Journal of Applied Botany and Food Quality 86, 47 - 54 (2013), DOI:10.5073/JABFQ.2013.086.007

Institute of Botany, Gottfried Wilhelm Leibniz University Hannover, Germany

Comparative analysis of methods analyzing effects of drought on the herbaceous plant *Lablab purpureus*

S. Guretzki, J. Papenbrock

(Received March 1, 2013)

Summary

Due to the changing climatic conditions, there is an enlargement of land areas with insufficient rainfall and therefore a reduction in the cultivated area for common crops. Hence, it is now important to find plants that are adapted to these drought conditions. The focus of our research was to apply and compare different methods to quantify the impact of drought stress on plants.

Lablab purpureus is considered to be drought tolerant. Therefore, we used *L. purpureus* genotypes from three continents CPI 36903 (Europe), CPI 52508 (Africa) and HA-4 (Asia) as examples for our study. All genotypes were screened for their tolerance to drought stress by various methods to obtain quantitative data on the drought stress tolerance of individual genotypes and to find out which methods are especially suitable for the measurement of drought tolerance. Classical methods such as leaf size, plant height, biomass, and plant water content were investigated. In addition, by chlorophyll fluorescence measurement effects of drought on the photosynthetic system were examined. Infrared thermography was used in order to make the changes in leaf temperature in plants stressed by drought compared to unstressed plants visible. The methods were complemented by the measurement of leaf conductivity.

Results indicate a difference in the usability of the methods for the determination of drought stress. Finally, a set of methods is assembled based on suitability for drought tolerance analysis in plants. The methods include classical growth parameters, including dry weight biomass, plant water content (PWC) and leaf size determination, as well as height measurements of the plants. The stomata behavior is analyzed by leaf conductivity and infrared thermography, both methods complete the set for drought tolerance identification.

Based on the results of these methods a ranking of the examined genotypes with respect to their drought tolerance is created.

Introduction

Because of global warming the climate conditions in e.g. Africa, South Asia and East Asia will become more arid (Dai, 2011). However, the precipitation amount is a key factor for the productivity of crops (Rosenzweig et al., 2001). Common crops have to deal with these new environmental conditions. The detection of new crop plants or genotypes of crop plants that are particularly adapted to more arid conditions is therefore a major objective to preserve the livelihood of the local populations. *Lablab purpureus* (L.) Sweet (synonyms: *Dolichos purpureus*, *Dolichos lablab* (NCBI-Taxonomy)), the experimental plant in this study, is referred to as drought tolerant in Maass et al. (2010). *L. purpureus* belongs to the Eudicots, Core Eudicots, Fabids order Fabales, family Fabaceae (The Angiosperm Phylogeny Group (APGIII), 2009). The herbaceous plant is perennial but is often grown as an annual plant. *L. purpureus* occur as bushy, semi-erect and prostate growth habit types. The stem is twining, the leaves are alternate and trifoliate. Flowers exist in different colors (white, pink, red, purple). Pods and seeds vary in color and size. Various parts of the plant are used as food (flowers, leaves, pods, root tubers, seeds) or fodder (www.lablab.org, University of Agricultural Sciences, Bangalore). *L. purpureus* is cultivated as a component of mixed cropping schemes or home gardens, wherein the plant is known especially in Africa, South Asia and South East Asia (Maass et al., 2010). Studies showed that this species is adapted to drought, but there are differences in terms of drought tolerance within the species as summarized in Maass et al. (2010). However, the differences in drought tolerance have not been quantified for this species so far. Also for other herbaceous species studies on the quantitative analysis to measure the impact of drought stress on plants are rare. There are the more traditional parameters measured, including the determination of the fresh weight and dry weight biomass, plant water content, as well as development of plant height and leaf size to investigate the effects of stress on plants. In addition, there are new non-destructive methods which allow a rapid screening of plants. This includes the determination of leaf chlorophyll fluorescence by PAM-Imaging in order to get information about the state of the photosynthetic system under stress conditions (Woo et al., 2008; Sperdouli and Moustakas, 2012). The behavior of stomatal conductivity can be observed over a large area by infrared thermography, a further rapid screening method (Grant et al., 2006). This makes the method interesting for phenotype screening and breeding programs (Chaves et al., 2003). Another option for stomatal conductivity measurements is the use of a porometer (Jones et al., 2002; Grant et al., 2006). The combination of infrared thermography and chlorophyll fluorescence for the observation of changes in transpiration rate and photosynthesis can be used for detection of early plant stress and stress tolerance screening (Chaerle et al., 2007, 2009). Munns et al. (2010) concluded that stomatal conductance is a growth rate indicator for plants under water stress and thermography is a good screening method. Together it is possible to find the best genotypes for different growth conditions. Berger et al. (2010) points out that chlorophyll fluorescence is helpful for the detection of servere drought stress. For early drought stress detection chlorophyll fluorescence seems only useful in combination with other methods to gain a more comprehensive picture of the plant stress response. The objective of this study is to find methods that are suitable for recognizing the impact of drought stress on plants and methods that are appropriate in a combined way for a reliable detection of drought-tolerant genotypes in the case of *L. purpureus* as an example for an herbaceous species. More precisely, to compile methods those allow the drought stress detection before the leaves wilt. The drought tolerance results correlated with the results of genetic analyses can be used to find the best genotypes for field experiments within future breeding programs.

Material and methods

Plant material, growth conditions and drought treatment

Seeds of *Lablab purpureus* (L.) Sweet were originally obtained from Dr. B.L. Maass, International Centre for Tropical Agriculture (CIAT), Nairobi, Kenya (CPI 36903, CPI 52508) and from Dr. M.B. Gowda,

University of Agricultural Sciences, Bangalore, India (HA-4). CPI 36903 (Southern Ukraine, Europe) and CPI 52508 (Mozambique, Africa) are semi-domesticated genotypes (MAASS and USONGO, 2007). The origin of HA-4 is Karnataka, Southern India, Asia. For germination, seeds were soaked in water at room temperature overnight and transferred on type CL T soil (Einheitserde, Sinntal-Altengronau, Germany) in pots of 12 cm diameter the following day. The soil contains 30% clay. Plants were grown in the greenhouse in a 12 h light/dark rhythm at a temperature of 22°C/22°C. When the outdoor light conditions did not ensure sufficient light intensity inside the greenhouse, additional light was switched on to obtain a constant quantum fluence rate of approx. 350 μmol m^{-2} s^{-1} (sodium vapor lamps, SON-T Agro 400, Philips, Amsterdam, Netherlands). Plants were grown in the greenhouse for five weeks under well-watered conditions.

The drought experiments started in week six under the same greenhouse conditions. On day one, the plants were watered for the last time before the plants were set to the experimental conditions. Experimental groups differ in soil volumetric water content (VWC); the control group (35% VWC) and the drought group (20% VWC) were used to reach a moisture content level near field capacity and near the permanent wilting point of the soil, respectively, in accordance to the manual of the used device. For the measurement of VWC time domain reflectometry (TDR) (Fieldscout, Spectrum Technologies, Plainfield, USA) was used. For daily irrigation, water was added based on water deficit calculation (D) of the Fieldscout, 1 mm = approx. 7.8 ml (experiment (exp.) 1) and approx. 8.0 ml (exp. 2) for a pot with approx. 710 cm^3 (exp. 1) and approx. 750 cm^3 (exp. 2) volume according to the formula of truncated cones. The water contained 0.25% Wuxal Top N fertilizer (Aglukon, Düsseldorf, Germany). Five plants per treatment were grown for every genotype with a distance of approx. 7.5 cm (exp. 1) and approx. 35 cm (exp. 2) between the pots.

Growth parameters

The growth parameters plant height, leaf size, biomass and plant water content (PWC) were determined. The plant height was measured with a folding yardstick; thereby the length of the longest shoot was used. The leaf of *L. purpureus* is divided into three leaflets. Instead of the leaf area, the approximate leaf size was calculated measuring the length of the paired leaflets and the terminal leaflet plus petiole (between paired leaflets and terminal leaflet). Afterwards, both values were multiplied to obtain the leaf size. The fresh and dry weight for biomass and PWC were determined by harvesting above-ground plant material, which was then dried in an incubator at approx. 110°C for 48 h. Plant material was weighed before drying for fresh weight (FW) and after drying for dry weight (DW). For biomass data DW was used. PWC was calculated using the formula PWC = (FW - DW)/ FW * 100.

Measurements for quantification of the drought stress effects

The effect of drought stress was examined by porometry, thermal imaging measurements and PAM-imaging. Porometry and thermal imaging measurements were done on attached leaves as non-destructive methods. Stomatal conductance (mmol m^{-2} s^{-1}) of leaves was determined by the use of the porometer AP4 (Delta-T Devices, Burwell, UK) in the morning. The measurements were performed on either of the two youngest fully expanded leaves, wherein only the higher value was used for further calculations (exp. 2) or one leaf was measured over the entire experimental period (exp. 1).

Thermal imaging investigation was carried out with the camera T360 (FLIR Systems, Wilsonville, USA) in accordance with GRANT et al. (2006) in the afternoon. To ensure consistent measurements, the camera was turned on at least 30 min before taking the first picture. The youngest fully expanded leaves were examined. One leaflet of the paired leaflets of a leaf was used as dry (T_{dry}) the other one as wet (T_{wet}) reference. Dry reference leaflets were covered with petroleum jelly and wet reference leaflets were wetted with water on both sides. The terminal leaflet of the same leaf was used as sample (T_{leaf}). T_{dry} was measured at least five min after the application of petroleum jelly, T_{wet} immediately after using the water. In addition to T_{leaf} a stomatal conductance measurement was performed on the terminal leaflet. The thermal imaging pictures were analyzed using the software FLIR QuickReport 1.2 SP2 (FLIR Systems, Wilsonville, USA). The object parameters were set for each image to emissivity 0.95, reflected apparent temperature 23°C, atmospheric temperature 23°C, relative humidity 45%/50% and distance 0.2 m. Based on the results the index I_G was calculated using the formula $I_G = (T_{dry} - T_{leaf})/ (T_{leaf} - T_{wet})$. Additionally the crop water stress index (CWSI) was calculated from $CSWI = (T_{dry} - T_{leaf})/ (T_{dry} - T_{wet})$.

Whether there is an influence of drought stress on photosynthesis was investigated by chlorophyll fluorescence using an Imaging PAM M series device and ImagingWin v2.32 software (Heinz Walz, Effeltrich, Germany). The measurements were performed either on a young fully expanded leaf (using cut off leaves; exp. 2) or one leaf was measured over the entire experimental period (using attached leaves; exp. 1) in the morning. For analysis of the photosynthetic system light curves were analyzed as presented by the manufacturer. Through the use of the filter plate IMAG-MAX/F the effective PAR values are about 15% lower. Before the measurement, the plants were dark adapted for 20 min. The parameters F_v/F_m (maximal PS II quantum yield), Y(II) (effective PS II quantum yield), Y(NPQ) (quantum yield of regulated energy dissipation), Y(NO) (quantum yield of non-regulated energy dissipation), NPQ/4 (non-photochemical quenching/4) and ETR (electron transport rate) were analyzed (for background information: BAKER, 2008; SPERDOULI and MOUSTAKAS, 2012). F_v/F_m values were obtained from the false-color images created by ImagingWin software. ETR values were determined using a mean value of PAR 396-801 µmol quanta m^{-2} s^{-1}. The other parameters were analyzed based on the PAR 396 (approx. growth light intensity) and 801 (approx. twice the growth light intensity) results.

Statistical analysis

All statistical analyzes were conducted with R 2.15.2 (www.r-project.org) in combination with R Studio v0.97.248 (RStudio, Boston, USA). Box plots were drawn using ggplot2 version 0.9.3 (WICKHAM, 2009). Significant differences (p <0.05) were determined by the Welch t-test analysis.

Results

Development of classical growth parameters under drought stress

The classic growth parameters, gain in biomass DW, plant water content, leaf size (exp. 1) and plant height growth (exp. 2) were analyzed (Fig. 1, 2). Drought stress results in a decrease of biomass DW by slower growth in stressed plants (Fig. 1A, 2A). In experiment set up 1 HA-4 was the least affected genotype among the groups. The strongest decrease in exp. 2 was found for genotype CPI 52508 (cg: 5.9 g; dg: 4.7 g), HA-4 showed no impact of drought stress (cg: 4.3 g; dg: 4.3 g). Significant differences (p<0.05) between stressed and unstressed plants for biomass dry weight measurements were found for CPI 36903 and CPI 52508. There is a reduction in plant water content under drought stress conditions, too (Fig. 1B, 2B). Significant differences (p<0.05) among the groups occurred only in genotype CPI 52508 exp. 2 (cg: 79.7%; dg: 78.3%). Generally, there

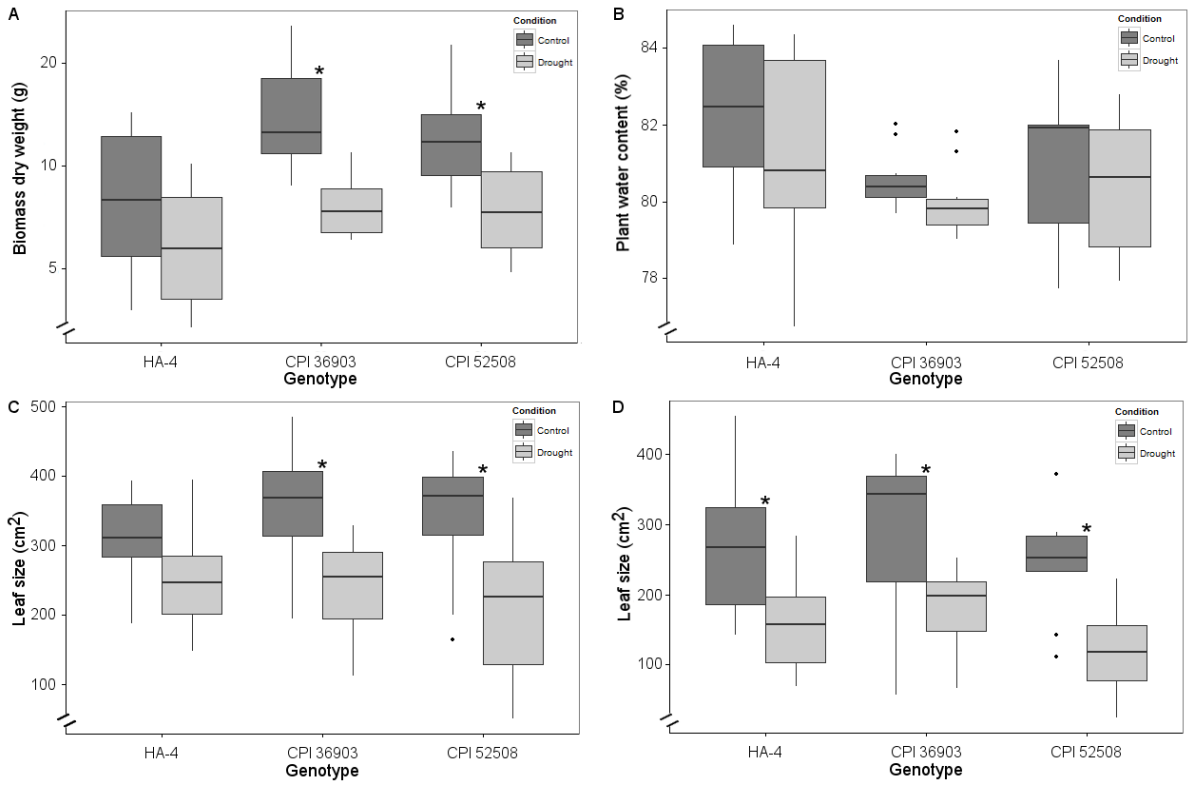

Fig. 1: Effects of water limitation after 15 days on (A) biomass dry weight (g), (B) plant water content (%) and after eight days on (C) size older leaf (cm^2) and (D) size younger leaf (cm^2); n=10, genotypes with * = $p<0.05$, exp. 1.

were only small changes in the drought stress plants in comparison to unstressed plants. There was also a considerable decrease in leaf size and plant growth, in the case of drought stressed plants. The strongest difference in leaf sizes between both groups (Fig. 1D) was found in CPI 52508 (cg: 253 cm^2; dg: 118 cm^2), HA-4 showed a smaller difference (cg: 267 cm^2; dg: 157 cm^2). The reduction of leaf size under drought stress is stronger in younger leaves (Fig. 1D) compared to older leaves (Fig. 1C). The decrease was in younger leaves between 21% (HA-4) and 39% (CPI 52508) and in older leaves between 41% (HA-4) and 53% (CPI 52508). The height growth of the plant was affected particularly in CPI 36903 (cg: 47%; dg: 31%). The slightest effect was found in HA-4 (cg: 31%; dg: 23%). However, it must be considered that *L. purpureus* is a twining plant and thereby the measurement of plant height to obtain the growth rate was impaired. In general, a large mean variation especially of the controls can be observed although the seed material was homogenous and also the plantlets had a homogenous phenotype. The conditions in the greenhouse might have local maxima and minima resulting in a large mean deviation, based on single measuring points collected from five different plants.

Determination of leaf conductance

The leaf conductivity was measured in the morning by porometry (Fig. 3). Drought stress led to a reduction in leaf conductance. In summary, the lowest difference between control and drought group showed CPI 52508 (exp. 2 - cg: 220 mmol m^{-2} s^{-1}; dg: 141 mmol m^{-2} s^{-1} (Fig. 3B)) in both experiments. HA-4 was most affected by drought stress (exp. 2 - cg: 212 mmol m^{-2} s^{-1}; dg: 84 mmol m^{-2} s^{-1} (Fig. 3B)). CPI 36903 behaved uneven; in exp. 1 the differences among the groups are similar to HA-4 and with larger space available per plant similar to CPI 52508 in exp. 2. Significant differences ($p<0.05$) among drought stressed and unstressed plants were found for all genotypes.

Analysis of the impact of drought stress on *L. purpureus* by infrared thermography

The influence of drought stress on surface temperature of plant leaves was analyzed by using an infrared thermography camera (Fig. 4). Drought stress leads to an increase in leaf temperature by closed stomata (Fig. 4A). Only HA-4 (cg: 30°C; dg: 28.8°C) showed a significant difference ($p<0.05$) between drought stressed and unstressed plants. CPI 36903 was stronger affected by drought stress (cg: 26.2°C; dg: 24.6°C). CWSI and I_G values drop under drought stress, wherein the results of both indices were homologous (Fig. 4B and 4D). The biggest differences between the two groups were measured in HA-4, followed by genotypes CPI 36903 and CPI 52508. The CWSI decrease for HA-4 (cg: 0.39; dg: 0.05) was 88% and in comparison only 28% for CPI 52508 (cg: 0.28; dg: 0.20). There were no significant differences ($p<0.05$) between the groups. Leaf conductance measured during the same time to substantiate the results of the infrared thermography camera showed lower values under drought stress (Fig. 4C). Drought stress led to a large decrease for CPI 36903 (cg: 158 mmol m^{-2} s^{-1}; dg: 44.5 mmol m^{-2} s^{-1}), the decreases for HA-4 and CPI 52508 were also above 50%. The leaf conductance confirms again that CPI 52508 was least affected by drought stress.

Measurement of various chlorophyll fluorescence factors during drought stress

Chlorophyll fluorescence was examined by factors ETR, F_v/F_m, NPQ/4, Y(II), Y(NPQ) and Y(NO) through a light curve (Fig. 5, 6).

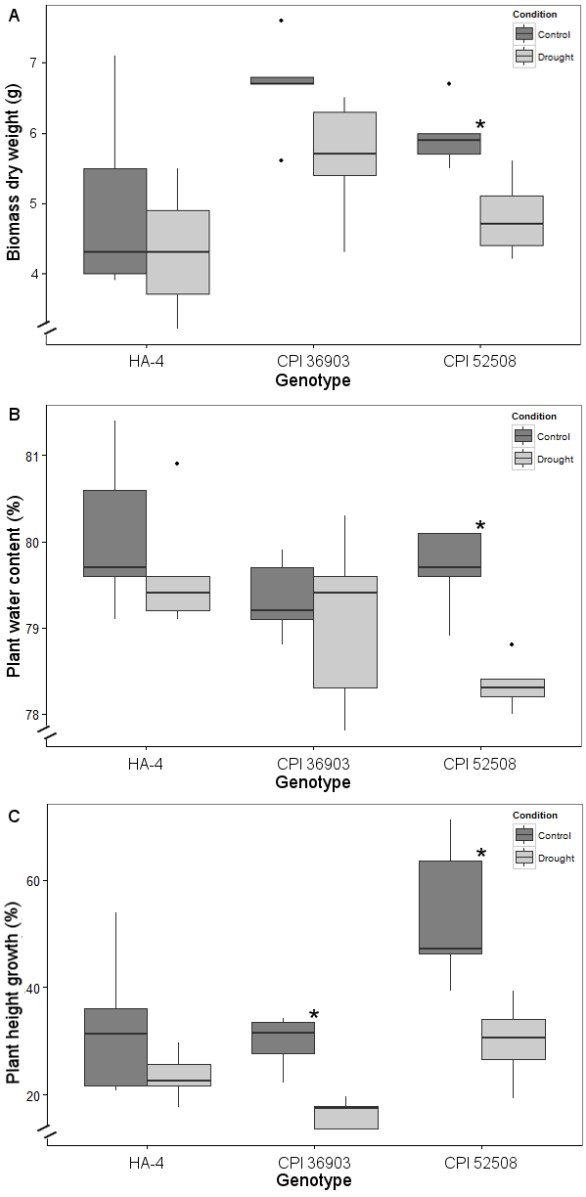

Fig. 2: Effects of water limitation after nine days on (A) biomass dry weight (g) (n=5), (B) plant water content (%) (n=5) and (C) plant height growth (%) (n=4-5); genotypes with * = $p<0.05$, exp. 2.

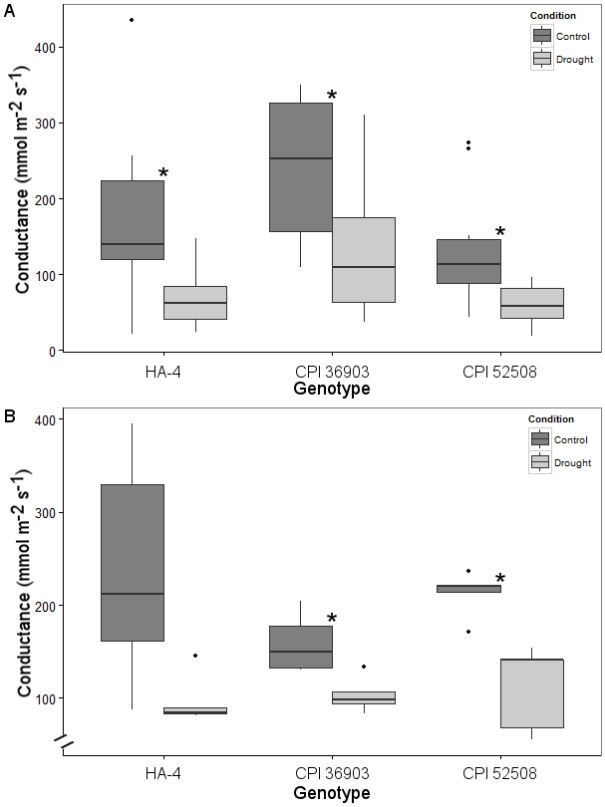

Fig. 3: Effects of water limitation after eight days on stomatal conductance through porometry in mmol m^{-2} s^{-1} (A) exp. 1 (n=10) and (B) exp. 2 (n=5); genotypes with * = $p<0.05$.

F_v/F_m showed no notable differences between control and drought groups for all genotypes (Fig. 5B, 6B), except a drought group increase for CPI 36903 in exp. 2 (cg: 0739; dg: 0788). Stress leads to F_v/F_m reduction. Drought stress caused also a decrease in rate of electron transport (Fig. 5A, 6A). ETR decreased under drought stress especially in HA-4 (exp. 1 - cg: 60.28; dg: 44.37; exp. 2 - cg: 63.75; dg 61.82), an increase under drought stress was found for CPI 36903 (exp. 1 - cg: 49.03; dg: 57.44)17%; exp. 2 - cg: 53.18; dg: 54.88). Factor NPQ/4 increased under stress conditions (Fig. 5C, 6C). Also that was particularly apparent for HA-4 (exp. 1 - cg: 0.345; dg: 0.453; exp. 2 - cg: 0.341; dg 0.384). Drought stress indicates a decrease in Y(II) and thereby a change in Y(NPQ) and Y(NO) (Fig. 5D, 6D). HA-4 was again most negatively affected by drought stress. Generally, there were only significant differences in CPI 36903. Based on the results CPI 36903 tends to be classified between the other two genotypes with respect to drought tolerance. HA-4 has a lower tolerance to drought. Overall, the results of chlorophyll fluorescence were very inconsistent and provide only evidences for drought tolerant genotypes.

Result summary of the different drought stress measurement techniques

Tab. 1 summarizes the results of the stress methods used in this study. A strong negative influence of drought stress on the genotype of a species is represented by a low score. A high score indicates that the influence of drought stress on the genotype is low. Genotypes with high scores are therefore more drought tolerant in comparison to the others. HA-4 (values 6 in the scale) appears to be the least drought tolerant genotype, CPI 36903 (8) is slightly more drought tolerant. CPI 52508 (10) is the most drought tolerant genotype of the three genotypes used in this study. It is noticeable that HA-4 is the most tolerant genotype, if the classical growth parameters are used. In the other methods HA-4 is the least adapted genotype. The results for CPI 52508 are exactly the opposite.

Discussion

The effectiveness of the determination of traditional growth parameters relating to drought tolerance investigations

The determination of classical growth parameters, e.g. leaf area measurements, is helpful in the screening of drought tolerance (Jones, 2007). The impact of drought leads to a reduced growth of plants. The limited availability of water leads to reduced turgor and restrictions in mitosis. This results in a lower rate of cell division and elongation, and therefore in reduced growth (Farooq et al.,

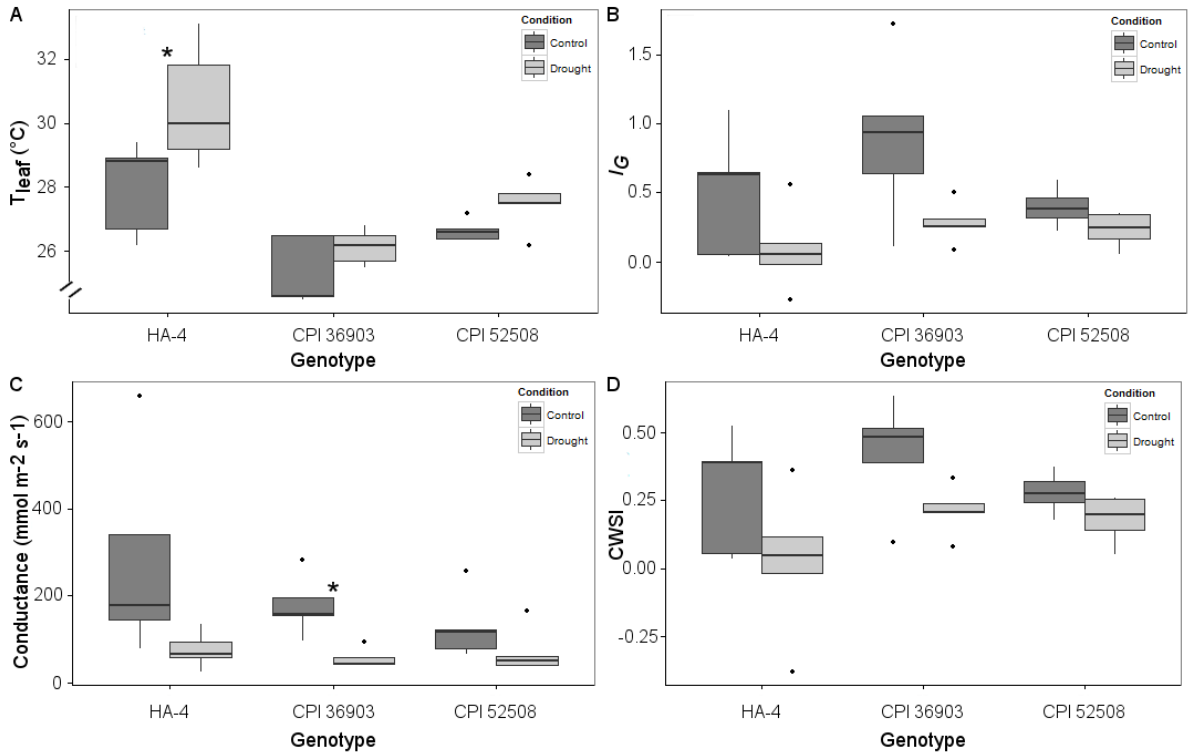

Fig. 4: Effects of water limitation after eight days on (A) leaf temperature (°C), (B) index I_G, (C) stomatal conductance (mmol m^{-2} s^{-1}) and (D) crop water stress index (CWSI) based on infrared thermography analysis; n=5, genotypes with * = p<0.05, exp. 2.

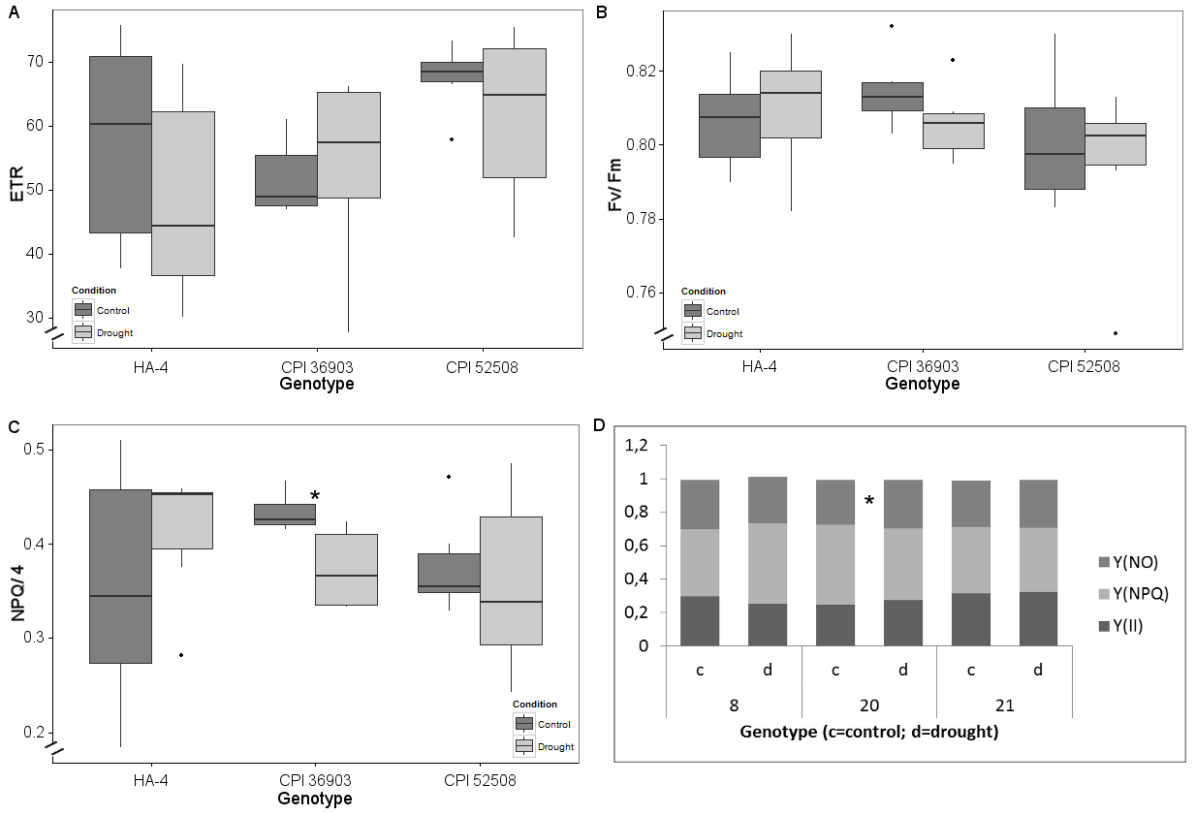

Fig. 5: Effects of water limitation after eight days on (A) ETR, (B) Fv/ Fm and under LL conditions through chlorophyll fluorescence measurements (C) NPQ/4, (D) Y(II) Y(NO) Y(NPQ); n=6, genotypes with * = p<0.05, exp. 1.

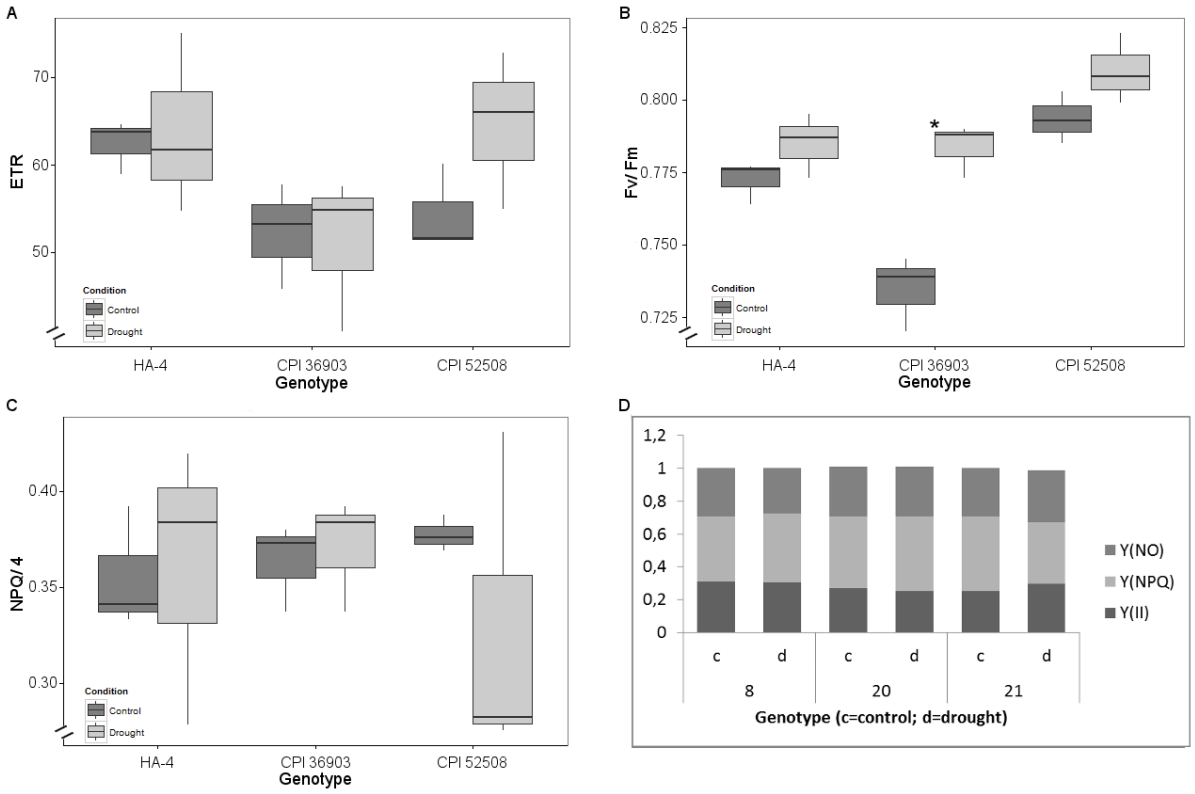

Fig. 6: Effects of water limitation after nine days on (A) ETR, (B) Fv/ Fm and under LL conditions through chlorophyll fluorescence analysis (C) NPQ/4, (D) Y(II) Y(NO) Y(NPQ); n=3, genotypes with * = $p<0.05$, exp. 2.

Tab. 1: Result summary of used stress methods. In this case each genotype is assigned to a value of one to three. Three corresponds to a low negative influence and one represents a strong negative influence of drought for the pants. Genotypes with similar values get the same rating.

Genotype	Growth parameters	Leaf conductance	Infrared thermography	Chlorophyll fluorescence	Sum
HA-4	3	1	1	1	6
CPI 36903	2	2	1	3	8
CPI 52508	1	3	3	3	10

2009). Biomass, PWC, leaf size and plant height decreased under drought conditions in *L. purpureus*. Especially genotypes CPI 36903 and CPI 52508 indicated significant differences between the control groups and drought treatment groups. Leaf size and plant height measurements combine the advantages of being non-destructive methods; biomass and PWC determination belong to the destructive methods. Reduction in leaf dry weight and stem dry weight, plant height and leaf area was already observed in faba bean (*Vicia faba* L.) under drought stress conditions (Zabawi and Dennett, 2010). A study on the effects of drought stress at different growth stages of the mung bean (*Vigna radiata* L.) also showed a reduction in plant height. Drought stress affected the measured plant height in different magnitude depending on the growth stage (Ranawake et al., 2011). A comparison of two common bean (*Phaseolus vulgaris* L.) varieties, one heavily influenced and the other one less affected by drought stress in yield, was done. A stronger decrease in relative water content and relative growth rate was shown in the variety that was more influenced by drought stress (Lizana et al., 2006). In conclusion, the results suggest that traditional growth factors can help to find drought tolerant genotypes in *L. purpureus*. But some of the techniques belong to the destructive methods. Furthermore results of the classical growth parameters reflect the impact of drought stress on the plant as a whole. It is therefore useful to study further non-destructive and non-invasive methods according to their suitability to detect drought tolerance and indicate specifically the impact of drought on the plants. For this we tested the turgor pressure probes for non-invasive online-monitoring of the water relations of intact leaves Zimmermann et al. (2008). However, problems arose in the operation of this system in terms of *L. purpureus*: the leaves were injured because they are too thin and grow very fast. With respect to genotype screening a lot of probes are required or the probes need to be repositioned frequently. This is in contrast to the aim that methods should allow a fast high-throughput screening of genotypes.

Infrared thermography as a valuable addition to the measurement of stomatal conductance

Stomatal conductance and infrared thermography are techniques that can be useful in an analysis of drought tolerances (Jones, 2007).

Under drought stress conditions stomata closure leads to a reduction of water loss for the plants. Conductivity and leaf temperature measurements allow the observation of stomata behavior. Both are non-destructive methods. Stomata conductivity measurements with a porometer have the disadvantage that the measurement is possible only punctually on a leaf. This results in a large mean variance of the data (Fig. 3). In contrast, it is possible to investigate the stomata behavior and thereby temperature changes of a leaf or even of complete plants with infrared thermography. The differences in stomatal conductance between drought and control groups in *L. purpureus* are significant. The differences in T_{leaf} measurements were significant, the thermal indices *I*g and CWSI showed non-significant differences between stressed and unstressed plants in *L. pupureus*. A study by GRANT et al. (2006) proves that the results of stomatal conductance measurements using a porometer correlate with the results of infrared thermography indices. For this grapevines (*Vitis vinifera* L.), french beans (*P. vulgaris*) and lupins (*Lupinus albus* L.) were examined. For *P. vulgaris* differences between well watered plants and drought stress plants were found for T_{leaf} and the thermal indices I_G and CWSI. It is suggested that measurements of T_{leaf} are probably sufficient for the comparison of different genotypes. For the calculations of the thermal indices I_G and CWSI measurements of the minimum temperature for a leaf (in this study T_{wet}), and the maximum achievable leaf temperature (in this study T_{dry}) is required at the same time as T_{leaf} measurements. The consequence is that the thermal indices are less susceptible to fluctuations in ambient conditions over a specific time period (IDSO et al., 1981; JONES, 1999). Otherwise, any changes in surface temperature may originate from changing environmental conditions. Thus, the thermal indices I_G and CWSI should be used to observe the stomatal behavior in long time experiments (GRANT et al., 2006). In conclusion, measurements of stomatal conductance in combination with T_{leaf} are the best for the identification of drought tolerant genotypes.

The suitability of chlorophyll fluorescence in drought tolerance screenings

Under mild to moderate drought stress, the closing of the stomata is the main reason for changes in photosynthesis as summarized by MEDRANO et al. (2002). The analysis of chlorophyll fluorescence in both experiments led to no meaningful results. The measurements of F_v/F_m, ETR, NPQ/4, Y(II), Y(NO) and Y(NPQ) showed almost no significant differences. Some of the drought stressed groups showed even better values in comparison to the control groups of the same genotypes. For example, F_v/F_m is considered as fast-measuring factor in the case of stress for plants. For non-stressed C_3 plants values of about 0.83 (BJÖRKMAN and DEMMIG, 1987) are expected. These approx. values were obtained with one exception in the analysis of the two experiments done here by both control groups and drought groups of the genotypes. Chlorophyll fluorescence does not appear to be sensitive enough to detect early symptoms of drought stress, at least in *L. purpureus*. This assumption is supported by a study with *Arabidopsis thaliana* (L.) Heynh. plants. Here, drought stress was initiated by complete withheld of water. A change in the measured values of F_v/F_m, NPQ and Y(II) occurred only after long-term (more than ten days) drought stress. ETR and Y(NO) measurements behaved similarly, at first there was no impact of stress and then both factors reflected strong signs of stress (WOO et al., 2008). Only a slightly decrease of F_v/F_m was found for *P. vulgaris* seven days after stopping irrigation (MIYASHITA et al., 2005). Another study with *A. thaliana* compared the behavior of the photosynthetic system under mild, moderate and severe drought stress. In this experiment, water was withheld until the soil water content reached 66-68% for mild drought stress, 50-52% for moderate drought stress and 43-45% for severe drought stress in comparison to the soil water content of the control group. Severe drought stress caused the strongest changes in comparison to the control group. But mild drought stress led in comparison to moderate drought stress to larger photosynthesis modifications. It was concluded that the response of the plant matches with the "Threshold for Tolerance Model" (SPERDOULI and MOUSTAKAS, 2012). According to this model, tolerance mechanisms are started with lag time or induced by threshold concentrations (BARCELÓ and POSCHENRIEDER, 2002). Moderate stress caused less damage to the plant, because stress adaptation processes and repair mechanisms started in the plant whereas during mild drought conditions the stress threshold was not reached. Therefore, the plants were more affected under mild drought stress reflected by stronger altered chlorophyll fluorescence values in comparison to the moderate group (LICHTENTHALER, 1998; SPERDOULI and MOUSTAKAS, 2012). The results of our study showed only small differences in ETR, F_v/F_m, NPQ/4, Y(II), Y(NO) and Y(NPQ) between the control and drought stress groups indicating a moderate stress level of the plant. Important for the characterization of drought tolerance in different genotypes is therefore the correct strength of drought stress, then chlorophyll fluorescence measurements might be used efficiently. New measurement protocols could provide more reliable data in early symptoms of drought stress on the photosynthetic system. BURKE et al. (2010) measured F_v/F_m at two time points by harvesting leaf punches. The chlorophyll fluorescence measurement thereby loses the advantage of being a non-destructive method. In summary, measurements of chlorophyll fluorescence in the case of *L. purpureus* are not advised without reservation. This is demonstrated by the measurement results: the differences between the unstressed and stressed plants are usually too low in order to make statements about the drought tolerance of the tested genotypes.

Conclusion

For the screening of drought-tolerant genotypes under moderate drought stress traditional methods such as leaf size measurements and biomass determination as well as new techniques like infrared thermography are suitable. Chlorophyll fluorescence is only appropriate to examine the impact of severe drought stress conditions or in recovery experiments. Because *L. purpureus* is a twining plant, the measurement of the more traditional growth parameter plant height is difficult. Overall, the combination of several methods is recommended. Based on the results a combination of infrared thermography and porometer measurements in conjunction with traditional growth parameter like biomass investigations is advisable for *L. purpureus* under greenhouse conditions. These methods are also suitable for other species because measurements are easy and quick to handle. Thereby the different effects of drought stress on the plant can be analyzed in order to filter out drought tolerant genotypes from a selection of genotypes. It must be considered that the selected genotypes of these greenhouse experiments have to be tested under field conditions. Finally, the yield of the required plant product of the selected genotypes in field conditions is most important for the growers.

Acknowledgements

L. purpureus seeds were kindly provided by Dr. B.L. Maass, International Centre for Tropical Agriculture (CIAT) Nairobi, Kenya, and by Dr. M.B. Gowda, University of Agricultural Sciences, Bangalore, India. We are also gratefully for valuable information on *L. purpureus* by Dr. B.L. Maass. We would like to thank the gardeners Yvonne Leye and Lutz Krüger for help in growing the plants.

References

Baker, N.R., 2008: Chlorophyll fluorescence: a probe of photosynthesis in vivo. Ann. Rev. Plant Biol. 59, 89-113.

Barcelo, J., Poschenrieder, C., 2002: Fast root growth responses, root exudates, and internal detoxification as clues to the mechanisms of aluminium toxicity and resistance: a review. Environ. Exp. Bot. 48, 75-92.

Berger, B., Parent, B., Tester, M., 2010: High-throughput shoot imaging to study drought responses. J. Exp. Bot. 61, 3519-3528.

Björkman, O., Demmig, B., 1987: Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77 K among vascular plants of diverse origins. Planta 170, 489-504.

Burke, J.J., Franks, C.D., Burow, G., Xin, Z., 2010: Selection system for the stay-green drought tolerance trait in sorghum germplasm. Agron. J. 102, 1118-1122.

Chaves, M.M., Maroco, J.P., Pereira, J.S., 2003: Understanding plant response to drought – from genes to whole plants. Funct. Plant Biol. 30, 239-264.

Chaerle, L., Leinonen, I., Jones, H.G., Van Der Straeten, D., 2007: Monitoring and screening plant populations with combined thermal and chlorophyll fluorescence imaging. J. Exp. Bot. 58, 773-784.

Chaerle, L., Lenk, S., Leinonen, I., Jones, H.G., Van Der Straeten, D., Buschmann, C., 2009: Multi-sensor plant imaging: Towards the development of a stress-catalogue. Biotechnol. J. 4, 1152-1167.

Dai, A., 2011: Drought under global warming: a review. WIREs Clim. Change 2, 45-65.

Farooq, M., Wahid, A., Kobayashi, N., Fujita, D., Basra, S.M.A., 2009: Plant drought stress: effects, mechanisms and management. Agron. Sust. Dev. 29, 185-212.

Grant, O.M., Chaves, M.M., Jones, H.G., 2006: Optimizing thermal imaging as a technique for detecting stomatal closure induced by drought stress under greenhouse conditions. Physiol. Plant. 127, 507-518.

Idso, S.B., Jackson, R.D., Pinter, P.J., Jr., Reginato, R.J., Hatfield, J.L., 1981: Normalizing the stress-degree-day parameter for environmental variability. Agr. Meteorol. 24, 45-55.

Jones, H.G., 1999: Use of infrared thermometry for estimation of stomatal conductance as a possible aid to irrigation scheduling. Agr. Forest Meteorol. 95, 139-149.

Jones, H.G., 2007: Monitoring plant and soil water status: established and novel methods revisited and their relevance to studies of drought tolerance. J. Exp. Bot. 58, 119-130.

Jones, H.G., Stoll, M., Santos, T., de Sousa, C., Chaves, M.M., Grant, O.M., 2002: Use of infrared thermography for monitoring stomatal closure in the field: application to grapevine. J. Exp. Bot. 53, 2249-2260.

Lichtenthaler, H.K., 1998: The stress concept in plants: An introduction. Ann. NY Acad. Sci. 851, 187-198.

Lizana, C., Wentworth, M., Martinez, J.P., Villegas, D., Meneses, R., Murchie, E.H., Pastenes, C., Lercari, B., Vernieri, P., Horton, P., Pinto, M., 2006: Differential adaptation of two varieties of common bean to abiotic stress: I. Effects of drought on yield and photosynthesis. J. Exp. Bot. 57, 685-697.

Maass, B.L., Usongo, M.F., 2007: Changes in seed characteristics during the domestication of the lablab bean (*Lablab purpureus* (L.) Sweet: Papilionoideae). Aust. J. Agr. Res. 58, 9-19.

Maass, B.L., Knox, M.R., Venkatesha, S.C., Angessa, T.T., Ramme, S., Bruce, C. Pengelly, B.C., 2010: *Lablab purpureus* – A crop lost for Africa? Trop. Plant Biol. 3, 123-135.

Medrano, H., Escalona, J.M., Bota, J., Gulías, J., Flexas, J., 2002: Regulation of photosynthesis of C_3 plants in response to progressive drought: Stomatal conductance as a reference parameter. Ann. Bot. 89, 895-905.

Miyashita, K., Tanakamaru, S., Maitani, T., Kimura, K., 2005: Recovery responses of photosynthesis, transpiration, and stomatal conductance in kidney bean following drought stress. Environ. Exp. Bot. 53, 205-214.

Munns, R., James, R.A., Sirault, X.R.R., Furbank, R.T., Jones, H.G., 2010: New phenotyping methods for screening wheat and barley for beneficial responses to water deficit. J. Exp. Bot. 61, 3499-3507.

Ranawake, A.L., Amarasingha, U.G.S., Rodrigo, W.D.R.J., Rodrigo, U.T.D., Dahanayaka, N., 2011: Effect of water stress on growth and yield of mung bean (*Vigna radiate* L.). Trop. Agr. Res. Ext. 14, 4-7.

Rosenzweig, C., Iglesias, A., Yang, X.B., Epstein, P.R., Chivian, E., 2001: Climate change and extreme weather events. GCHH 2, 90-104.

Sperdouli, I., Moustakas, M., 2012: Spatio-temporal heterogeneity in *Arabidopsis thaliana* leaves under drought stress. Plant Biol. 14, 118-128.

The Angiosperm Phylogeny Group, 2009: An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG III. Bot. J. Linn. Soc. 161, 105-121.

Wickham, H., 2009: ggplot2: Elegant graphics for data analysis. Springer, New York.

Woo, N.S., Badger, M.R., Pogson, B.J., 2008: A rapid, non-invasive procedure for quantitative assessment of drought survival using chlorophyll fluorescence. Plant Meth. 4, 27.

Zabawi, A.G.M., Dennett, M.D.D., 2010: Responses of faba bean (*Vicia faba*) to different levels of plant available water: I. Phenology, growth and biomass partitioning. J. Trop. Agr. Food Sci. 38, 11-19.

Zimmermann, D., Reuss, R., Westhoff, M., Gessner, P., Bauer, W., Bamberg, E., Bentrup, F-W., Zimmermann, U., 2008: A novel, non-invasive, online-monitoring, versatile and easy plant-based probe for measuring leaf water status. J. Exp. Bot. 59, 3157-3167.

Address of the authors:

Sebastian Guretzki, Jutta Papenbrock (corresponding author), Institute of Botany, Leibniz University Hannover, Herrenhäuserstr. 2, 30419 Hannover, Germany.

E-mail: Jutta.Papenbrock@botanik.uni-hannover.de