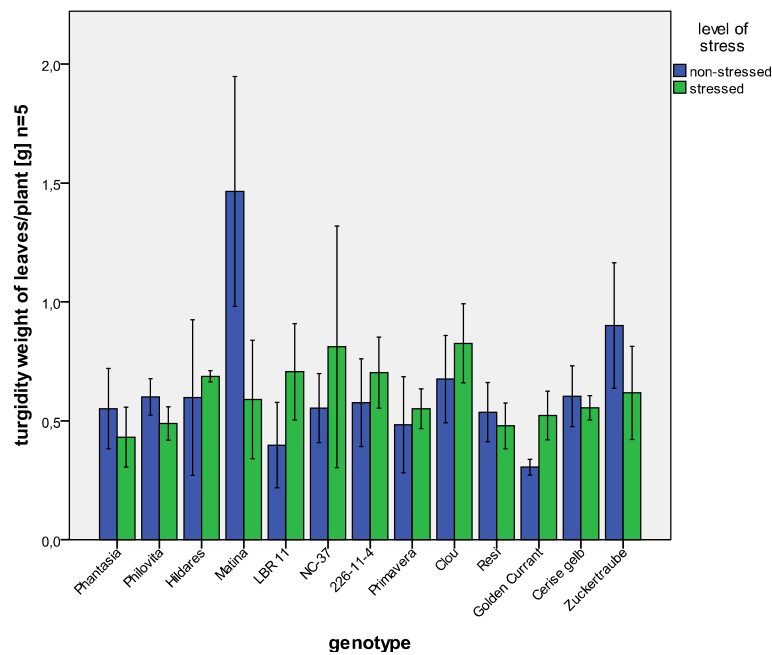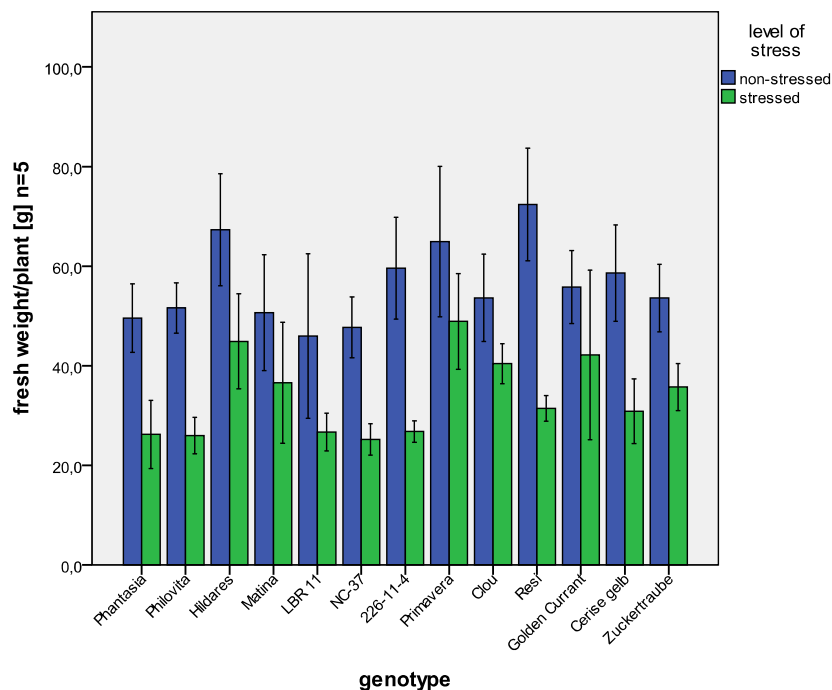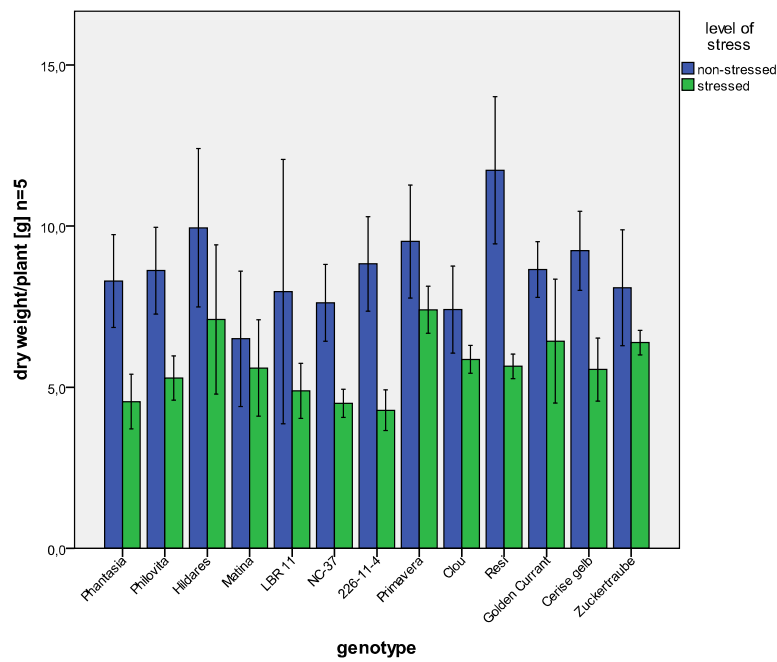
bars show the confidence interval at 95%)

Figure 14: Dry weight of leaves of the 13 genotypes,

non-stressed and stressed separately (average of five replications each, error

bars show the confidence interval at 95%)

Figure 15: Saturation weight of leaves of the 13 genotypes, non-stressed and stressed separately (average of five replications each, error bars show the confidence interval at 95%)

Figure 16: Fresh weight of plants of the 13 genotypes, non-stressed and stressed separately (average of five replications each, error bars show the confidence interval at 95%)
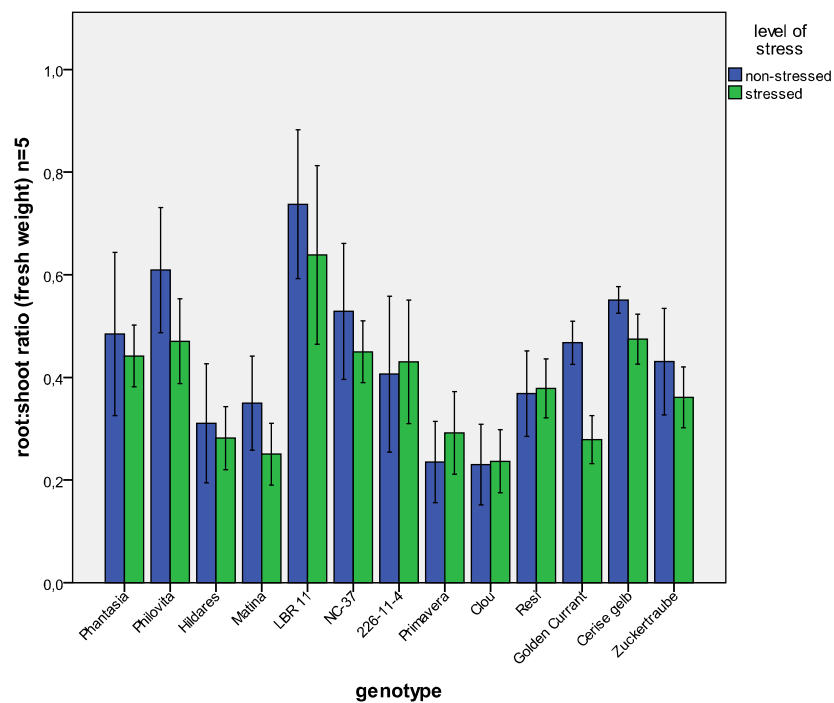
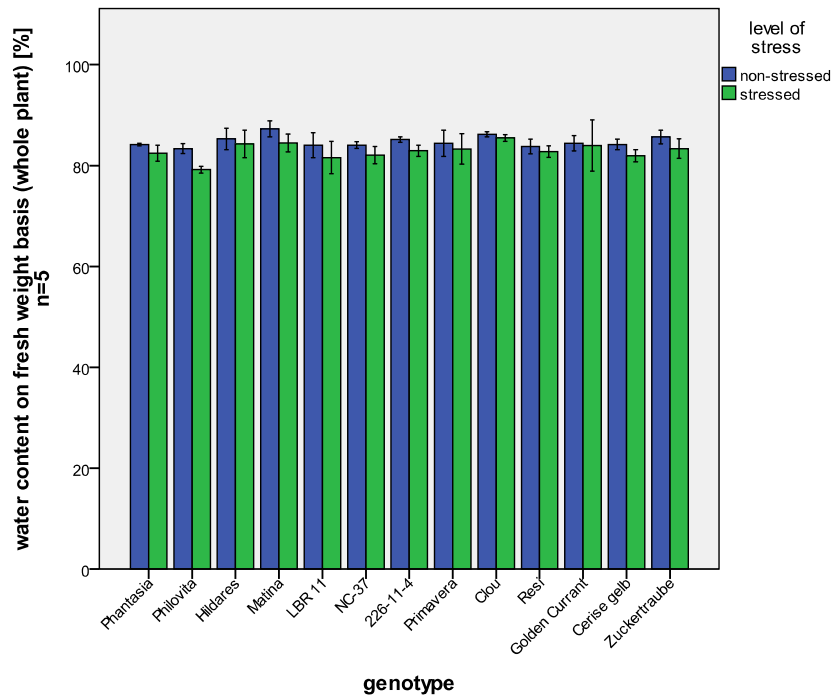**Figure 17: Dry weight of plants of the 13 genotypes,**

non-stressed and stressed separately (average of five replications each, error

bars show the confidence interval at 95%)

Figure 18: Root/shoot ratios (on fresh weight basis) of the 13 genotypes,

non-stressed and stressed separately (average of five replications each, error

bars show the confidence interval at 95%)

Figure 19: Water content of plants (on fresh weight basis) of the 13 genotypes, non-stressed and stressed separately (average of five replications each, error bars show the confidence interval at 95%)
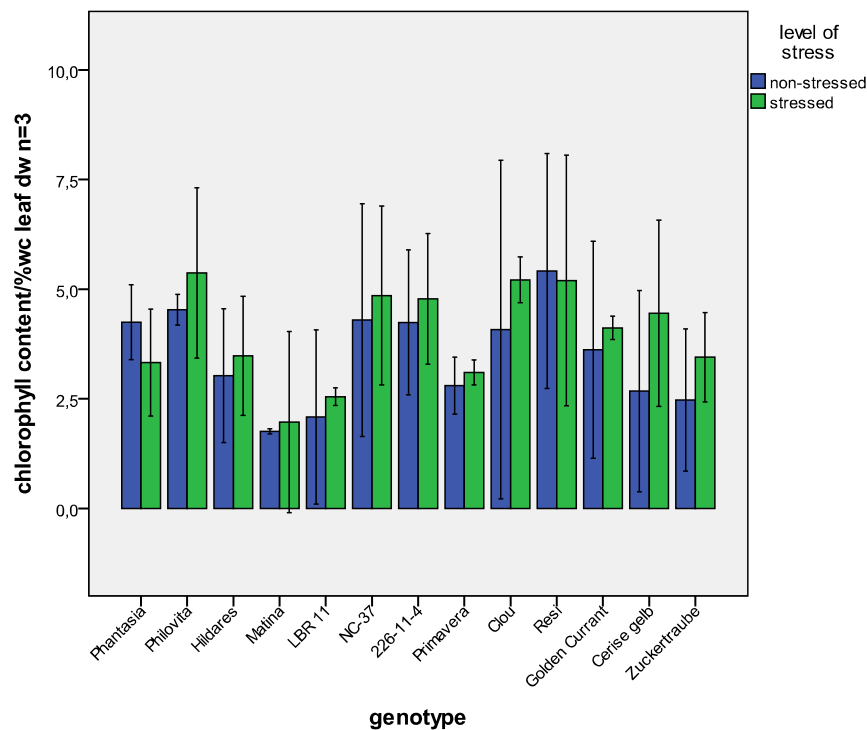

Figure 20: Chlorophyll content/% water content of leaves (on dry weight basis), non-stressed and stressed plants separately (average of three replications each, error bars show the confidence interval at 95%)
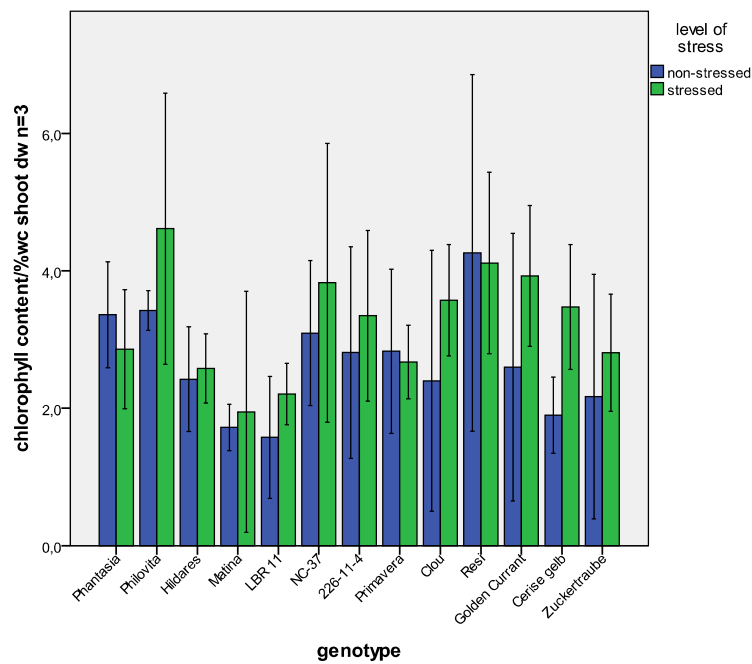
Figure 21: Chlorophyll content/% water content of shoots (on dry weight basis), non-stressed and stressed plants separately (average of three replications each, error bars show the confidence interval at 95%)
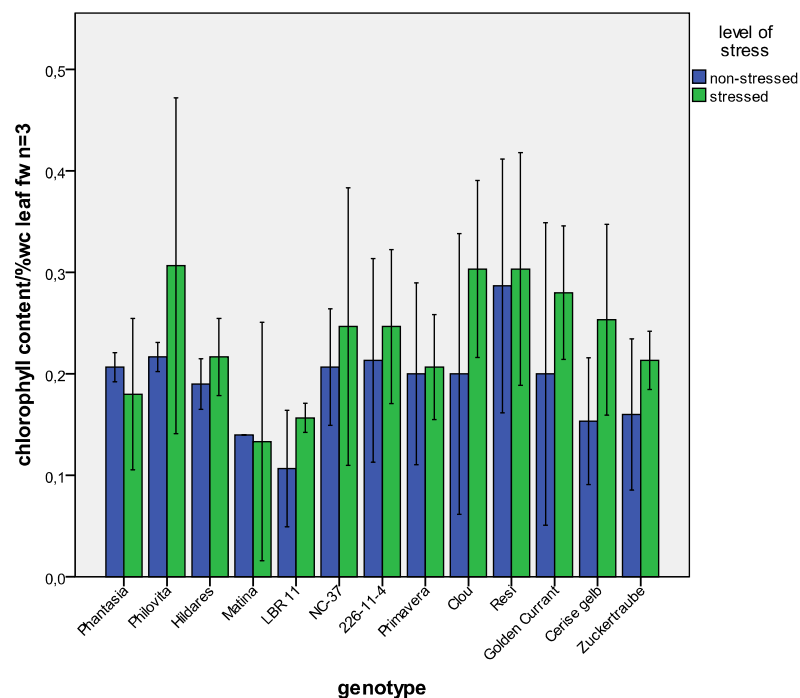

Figure 22: Chlorophyll content/% water content of leaves (fresh weight basis), non-stressed and stressed plants separately (average of three replications each, error bars show the confidence interval at 95%)
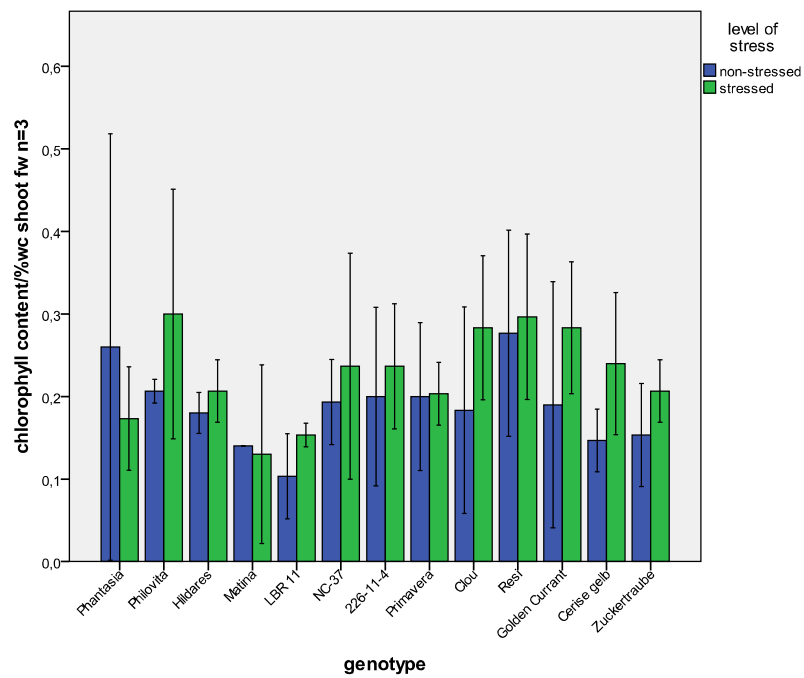
Figure 23: Chlorophyll content/% water content of shoots (fresh weight basis), non-stressed and stressed plants separately (average of three replications each, error bars show the confidence interval at 95%)

Eidesstattliche Erklärung

Hiermit versichere ich an Eides statt und durch meine Unterschrift, dass die vorliegende Arbeit von mir selbstständig, ohne fremde Hilfe angefertigt worden ist. Inhalte und Passagen, die aus fremden Quellen stammen und direkt oder indirekt übernommen worden sind, wurden als solche kenntlich gemacht. Ferner versichere ich, dass ich keine andere, außer der im Literaturverzeichnis angegebenen Literatur verwendet habe. Diese Versicherung bezieht sich sowohl auf Textinhalte sowie alle enthaltenden Abbildungen, Skizzen und Tabellen. Die Arbeit wurde bisher keiner Prüfungsbehörde vorgelegt und auch noch nicht veröffentlicht.

Feldkirchen an der Donau, Februar 2014