Transpiration Efficiency of Tropical Maize (Zea Mays L.)

A dissertation submitted to the Swiss Federal Institute of Technology Zürich for the degree of Doctor of Natural Sciences

presented by

Karl-Heinz Camp
Dipl. Ing. Agr., Universität Kiel
born January 13, 1964
in Ratingen (Germany)

accepted on the recommendation of

Prof. Dr. Peter Stamp
Dr. Urs Schmidhalter
Dr. Christian R. Jensen

Zürich 1996
Contents

1. General Introduction .......................................................... 1
   1.1 Drought tolerance and transpiration efficiency ...................... 1
   1.2 Outline of the thesis .................................................... 2

2. Estimation of Maize Canopy Transpiration Based on Porometric
   Measurements ............................................................... 3
   2.1 Abstract ..................................................................... 3
   2.2 Introduction ............................................................... 3
   2.3 Materials and Methods ................................................... 4
       2.3.1 Technical equipment used in field and growth chamber
            experiments ................................................................. 4
       2.3.2 Growth chamber experiments ........................................ 5
       2.3.3 Field experiments .................................................... 6
   2.4 Results and Discussion .................................................. 10
       2.4.1 Deviations in transpiration rates between cuvette enclosed
            and non-enclosed maize leaves ........................................ 10
           2.4.1.1 Temperature effects ............................................ 11
           2.4.1.2 Boundary layer effects ......................................... 12
       2.4.2 A sampling design for porometer measurements to
            compare cultivars and extrapolate to canopy transpiration
            in field experiments ................................................... 13
           2.4.2.1 Contribution of adaxial and abaxial leaf surfaces
                   to whole leaf transpiration ......................................... 14
           2.4.2.2 Vertical distribution of transpiration rates ............... 15
           2.4.2.3 Diurnal course of transpiration rates ..................... 17
       2.4.3 Comparison of porometrically derived transpiration rates
            with reference methods in growth chamber and field
            experiments .............................................................. 19
       2.4.4 Variability of transpiration rates and stomatal conductance
            in the field and limits to detect genotypic differences ........ 21
   2.5 Conclusions .................................................................. 23

3. Mineral Element Content in Leaf Tissue as an Indicator of
   Transpiration and Transpiration Efficiency in Maize with
   Particular Consideration of Silicon ........................................ 25
   3.1 Abstract ..................................................................... 25
   3.2 Introduction ............................................................... 25
   3.3 Materials and Methods ................................................... 27
3.3.1 Growth conditions and experimental layout ............... 27
3.3.2 Analysis of mineral elements .................................. 28

3.4 Results .............................................................. 29
3.4.1 Pot experiments .................................................. 29
3.4.2 Field experiments ................................................ 31

3.5 Discussion .......................................................... 34

4. Growth, Stomatal Conductance, Transpiration and Transpiration Efficiency of Tropical Maize Cultivars and Responses to Drought Stress at Vegetative Stages .............. 39
4.1 Abstract .............................................................. 39
4.2 Introduction .......................................................... 40
4.3 Materials and Methods .............................................. 41
4.3.1 Layout of the experiments ..................................... 41
4.3.2 Genotypes .......................................................... 42
4.3.3 Soil ................................................................. 42
4.3.4 Meteorological data .............................................. 42
4.3.5 Cultural practices ............................................... 43
4.3.6 Soil water parameters .......................................... 43
4.3.7 Plant parameters ................................................ 44
  4.3.7.1 Dry matter accumulation and grain yield ............ 44
  4.3.7.2 Stomatal conductance and transpiration rates ....... 45
4.3.8 Statistical analyses and calculations ......................... 45

4.4 Results .............................................................. 46
4.4.1 Total shoot biomass and shoot biomass accumulation .... 46
4.4.2 Grain yield and harvest index ................................ 50
4.4.3 Stomatal conductance during vegetative growth .......... 51
  4.4.3.1 Response of stomatal conductance to soil water limitation ................ 51
  4.4.3.2 Response of stomatal conductance to atmospheric humidity .......... 53
  4.4.3.3 Genotypic variability in stomatal conductance .......... 55
4.4.4 Canopy transpiration ............................................ 60
4.4.5 Transpiration efficiency ....................................... 65
  4.4.5.1 Transpiration efficiency and water limitation ....... 65
  4.4.5.2 Genotypic variability for transpiration efficiency .... 66
4.4.6 Relations between transpiration efficiency, stomatal conductance and yield parameters ......................... 69

4.5 Discussion .......................................................... 70
4.5.1 Shoot biomass and grain yield ................................ 70
4.5.2 Stomatal conductance ........................................... 72
4.5.3 Canopy transpiration ............................................ 74
4.5.4 Transpiration efficiency under drought ...................... 74
1 General Introduction

1.1 Drought Tolerance and Transpiration Efficiency

Insufficient water supply is one of the major limitations to maize production in the tropical and subtropical regions, and the use of adapted varieties may be the only affordable option for small farmholders on rainfed cultivated areas. In the semiarid regions unpredictable spells of drought occur frequently during the growing season, hence, drought escape by choice of sowing dates or early maturing varieties is not suitable as e.g. in the Mediterranean climate with typically terminal drought events. In the range of physiological traits related to the ability of crops to cope with water stress, water-use efficiency, as an obvious determinant of growth with limited water supply, has received early scientific interest (see Briggs and Shantz 1913). While water-use efficiency in a hydrological or agronomical meaning is commonly defined as the ratio of above-ground biomass accumulation to the total water input, we will refer in the following to transpiration efficiency in a physiological meaning as the ratio of above-ground biomass accumulation to the amount of water transpired.

The efficiency of water use is of particular relevance in non-irrigated crops but nonetheless increasingly emphasized in irrigated land with respect to ecological and economic effects like groundwater depletion and increasing pumping energy costs. Water-use efficiency might be enhanced by agronomic means such as, planting dates, irrigation technique and scheduling, nutrient supply etc., but as well as by breeding, since genotypical variation for transpiration efficiency has been shown in different species (see Boyer 1996). Significant genotypic variation for transpiration efficiency in maize was found either with gas-exchange measurements or gravimetric determination of transpiration in greenhouse studies (Sobrado 1990b, Siri 1993). In the latter study transpiration efficiency under water stress varied between 3.2 and 4.2 g kg⁻¹ within 12 varieties and showed a high and positive correlation to the dry weight accumulation. This investigation has been done with potted maize seedlings. Due to methodological problems, little information is currently available about genotypic variation for transpiration efficiency and the relevance for drought tolerance under field conditions. Hence, we investigated in this thesis two aspects, which have to be considered for the improvement of drought tolerance in tropical maize by enhancing transpiration efficiency: (i) to find reliable screening methods for transpiration efficiency suitable for field experiments and (ii) to determine if there is genotypical variability for transpiration efficiency worth of being included in breeding programs.
1.2 Outline of the Thesis

Transpiration is strongly controlled by stomatal conductance at the leaf level. Due to the close link between carbon dioxide and water flux, stomatal conductance will also affect transpiration efficiency. At the whole-plant and canopy level stomatal control of both is mediated by aerodynamic conductances, which are partly dependent on morphological characteristics, like canopy structure, but mainly on environmental conditions. However, stomatal conductance and stomatal response to water deficits are potential traits for selection in a range of cultivars. At first, we will investigate the possibility and reliability of canopy transpiration estimates based on porometric measurements of stomatal conductance. The gravimetric determination of transpiration in potted plants and the transpiration determined by a soil water balance in field experiments will serve as reference methods (Chapter 2).

In the next chapter, we will study the potential of an integrative approach for estimating transpiration and transpiration efficiency by an analysis of mineral element contents in plant tissue. Based on the hypothesis that mineral elements, in particular silicon, are taken up and transported to the shoot by the transpiration stream, the concentration as the ratio of cumulated mineral uptake to biomass produced should be correlated to the transpiration efficiency (Chapter 3).

Eight tropical maize varieties, exposed to water stress at the early and late vegetative stage, were investigated in two year field experiments during the dry season in Thailand. Genotypic variability and adaptations to limited water supply in growth, stomatal conductance, transpiration, depth of water uptake and transpiration efficiency were evaluated. The prospects of a selection for stomatal conductance, depth of water uptake and transpiration efficiency for improving the drought tolerance in maize will be discussed (Chapter 4).

Leaf water relations are closely involved in the regulation of stomatal conductance. Genotypic variability in the interdependencies of leaf water status and stomatal conductance and the adaptations under drought, e.g. in the osmotic potential, will be examined with respect to their significance for improving drought tolerance of maize (Chapter 5).
2 Estimation of Maize Canopy Transpiration Based on Porometric Measurements

2.1 Abstract

Transpiration efficiency as the ratio of biomass accumulation to transpiration is an important plant attribute to produce high yield with limited water supply, but a sufficiently precise determination of transpiration of different genotypes is difficult to achieve in field experiments. In growth chamber and field experiments with well-watered and water stressed maize plants the estimation of whole plant and canopy transpiration, based on porometric measurement of stomatal conductance and leaf area determination was investigated. The porometrically determined transpiration was compared to gravimetric measurements of transpiration of potted plants. Reference transpiration in the field was calculated from a soil water balance, based on neutron probe measurements of soil water content.

The deviation in leaf and air temperature due to cuvette enclosure of leaves resulted in an overestimation of canopy transpiration of about 10% in the field. Whole plant transpiration in a maize stand could be described as a function of whole plant leaf area, the noon transpiration rate of the youngest fully developed leaf at the abaxial leaf surface, and a coefficient describing the vertical distribution of transpiration rate and leaf area. The spherical distribution of leaf area in the canopy and the cuvette temperature effect caused some limitations in the estimation of canopy transpiration under field conditions. The variability of transpiration rate was high, with CV values of 10% in well-watered plants, increasing to more than 30% in water stressed plants. Porometric measurements may hence allow detection of genotypic differences in transpiration rates in well-watered plants. For water-stressed plants, however, the method is not likely to detect potentially important genotypic differences in transpiration and, consequently, transpiration efficiency in common designs of field experiments.

2.2 Introduction

Transpiration efficiency, the ratio of biomass accumulation to the amount of water transpired, is an important plant attribute to produce high yield with limited water supply. Theoretical derivation of its relevance was given by Passioura (1977), who expressed yield as the result of the three traits: water used, transpiration efficiency and harvest index. An increase in any of these, presumably independent factors should result in a better performance of crops or varieties under drought conditions. Several authors have argued that there is little prospect in finding genotypic differences in transpiration efficiency within one species because of the close relation between
carbon assimilation and transpiration (Krieg 1983b, Sinclair et al. 1984, Jones 1993). In field experiments, the small but nevertheless potentially important genotypic differences may be masked by the high variability in the estimates of transpiration.

Over the previous years, carbon isotope discrimination has successfully been used to reveal genotypic variability for transpiration efficiency in species with C3 assimilation pathway (Farquhar and Richards 1984, Hubick and Farquhar 1989, Martin and Thorstenson 1988, Ismail and Hall 1992). However, for C4 plants like maize, the discrimination is low and a precise method to screen for differences in transpiration in the field is still lacking. Porometry is a widely used method to measure transpiration rate and stomatal conductance in the field. Modern diffusion porometers are portable, fast and easy to use. To minimize the effect of increasing humidity on the stomatal aperture during the measurement, steady-state porometers have been developed. These porometers balance the transpiration with a stream of dry air into the cuvette, so that an initially set relative humidity is maintained. Different designs of porometers have been described and possible errors due to miscalibrations and operational problems have thoroughly been discussed elsewhere (McDermitt 1990, Monteith and Campbell 1988). Objective of this study was to develop a method to extrapolate porometric measurements of stomatal conductance to whole plant and canopy transpiration. The method should be precise enough to reveal genotypic differences in transpiration, which we expected to be important in the range of 5-10%. A high capacity in order to compare a reasonable number of entries is required. The method should be applicable in well-watered and water stressed plants, since the response in transpiration efficiency to limited water supply might in particular express the genotypical adaptability. We evaluated the precision of estimated transpiration rates with respect to the level of significance for genotypic differences and suggest a sampling procedure to determine transpiration and transpiration efficiency of maize varieties in field experiments.

2.3 Materials and Methods

2.3.1 Technical Equipment and Procedures Used in Field and Growth Chamber Experiments

All porometric measurements were conducted with a LI-1600 steady-state porometer (Li-Cor, Lincoln, Neb. USA). Leaf temperatures were measured with thermocouples (alumel/chromel 0.2 mm), air temperature and humidity with a portable thermometer/hygrometer device (Rotronic AG, Zürich, Switzerland). Thermometers and thermocouples were equalized with a temperature calibrator (Jofra Calibrator D40, Ametek Instruments, Farum, Denmark).

Leaf boundary layer conductances within the canopy were estimated using leaf replicas of double folded wet filter paper (Whatman No.4) connected to a water reservoir and mounted on a tripod. Transpiration rate $E$ (mmol m$^{-2}$ s$^{-1}$) was determined
gravimetrically and boundary layer conductance $g_{bi}$ (mmol m$^{-2}$ s$^{-1}$) was calculated from:

$$g_{bi} = \frac{P \cdot E}{(e_{wl} - e_{wa})},$$

(2.1)

where $e_{wl}$, $e_{wa}$ and $P$ are leaf and air water vapour pressure and atmospheric pressure (kPa), respectively. Leaf and air water vapour pressure was calculated from leaf and air temperatures and relative air humidity. Boundary layer conductance for the cuvette enclosed leaf segment was determined analogously.

### 2.3.2 Growth Chamber Experiments

Maize plants were grown with one plant per 1.5 L pot up to the fifth to sixth leaf stage in growth chambers (400 μmol m$^{-2}$ s$^{-1}$ PPFD, 12 h photoperiod, 24/20°C day/night temperature, 50/80% day/night rel. humidity). The soil was illitic-chloritic silt loam (fine mixed mesic Aquic Ustifluvent) (Schmidhalter et al. 1994). For half of the pots watering was withheld nine days after planting at the three leaf stage to induce variability in transpiration. The pots were covered with polyethylene foil to avoid evaporation from the soil. Plants were widely spaced in the growth chamber to prevent gradients of light intensity and humidity at different leaf levels. During porometric measurements the exhaled air of the experimenter was ejected out of the growth chamber to prevent stomatal closure and decreased transpiration rates due to an increase in CO$_2$ concentration in the growth chamber (Camp and Schmidhalter 1994).

### Determination of transpiration from adaxial and abaxial leaf surfaces

For the gravimetric determination of one-sided leaf transpiration rates, all but one leaf of maize plants were wrapped with aluminium foil to prevent transpiration. We measured then gravimetrically the transpiration rate of the single uncovered leaf and afterwards the transpiration of the leaf with either one of the leaf surfaces sealed with varnish, each period lasting for two to three hours. The porometric measurements were done for both leaf surfaces on the same leaf. The dimensionless factor $C_{ab}$, relating the abaxial transpiration rate to whole leaf transpiration was calculated from:

$$C_{ab} = \frac{E_{ad} \cdot E_{ab}}{E_{ab} \cdot E_{ad}} \times \frac{E_{ad}}{E_{ab}}$$

(2.2)

where $E_{ad}$ and $E_{ab}$ are the relative contribution of adaxial and abaxial transpiration to whole leaf transpiration rate.

### Comparison of porometric and gravimetric transpiration rates

During a period of three to five hours transpiration rate of the abaxial leaf surface
was measured three times with the porometer on all leaves except the oldest two, which were too small to cover the aperture of the cuvette. Additionally, transpiration was measured gravimetrically by weighing the pots at the beginning and the end of the experiment. Leaf area was determined from ruler measurements of length and maximum width of single leaves, using a factor of 0.7 to calculate leaf area. This factor was determined in previous experiments under the same growth conditions by destructive leaf area measurements, using a leaf area meter (Li-3000 A, Li-Cor, Lincoln, Nebraska, USA). An total mean of all measured transpiration rates of abaxial leaf surfaces per single plant (9 to 12 measurements: three times on three to four leaves) was used to calculate whole plant transpiration according to

\[ E_{\text{plant}} = \bar{E}_{ab} \cdot LA_{\text{plant}} \cdot C_{ab} \]  

where \( E_{\text{plant}} \), \( \bar{E}_{ab} \), \( LA_{\text{plant}} \) and \( C_{ab} \) are the transpiration rate of the whole plant (\( \text{mmol s}^{-1} \)), mean transpiration rate of the abaxial leaf surfaces (\( \text{mmol m}^{-2} \text{s}^{-1} \)), whole plant leaf area (\( \text{m}^{2} \)) and the dimensionless coefficient \( C_{ab} \), relating the abaxial leaf surface transpiration rate to whole leaf transpiration rate.

2.3.3 Field Experiments
Experimental site and layout
The field experiments were conducted at the National Corn and Sorghum Research Center, Farm Suwan, Kasetsart University in Thailand (14.5° N lat.). The climate in this area is semi-arid with low average rainfall from November to March. The soil was an ustic, isohyperthermic, kaolinitic oxisol. Clay content was 56, 85 and 88% in 5, 30 and 60 cm, respectively (Neidhart 1994). The plant available water was 12 to 15 vol.% in the range of soil matric potential from -5 to -1400 kPa, as determined from water retention curves (see Chapter 4, Fig. 4.1). Four experiments were conducted during the dry seasons 1993/94 and 1994/95. Eight tropical maize varieties and two water regimes were arranged in a split-plot design with four replications. Detailed information about the varieties and cultural practises are given in Chapter 4. A brief description is given below. Each year water stress was induced by withholding water at the early vegetative stage (early stress experiments) and in a second experiment at the late vegetative stage (late stress experiments). The control plots received weekly furrow irrigation of estimated 70 mm. Observations were started after the first irrigation in the control, that means when water was withheld on stressed plots for at least one week.

Plant parameters
Six to twelve plants per plot and week were harvested and dry matter weight of
green leaves and stem/senescent leaves were determined. Leaf area was measured with a portable, non-destructive leaf area meter (LI-3000 A, Li-Cor, Lincoln, Nebraska, USA) on the harvested plants in one replication. Specific leaf weight of green leaves was determined and green leaf area for the other replications was calculated based on this specific leaf weight.

Porometric measurements for genotypic comparisons were made from 12:00 to 13:00 h in 1993/94 and from 12:00 to 14:00 h local time in 1994/95. Sampling techniques were as follows: (S1) In 1994/95 we measured on two genotypes in the early stress and on four genotypes in the late stress experiment with six measurements per plot in four replications, four to six days per week. (S2) In the early stress experiment 1994/95 six genotypes were measured with two measurements per plot in three replications on four to six days per week. (S3) In 1993/94 we measured eight genotypes with one replication per day at four to five days each week in the early and late stress experiments. The factor replication was replaced in this case by a replication/day (rep/day) combination. Six measurements on randomly chosen plants were made in each plot.

Leaf boundary layer conductance was measured daily for the whole experiment during the period of porometric measurements from 12:00 to 14:00 h, and was previously determined for the porometer cuvette. Transpiration rate \( E \) (mmol m\(^{-2}\) s\(^{-1}\)) was recalculated from the stomatal conductance \( g_s \) to account for leaf boundary layer conductance in situ \( g_{bl} \) according to:

\[
E = \frac{1}{\frac{1}{g_{bl}} - \frac{1}{g_s}} \cdot \frac{\left( e_{wL} - e_{wA} \right)}{P},
\]

where \( g_{bl}, g_s, e_{wL}, e_{wA} \) and \( P \) are leaf boundary layer and stomatal conductance (both in mmol m\(^{-2}\) s\(^{-1}\)), leaf and air water vapour pressure and atmospheric pressure (both in kPa), respectively.

**Determination of vertical distribution of transpiration rate and leaf area**

The vertical distribution of transpiration rate and leaf area was determined in the field experiment at 52 DAE (days after emergence). The transpiration rate of each leaf position was measured porometrically on 15 plants of each water supply treatment arranged in three replications. The leaf area of each leaf position was measured destructively on six plants per genotype and water supply treatment in four replications. An average leaf area distribution of all genotypes in the water supply treatment was used to calculate the dimensionless coefficient \( C_{vd} \) describing the vertical distribution of transpiration. Whole plant transpiration \( E_{plant} \) can be described as a function of whole plant leaf area \( L_{A,plant} \), the transpiration rate of the youngest fully developed leaf \( E_0 \) and the coefficient \( C_{vd} \). This coefficient is the sum of the relative transpiration rates
weighted by the fractional leaf area for all leaf positions:

$$E_{\text{plant}} = E_0 \cdot L_{\text{plant}} \cdot C_{\text{vd}}$$  \hspace{1cm} (2.5)

with

$$C_{\text{vd}} = \sum_i \left( E_{\text{ref}(i)} \cdot \frac{L_i}{L_{\text{plant}}} \right)$$  \hspace{1cm} (2.6)

where $E_{\text{ref}(i)}$ and $L_i$ are transpiration rate of leaf $(i)$, relative to transpiration rate on the youngest fully developed leaf, and leaf area of leaf $(i)$, respectively.

**Soil water parameters**

Soil matric potentials were monitored at 20, 40, 60 and 80 cm soil depth using tensiometers. Volumetric water content was measured with a neutron probe (Model 503DR, CPN Co., Marneze, California, USA) in 20 cm increments down to 100 cm soil depths on 32 plots with one access tube per plot in the late stress experiment 1994/95. Measurements were made three times per week in stressed plots, in watered plots just before and one day after irrigation and additionally at DAE 53 after a rainfall event. Evaporation was estimated gravimetrically with small lysimeters (12 cm diameter, 20 cm depth), which received weekly 60 ml water in the well-watered plot treatment. Deep percolation was estimated by monitoring the soil water depletion on six experimental plots, where water loss by evapotranspiration was prevented. On this plots, the maize plants where removed and the soil surface was covered immediately after an irrigation. Soil water content was then measured for the next five days with the neutron probe.

**Statistical analysis of error margins**

Tables for analysis of variance including plot sampling are given below. Expected mean squares were derived according to Schultz (1955).

**Table 2.1** Table of analysis of variance for weekly means of transpiration rates in 1993/94, separately for each water treatment.

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>MQ</th>
<th>Expected mean square</th>
</tr>
</thead>
<tbody>
<tr>
<td>rep/day (r)</td>
<td>r-1</td>
<td>$M_Q_r$</td>
<td>$\sigma_r^2 + \sigma_{gr}^2 + \sigma_{n}^2$</td>
</tr>
<tr>
<td>genotype (g)</td>
<td>g-1</td>
<td>$M_Q_g$</td>
<td>$\sigma_g^2 + \sigma_{gr}^2 + \sigma_{n}^2$</td>
</tr>
<tr>
<td>genotype*rep/day (g-1)(r-1)</td>
<td>(g-1)(r-1)</td>
<td>$M_Q_{gr}$</td>
<td>$\sigma_n^2 + \sigma_{gr}^2$</td>
</tr>
<tr>
<td>plants within plots (n)</td>
<td>(n-1)g</td>
<td>$M_Q_n$</td>
<td>$\sigma_n^2$</td>
</tr>
<tr>
<td>Total</td>
<td>g(r-1)</td>
<td>$M_Q_n$</td>
<td>$\sigma_n^2$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2.2 Table of analysis of variance for weekly means of transpiration rate in 1994/95 separately for each water treatment with plot sampling. The repeated measurements are arranged in a split-plot design (genotype as mainplot and day as subplot factor).

<table>
<thead>
<tr>
<th>Source</th>
<th>d.f.</th>
<th>MQ</th>
<th>Expected mean square</th>
</tr>
</thead>
<tbody>
<tr>
<td>replication (r)</td>
<td>r-1</td>
<td>MQ$_{r}$</td>
<td>$\sigma^2_r + \text{no}^2_{rd(g)} + \text{ngdo}^2_{d}r$</td>
</tr>
<tr>
<td>genotype (g)</td>
<td>g-1</td>
<td>MQ$_{g}$</td>
<td>$\sigma^2_r + \text{no}^2_{rd(g)} + \text{nro}^2_{gd} + \text{ndo}^2_{rg}$</td>
</tr>
<tr>
<td>replication*genotype</td>
<td>(r-1)(g-1)</td>
<td>MQ$_{rg}$</td>
<td>$\sigma^2_r + \text{no}^2_{rd(g)} + \text{nro}^2_{gd}$</td>
</tr>
<tr>
<td>day (d)</td>
<td>d-1</td>
<td>MQ$_{d}$</td>
<td>$\sigma^2_r + \text{no}^2_{rd(g)} + \text{ndt}^2_{gd}$</td>
</tr>
<tr>
<td>genotype*day</td>
<td>(g-1)(d-1)</td>
<td>MQ$_{gd}$</td>
<td>$\sigma^2_r + \text{no}^2_{rd(g)} + \text{nro}^2_{gd}$</td>
</tr>
<tr>
<td>replication*day within genotype</td>
<td>(r-1)(d-1)g</td>
<td>MQ$_{rd(g)}$</td>
<td>$\sigma^2_r + \text{no}^2_{rd(g)}$</td>
</tr>
<tr>
<td>plants within plots (n)</td>
<td>(n-1)grd</td>
<td>MQ$_{n}$</td>
<td>$\sigma^2_r$</td>
</tr>
<tr>
<td>Total</td>
<td>ngr-1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

According to equations (2.7) and (2.8) we determined the margins of error (Gomez and Gomez 1984) $D_{93/94}$ and $D_{94/95}$ for the estimated genotypic weekly mean value of transpiration rate:

$$D_{93/94}^2 = \bar{x}^2 - \frac{\sigma^2_n}{n^2} - \frac{\sigma^2_{rd(g)}}{dr} - \frac{\sigma^2_{d}}{r}$$ \hspace{1cm} (2.7)

where

- $D$ margin of error for treatment mean estimate as a fraction of mean,
- $\bar{x}$ total mean within the water treatment,
- $n$ number of measurements per plot,
- $Z_{\alpha}$ standardized normal variate at $\alpha$ level of significance,
- $\sigma^2_n, \sigma^2_{rd(g)}, \sigma^2_{d}$ estimated components of variance,
- $r$ number of replications (94/95) or number of rep/day combinations (93/94),
- $d$ number of days per week on which measurements were made,
- $g$ number of genotypes.

The components of variance were estimated according to a restricted maximum-likelihood method (PROC VARCOMP, SAS Institute Inc. 1988). The error margin $D$ is related to the least significant difference for genotypic differences according to:
where $t_{\alpha/2}$ is the $\alpha/2$-percentile of the t-distribution with the degrees of freedom of the respective error term according to the table of analysis of variance.

2.4 Results and Discussion

2.4.1 Deviations in Transpiration Rates between Cuvette Enclosed and Non-Enclosed Maize Leaves

Water vapour loss from a leaf placed in the porometer cuvette is determined as the transpiration rate $E$ by measuring the flow rate of dry air, which is necessary to maintain a constant relative humidity inside the cuvette. The ambient relative humidity is commonly used as the reference null-point. Leaf and air temperature and relative humidity are measured and water vapour pressure is calculated for leaf and air assuming 100% humidity for the leaf intercellular space. Stomatal conductance $g_s$ can be calculated from the equation (2.4). Deviations between the cuvette and the non-enclosed leaf environment in temperature and humidity gradients, wind speed, and eventually perceived radiation will lead to errors when we extrapolate measured transpiration rates for non-enclosed leaves.

Assuming that stomatal conductance is not affected by placing the leaf into the porometer cuvette, transpiration rates for non cuvette enclosed leaves can be recalculated from air humidity, leaf and air temperature and boundary layer conductance for the non cuvette enclosed leaf. However, in field experiments monitoring of leaf and air temperature and humidity gradients independently of the porometer is impractical for a huge number of measurements. Therefore quantitative information about the possible deviations under field conditions is needed.

Alterations in stomatal aperture, mostly as stomatal closure in response to the enclosure of the leaf into the ventilated cuvette, has been observed (McDermitt 1990). Stomatal closure is evidenced during the measurement by the inability to stabilize the flow of dry air into the cuvette although the reference humidity is reached. We observed stomatal closure for the cuvette enclosed leaf of maize plants occasionally in growth chambers, but seldom in the field. The frequent experience of strong winds and mechanical stress in the field might cause an adaptation. The ability of hardening in this respect has not been systematically investigated, but for the growth chamber grown plants the increased ventilation in the porometer cuvette was probably much more exceptional than for field grown maize plants. For the older and mature leaves in maize the higher frequency of hairs might also be a factor to explain the lower sensitivity in comparison to growth chamber grown maize plants. Hairs are an effective way to
increase boundary layer thickness and hence may reduce stomatal responses to fluctuations in humidity and wind speed (Schuepp 1993).

2.4.1.1 Temperature Effects

McDermitt (1990) showed relative errors in estimated stomatal conductance as functions of errors in leaf and air temperature. Estimated stomatal conductance is particularly susceptible to errors in the leaf to air temperature gradient. Therefore, temperature differences between cuvette enclosed and non-enclosed leaves were measured under field conditions on several days during the experiment. Air and leaf temperatures were increased by 2-3°C for porometer enclosed leaves, with comparatively small effects on the measured leaf to air temperature gradient (Fig. 2.1 A, B, C). For severely stressed plants, when leaves were much warmer than the surrounding air (ΔT > 2°C), the effective temperature gradient was lowered after enclosure into the porometer cuvette. Due to the higher temperatures in the cuvette the leaf to air water vapour pressure gradient was higher for cuvette enclosed leaves. Independent of the water supply the gradient was overestimated by about 10% using the temperatures measured by the porometer (see Fig. 2.1 D). According to equation (2.4) the transpiration rate is proportional to the leaf to air water vapour pressure gradient, hence we must expect for the investigated measurements an overestimation of the transpiration rate by the same extent.

Idso et al. (1988) reported much higher deviations of temperature gradients for cuvette enclosed and non-enclosed leaves and the deviation increased with air saturation deficit. Our observations were made in a range of saturation deficits from 1.8 to 3.2 kPa, but we neither detected a significant effect of the saturation deficit in the air nor of the leaf to air water vapour pressure difference. McDermitt (1990) emphasized the possible warming of the cuvette during continuous use in full sun. Before measurements were taken the porometer cuvette was allowed to equilibrate with air temperature and humidity within the canopy. Nevertheless, the cuvette temperature was considerably increased by radiation load in comparison to the canopy air temperature. After equilibration no significant rise of cuvette temperatures with operating time was detected.

Hence, we conclude that the temperature deviations affect the extrapolated transpiration rate for the non-enclosed leaf environment, but do not distort the comparison of genotypes or treatments within the same experiment.
2.4.1.2 Boundary Layer Effects

Porometers are well ventilated to prevent that differences in stomatal conductance between plants are concealed by high aerodynamic resistances. Leaf boundary layer resistances can be calculated from the energy balance according to the Penman-Monteith equation (Monteith 1965), from measurements of wind speed and dimensions of the leaf (Nobel 1983), or empirically from transpiration with leaf replicas made of wet filter paper. The wet filter paper method allows one to determine analogously the aerodynamic resistances for the compared environments.

Fig. 2.1  Air temperature (A), leaf temperature (B), leaf to air temperature gradients (C) and water vapor pressure gradients (D) of maize leaves as measured in situ and enclosed in a porometer cuvette in well-watered and water stressed plants. Dashed lines indicate equality; the solid line in (D) refers to the regression.
In growth chambers we measured a boundary layer conductance for leaf replicas, which was less than one sixth of the boundary layer conductance for a leaf segment inside the cuvette (growth chamber $g_{bl} = 0.3 \text{ mol m}^{-2} \text{ s}^{-1}$, cuvette $g_{bl} = 2 \text{ mol m}^{-2} \text{ s}^{-1}$). Estimated leaf boundary layer conductance in the field was in the range of 1 to 0.5 mol m$^{-2}$ s$^{-1}$ and showed high variability for the early stage, but with the more closed canopy in the late vegetative stage both the mean and the day to day variability decreased (Fig. 2.2). In contrast to temperature effects the different boundary layer conductances resulted in a non-proportional bias in extrapolated transpiration rates. Stomatal resistance and boundary layer resistance are additive (see eq. 2.4). For a water stressed plant with rather high stomatal resistance the contribution of the comparatively low boundary layer resistance to the leaf resistance is low. Therefore, the transpiration rate for the non-chamber enclosed leaf is for a water stressed plant not severely affected by the difference in the boundary layer resistance. However, for well-watered plants with stomatal resistance being closer to the magnitude of boundary layer resistance, the in situ transpiration rate differs from that in the porometer cuvette. For both environments, growth chamber and field, the overestimation of transpiration in the observed range of stomatal conductances increased from 5% for severely stressed plants to more than 25% for highly transpiring well-watered plants. Hence, the adjustment of transpiration rates and recalculation of stomatal conductance is necessary, even if only relative comparisons are intended and not the extrapolation of transpiration rates.

For the field environment, effective aerodynamic resistance to transpiration includes additionally to the leaf boundary layer resistance an canopy boundary layer resistance to water vapour transfer. The estimation of leaf boundary layer conductance enables one to correct the transpiration for the pathway between leaf and canopy air. The extrapolated transpiration to canopy level is valid under the assumption of a constant capacitance of the canopy for water vapour, indicating that the flow through the leaf boundary layer and canopy boundary layer is continuous.

### 2.4.2 A Sampling Design for Porometer Measurements to Compare Cultivars and Extrapolate to Canopy Transpiration in Field Experiments

For comparisons of genotypes the environmental effects on transpiration rates
must be minimized. Therefore, we chose the transpiration rate for a period around noon (12:00 to 14:00 h) of a non-shaded leaf segment on the abaxial leaf surface of the youngest fully developed leaf (leafpos=0) as the definition of the reference leaf segment. Canopy transpiration might be described based on this well-defined transpiration rate assuming interrelations between transpiration from different leaf surfaces, for different levels in the canopy, and for different times of the day. The crop and experimental site specific coefficients of the above relations were determined with respect to eventual modifications under water stress. Transpiration of genotypes might differ because of different transpiration rates on the reference leaf segment, but also because of differences in spherical leaf angle distribution or ratio of adaxial to abaxial transpiration. Although these differences eventually influence the relation between measured leaf segment and canopy transpiration, the coefficients for upscaling were not systematically investigated for genotypic differences.

2.4.2.1 Contribution of Adaxial and Abaxial Leaf Surfaces to Whole Leaf Transpiration

The contribution of adaxial and abaxial leaf surfaces to whole leaf transpiration are about one third and two thirds, respectively (Table 2.3). As an average \( C_{ab} \) (eq. 2 we used 1.5 in our calculations. The porometric measurements showed a somewhat lower contribution of the adaxial leaf surface in comparison with the gravimetric method.

<table>
<thead>
<tr>
<th>Method of determination</th>
<th>adaxial leaf surface</th>
<th>abaxial leaf surface</th>
</tr>
</thead>
<tbody>
<tr>
<td>gravimetry - growth chamber</td>
<td>34.8 ± 7.7</td>
<td>65.2 ± 8.9</td>
</tr>
<tr>
<td>porometry - growth chamber</td>
<td>28.6 ± 2.9</td>
<td>71.4 ± 2.9</td>
</tr>
<tr>
<td>porometry - field</td>
<td>31.6 ± 14.0</td>
<td>68.4 ± 14.0</td>
</tr>
<tr>
<td>porometry - field, leaf inversion*</td>
<td>41.7 ± 6.0</td>
<td>58.3 ± 6.0</td>
</tr>
</tbody>
</table>

*the leaf was inverted for measurement of the adaxial leaf surface

Porometric measurement of the adaxial leaf surface requires either shading of the measured leaf segment with the porometer cuvette or inverting the leaf, what likewise means a change in the light interception of the measured leaf segment. Transpiration rate of maize leaves decreased rapidly after shading (in less than 30 s) and increased within one minute after increasing light intensity as could be shown gravimetrically (data not shown). This susceptibility of the measured leaf segment to changes in light environment might explain the reduced contribution of the adaxial leaf surface to whole leaf transpiration if it is measured in situ position, and an increased contribution if the leaf is inverted. Leaves of control plants, which had half of the upper and half of the
lower leaf surface sealed, transpired about half as much as leaves without any sealing \((49 \pm 13.5\%, \text{mean} \pm \text{one standard deviation})\). Moreover, no deleterious side effects of the sealing were found, since transpiration was constant for several hours after varnishing.

The ratio of adaxial and abaxial transpiration is well supported by anatomic investigations of stomata frequencies and dimensions of maize leaves of comparable tropical cultivars (Sin 1993). About 40% of the stomata were found on the adaxial and 60% on the abaxial leaf surface with minor changes under limited water supply (42 and 58%, respectively). Stomata were approximately 10% smaller on the adaxial leaf surface for both water supply treatments. Pospíšilová and Solárová (1980) concluded, that the ratio of adaxial and abaxial transpiration is not significantly changed under water stress for a range of species.

### 2.4.2.2 Vertical Distribution of Transpiration Rates

In a dense canopy, gradients in light interception and humidity will lead to different transpiration rates on different leaf positions. By comparing the transpiration rates at different leaf positions, accounting for the leaf area at the respective position, we can describe the vertical distribution of transpiration within a canopy. Vertical distribution of transpiration rate and leaf area for maize plants in a droughted and a non-droughted canopy is depicted in Fig. 2.3. For both treatments the transpiration rate increased with higher light interception at higher levels in the canopy. The increase was more pronounced in well-watered plants compared to water stressed plants.

With increasing water stress transpiration rate and increase of transpiration rates at higher leaf positions decreased. In severely stressed plants transpiration rates are very low except for the very youngest leaves (data not shown). The high transpiration rate of the youngest fully developed leaf \((\text{leafpos}=0)\) is due to the definition of the reference position as a non-shaded leaf segment. Calculated relative transpiration rates, relating transpiration rates on each leaf position to the transpiration rate on the youngest fully developed leaf, showed that increasing senescence led to lower relative transpiration rates of the older leaves, while relative transpiration rates were higher on the youngest leaves.

Water stress changes the architecture of the canopy by enhancing senescence of the older leaves and by depressing elongation of the growing leaves (Acevedo et al. 1979). Thirteen days after withholding water, the whole plant leaf area was not very much reduced, except the leaf area of the youngest leaves (Fig. 2.3 B). Changes in the distribution of transpiration rate and leaf area together resulted in a higher fraction of whole plant transpiration, contributed by leaves at positions around the youngest fully developed leaf. However, the pattern of the vertical distribution of transpiration was similar for both water regimes (Fig. 2.4).
Fig. 2.3 Vertical distribution of transpiration rates (A) and leaf area (B) of well-watered (ww) and water stressed (ws) field-grown maize plants at DAE 52. For water stressed plants irrigation was withheld since DAE 38. Error bars denote one standard deviation. Leaf position 0 marks the youngest fully developed leaf.

Fig. 2.4 Vertical distribution of absolute transpiration rates (A) and expressed as fractions of whole plant transpiration rate (B) in well-watered (ww) and water stressed (ws) field-grown maize at DAE 52. For water stressed plants irrigation was withheld since DAE 38. Leaf position 0 marks the youngest fully developed leaf.
We determined a $C_{vd}$ of 0.72 (see eq. 6) as a mean of both water supply treatments (in well-watered $C_{vd} = 0.75 \pm 0.05$ and in water stressed plants $0.69 \pm 0.09$, mean $\pm$ one standard deviation) at DAE 52, which was in midst of the late stress period. Vertical distribution of leaf area and transpiration rate is dynamic and has to be considered with respect to the developmental stage, and intensity and history of the water stress. The average transpiration rate of water-stressed plants was at this time decreased by 50% in comparison to the control. With more severe stress situations this coefficient may change.

Rochette et al. (1991) compared different models for the upscaling of stomatal conductance to canopy conductance. The best performing model included, besides the vertical distribution of leaf area in the canopy, the assumption of spherical distribution of leaf angles. Canopy transpiration is therefore likely to be overestimated, when the distribution of leaf area with respect to the leaf angles is not accounted for in the calculation. However, information about the actual distribution, which is likely to vary between genotypes of erect and lax leaf position, is hardly available. Including the assumption of spherical leaf angle distribution is thus not expected to fortify the comparison of genotypes.

2.4.2.3 Diurnal Course of Transpiration Rates

Comparisons of transpiration rate and stomatal conductance for a range of varieties in field experiments are hampered by fast changes of atmospheric conditions. Most studies comparing stomatal conductance or gas-exchange of genotypes or treatments in the field are conducted around mid-day because light intensity and temperature during this period of the day is rather stable.

For both years diurnal changes of transpiration rates, stomatal conductance and the meteorological parameters radiation, leaf and air temperature, and humidity were recorded from DAE 15 to 60 with different intensities of water stress to establish a relation between the transpiration rate at noon and a transpiration rate integrated over the day. Typical patterns of the diurnal course of stomatal conductance, transpiration rate, and leaf to air water vapour pressure gradient for well-watered and water stressed plants are shown in Fig. 2.5. The highest stomatal conductance was observed in the morning when the water vapour pressure difference from leaf to air was still low. Stomatal conductance decreased throughout the day for water stressed and well-watered plants. Transpiration rates showed a more symmetrical pattern, with the peak of transpiration occurring at noon. Transpiration rates of severely water stressed plants reached the maximum earlier and decreased gradually throughout the day. Diurnal courses have been established for different genotypes, but the pattern of stomatal conductance throughout the course of the day was similar (compare also Chapter 5).
Fig. 2.5 Diurnal change of stomatal conductance (A), transpiration rate (B) and leaf to air water vapour pressure difference VPD (C) of field-grown maize plants with different water supply. Error bars indicate one standard error of mean (n=6).

The whole day transpiration rates were linearly related to the noon transpiration rates. We found a unique relation independent of water supply, genotype, age of the crop and year (Fig. 2.6), which was used to calculate daily integrates of transpiration rate. No interaction of diurnal courses and canopy architecture on transpiration has been tested. Turner and Begg (1973) and Turner (1974) observed diurnal changes for leaves in all levels of the canopy, but the range was smaller for the lower levels. Since the contribution of the lower leaf levels to whole plant transpiration was relatively small, the assumption of no interaction is not likely to produce a big error in the calculation of canopy transpiration.
Fig. 2.6 Regression between transpiration rate at noon and integrated daily transpiration rate of well-watered (ww) and water stressed (ws) maize at different days (20 to 60 DAE). The experiments were conducted over two years.

2.4.3 Comparison of Porometrically Derived Transpiration Rates with Reference Methods in Growth Chamber and Field Experiments

Gravimetric and porometric determination of transpiration for maize plants in growth chambers agreed well when in the calculation was accounted for the differences in leaf boundary layer conductance between porometer cuvette and growth chamber (Fig. 2.7). For field experiments the correlation of transpiration assessed by soil water balance and measurements of noon transpiration rates for a seventeen-day-period is depicted in Fig. 2.8. Porometric transpiration is based on six measurements per plot at each of ten days during this period. The correlation revealed a systematic overestimation with porometry. Errors in the calculation of the soil water balance, especially the assumptions about water uptake from the top soil, deep percolation and/or water uptake from more than 1 m depth, may have contributed to the different estimates of transpiration in the field.

Profiles of soil water uptake indicated, at least for one genotype in the stressed treatment, water uptake from more than one meter soil depth (compare Chapter 4, Fig. 4.10). When we estimated the deep percolation decrease of both volumetric water content and soil matric potential was found for more than five days in the percolation plots. This indicates that there is no well-defined field capacity threshold in this soil.
Moreover, there was no difference in the soil water depletion under a maize canopy and the covered percolation plots for the first five days after irrigation. For calculation of the soil water balance we assumed therefore, that all water remaining in the soil 24 hours after irrigation was, although slowly draining, fully available for plant water uptake. This assumption was supported by the characteristics of the soil hydraulic conductivity. While hydraulic conductivity was high in saturated soil (11 ± 9.7 cm day⁻¹; mean ± one standard deviation), it decreased rapidly when the soil moisture decreased (see Chapter 4, Fig. 4.2). The lack of a well-defined field capacity has been observed in many soils and is a common problem in the estimation of evapotranspiration from soil water records (McGowan and Williams 1980). It is of particular relevance for rather short term observations with frequent and high water input, as the conditions have been in the well-watered treatment of our experiments.

Neutron probe measurements of soil water depletion are commonly carried out with one access tube installed per plot. The repeated measurements on this tube describe mainly the soil water uptake of the two surrounding maize plants, which have to represent the whole plot. In contrast, porometric measurements have been carried out on 30 to 50 plants throughout the experimental period. Spatial heterogeneity in the soil water depletion within one plot was revealed by measurements of soil water potential using tensiometers, where two for each depth were installed within a distance of 0.75 cm. The averaged CV for the soil matric potential between these two adjacent sites was 31%.

In calibrating the neutron probe, we found a rather strong correlation of the count ratio to bulk soil density. Since not for every plot the bulk soil density was measured, an averaging calibration curve for all plots had to be used. Most of the scatter in the
comparison of transpiration estimated by porometry and soil water balance is therefore likely to be caused by the latter method.

2.4.4 Variability of Transpiration Rates and Stomatal Conductance in the Field and Limits to Detect Genotypic Differences

Although we predefined the measured leaf segment with respect to time of day, developmental stage and position of the leaf in the canopy, the variability of transpiration rate and stomatal conductance of leaf segments was high, with CV values of 10% in well-watered plants, increasing to more than 30% in water stressed plants. This increased variability will cause a decrease in the gain of selection, when these traits will be selected for under water stress. The precision of the estimates of plot and genotypic mean transpiration rates depends on the sampling technique and the experimental layout. The number of entries that can be evaluated for transpiration is limited by the required time for measurements with rapidly changing environmental conditions in the field. Hence one has to find an optimum for the number of entries and the desired precision of estimates. We performed a statistical analysis to estimate the experimental error variance and the variance between plants within one plot. We determined the precision of the estimates of genotype means in different sampling designs, which were partly chosen on the basis of the first year results, partly because of break-down of the equipment. Since breeders are mainly interested in relative differences precision was expressed as a fraction of the mean.

Calculations were conducted for each water treatment separately, because non-homogeneity of variances was found for the water treatments. For stomatal conductance variation within plots as well as experimental error variance was higher in well-watered treatments, while for the transpiration rate the higher variance within plots was often found for water stressed plants. No heterogeneity of variances was found for genotypes, indicating that comparisons between genotypes can be done with standard procedures on original data.

The error margin $D$, as proposed by Gomez and Gomez (1984) describes the precision for the estimated means, based on experimental dimensions. Error margins of mean transpiration rates are given for all experimental periods and different sampling dimensions in Table 2.4. An error margin of 10% means that with a 95% probability the population mean transpiration rate of the genotype is within a range of $\pm$ 10% of the estimate. The LSD for the comparison of two genotypes (in e.g. sampling design (S1), including four genotypes) is then about 16 %, as approximated from equation (2.9).

Precision of estimated mean stomatal conductance was in general lower and showed similar trends for treatments and experimental periods. For water stressed plants the error margins were higher and increased with increasing stress intensity. This effect was pronounced for the late stress experiments. Decrease of precision, expressed as the fraction of mean was not primarily due to increasing variances but due to
decreasing means of transpiration rate and stomatal conductance (see Chapter 4, Fig. 4.5). The dimensions of sampling affected the precision of estimates for water stressed plants more strongly than for well-watered plants.

Table 2.4 Margins of error $D_{94/95}$ and $D_{93/94}$ in percent of the estimated genotypic mean transpiration rate for experimental periods and different sampling dimensions in well-watered (ww) and water stressed (ws) maize plants.

<table>
<thead>
<tr>
<th>sampling design</th>
<th>water treatment</th>
<th>early stress</th>
<th>late stress</th>
</tr>
</thead>
<tbody>
<tr>
<td>(S1) 1994/95</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n=6, d=4, r=4</td>
<td>ww</td>
<td>4.4</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td>ws</td>
<td>9.4</td>
<td>12</td>
</tr>
<tr>
<td>(S2) 1994/95</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n=2, d=4, r=3</td>
<td>ww</td>
<td>5.9</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>ws</td>
<td>16</td>
<td>20</td>
</tr>
<tr>
<td>(S3) 1993/94</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n=6, d/r=4</td>
<td>ww</td>
<td>6.3</td>
<td>5.3</td>
</tr>
<tr>
<td></td>
<td>ws</td>
<td>11.8</td>
<td>11.2</td>
</tr>
<tr>
<td>(S1) 1994/95</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n=6, d=4, r=4</td>
<td>ww</td>
<td>4.4</td>
<td>4.8</td>
</tr>
<tr>
<td></td>
<td>ws</td>
<td>9.4</td>
<td>12</td>
</tr>
<tr>
<td>(S2) 1994/95</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n=2, d=4, r=3</td>
<td>ww</td>
<td>5.9</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>ws</td>
<td>16</td>
<td>20</td>
</tr>
<tr>
<td>(S3) 1993/94</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n=6, d/r=4</td>
<td>ww</td>
<td>6.3</td>
<td>5.3</td>
</tr>
<tr>
<td></td>
<td>ws</td>
<td>11.8</td>
<td>11.2</td>
</tr>
</tbody>
</table>

1 n = number of measurements per plot, d = number of days per week, r = number of replications
2 d/r = number of rep/day combinations

From the definition of the error margin $D$ (eq. 2.7 and 2.8) it can be seen that the most efficient way to optimize precision for genotypic comparisons of transpiration rates is to increase the number of replications for the experiment and secondly the number of days on which measurements are conducted. Increase of the number of measurements within the plot is, as far as we are mostly interested in a precise genotype information, much less promising. Expected error margins for the genotype means of transpiration rate with different experimental dimensions based on the estimated components of variance are shown in Fig. 2.9. It is evidenced, that for reliable estimates of canopy transpiration efficiency which requires observations for a longer period of growth it is necessary to measure frequently, at best every day. However, also for experiments including six replications the error margin for water stressed plants is not likely to be smaller than 14%.

The error margin for a plot mean transpiration rate is more dependent on the number of measurements per plot. If we intend to compare water consumption on a plot level, as we have done in the comparison of soil water balance and porometry, it is however reasonable to increase the precision by a high number of measurements per plot.
2.5 Conclusions

Porometrically determined transpiration deviated only under field conditions from the transpiration assessed by reference methods. Two reasons for the porometric overestimation of canopy transpiration have been identified: in the calculation, we did not take account of the spherical leaf area distribution in the maize stand and the increased temperature in the porometer cuvette. Both are of particular relevance for field experiments. Heating of the cuvette was insignificant in the growth chamber with rather low radiation. Reliability of the correction of transpiration rates to the non-cuvette environment concerning the higher boundary layer resistance was evidenced by the high agreement between porometric and gravimetric determined transpiration rates of maize plants in growth chambers. A great fraction of radiation in a small growth chamber has been reflected at the walls. This should diminish the effect of the leaf angle distribution on transpiration rates.

Hence, whole plant transpiration can be described with measuring transpiration rates on a well-defined leaf position, since consistent coefficients for the extrapolation have been found. However, limitations exist under field conditions where we are likely to overestimate canopy transpiration by extrapolating porometric measurements. Relative comparisons of genotypes or treatments should nevertheless be reliable if transpiration rates are adjusted to the aerodynamic conductances in the field.
Porometric measurements allow the detection of genotypic differences in transpiration rates in the magnitude of 10% for well-watered maize plants. However, for water-stressed plants and common designs of field experiments with 4 to 6 replications, differences between genotypes must exceed 20% to be significant, which might be beyond reasonable expectations. With respect to the high variability found for leaf transpiration rates, the porometric method is not likely to detect potentially important genotypic differences in transpiration efficiency in field experiments.
3 Mineral Element Content in Leaf Tissue as an Indicator of Transpiration and Transpiration Efficiency in Maize with Particular Consideration of Silicon

3.1 Abstract

An integrative method of determining transpiration efficiency is still lacking particularly in C4 crops, where carbon isotope discrimination is low. Therefore, the relations between concentrations of mineral elements and HCl non-soluble ash (nHCl-ash), and transpiration and transpiration efficiency were investigated in maize in pot and field experiments. Maize plants, grown with different water supply, were analyzed for mineral elements and nHCl-ash, as an indicator of Si. Transpiration was measured gravimetrically for potted maize plants and porometrically in the field experiments including four tropical maize varieties. In the pot experiment transpiration was highly correlated with total K and Si content in the plant, but mass flow could only be assumed for Si. Transpiration efficiency was only weakly correlated to the concentration of mineral elements in the pot experiments. A high, non-linear correlation was obtained between nHCl-ash concentration in leaves and transpiration rate during a previous period of water stress in the field experiments. No genotypic differences were found in this relation, whereas K and Ca concentrations varied genotypically and transpiration rate was highly correlated to the respective concentration only within the genotypes. In contrast to nHCl-ash and Ca, the concentration of K was negatively correlated with transpiration rate. Transpiration efficiency was negatively correlated to nHCl-ash concentration on the basis of water treatment and replication means. Conclusively, the concentrations of most mineral nutrient elements in the plant tissue are not a suitable indicator of transpiration and transpiration efficiency due to the non-homogeneous distribution in the soil, the selective and specific uptake kinetics, and genotypic differences in the uptake. However, the concentration of nHCl-ash, implicitly Si, showed some potential to describe transpiration and transpiration efficiency in maize. Further evaluation of the method is needed before it can be used to evaluate transpiration efficiency for a broader range of maize genotypes.

3.2 Introduction

Estimation of transpiration is essential to describe water uptake and transpiration efficiency, and both are major factors for the evaluation of drought tolerance in a range of cultivars. Porometric measurements of transpiration are tedious, extremely time-consuming and difficult to extrapolate for longer experimental periods. Soil water
balance or sapflow measurements are often impractical in field experiments with a large number of entries. Over previous years extensive use has been made of carbon isotope discrimination to estimate transpiration efficiency. This method, which is integrative over the growing period, has revealed genotypic variability for transpiration efficiency in several plant species (Farquhar and Richards 1984, Hubick and Farquhar 1989, Martin and Thorstenson 1988, Ismail and Hall 1992). Discrimination is low for C4 plants and has not been shown to be correlated to transpiration efficiency.

A comparable integrative approach to carbon isotope discrimination may be provided by the mineral element concentration in plant tissue. Mass flow and diffusion are the main mechanisms for movements of nutrients from the soil solution to the root cell walls and to the actual place of ion uptake at the plasma membrane. Mineral elements are further transported from the root to the shoot via the transpiration driven mass flow. Therefore an effect of transpiration rate on the mineral content in the shoot is likely and may provide a means to measure transpiration. Ion uptake is characterized by selectivity and certain minerals are taken up preferentially, while others are discriminated or almost excluded (Marschner 1995). Hence expected relations of ash or mineral element concentration to transpiration depend on the plant species and ion specific kinetics of transport and uptake.

Controversial results of correlations between ash or mineral element concentration and transpiration or transpiration efficiency have been reported. Correlations between leaf mineral content and transpiration ratio were found in greenhouse studies with wheat, sunflower and sorghum (Masle et al. 1992). For oat (Jones and Handreck 1965) and wheat (Hutton and Norris 1974) high correlations between transpiration and Si uptake or concentration were reported. Mayland et al. (1991) showed partial active uptake of Si for wheat and hence concluded, that Si cannot be used to estimate water use in wheat. Stronger correlations between Si concentration and transpiration efficiency of barley in the field, as compared to the greenhouse, emphasized environmental effects on the characteristics of Si uptake (Walker and Lance 1991).

Since an integrative method of determining transpiration efficiency is of particular importance in C4 crops we investigated the potential of ash and mineral element concentration to indicate transpiration and/or transpiration efficiency of maize under well-watered and water stressed conditions in pot and field experiments. Results of poromectrically determined transpiration efficiency indicated no significant genotypic variation. Therefore, environmentally induced variation in transpiration efficiency was also investigated.
3.3 Materials and Methods

3.3.1 Growth Conditions and Experimental Layout

Pot experiments

Maize (cv. KTX 3101) was grown with one plant per 1.5 L pots containing an illitic-chloritic silt loam up to the fifth to sixth leaf stage. These pots were either placed in growth chambers (400 μmol m⁻² s⁻¹ PPFD, 12 h photoperiod, 24/20 °C day/night temperature, 60/80% day/night rel. humidity) or outdoor (except for rainy days, when they were placed in the greenhouse), where the minimum and maximum daily air temperature averaged 12.1 and 21.5°C and the minimum and maximum daily air humidity averaged 67 and 93%, respectively. In each environment water was withheld nine days after emergence for half of the pots. The pots were covered with polyethylene foil to avoid evaporation. Transpiration was measured gravimetrically. Above-ground biomass was determined 18 or 22 days after emergence (DAE). Analysis of mineral element content was conducted separately for leaves and stem and the average concentration in the plant biomass was calculated. The environmental conditions of growth were varied to create variation in transpiration and transpiration efficiency. Only the results of the correlation analysis which was carried out on single plant values, irrespective of water treatment, growth environment and age, are reported.

Field experiments

The field experiments were performed at the National Corn and Sorghum Research Center, Farm Suwan, Kasetsart University in Thailand (14.5 °N lat.), on an ustic, isohyperthermic, kaolinitic oxisol. More detailed information about meteorological data, cultural and experimental practises and genotypes are given in Chapter 4. A brief description of the experiments is given below. Two experiments were conducted during the dry season 1994/95 with four replications including two water regimes arranged in a split-plot design. In all experiments water stress was induced by withholding water for approximately four weeks at the early vegetative stage (early stress, from DAE 9 to 37) or at the late vegetative stage (late stress, from DAE 37 to 61). Observations were started after the first irrigation in the control, when water had been withheld on stressed plots for at least one week. The control plots received weekly furrow irrigation of about 50 mm. The plots included six rows, each of which were 0.75 m apart and the plot size was 36 m². All measurements were restricted to the inner four rows. The experiments included two tropical maize cultivars in the early stress experiment (KS 6 and DK 888) and four in the late stress experiment (additionally Cargill 922 and Tuxpeño seq. C₀).

Dry matter weight of leaves and stems as well as leaf area were determined weekly on 6 and 12 well-bordered plants for the well-watered and water stressed treatment, respectively. Mean leaf area and dry matter accumulation were calculated for weekly periods. Porometric measurements of transpiration rates were conducted from 12:00 to 14:00 h with a LI-1600 steady-state porometer (Li-Cor, Lincoln, Nebraska, USA). Only abaxial transpiration rates of the youngest fully developed leaf was
measured. Six measurements on randomly chosen plants per plot were performed on
four to six days per week and averaged to weekly mean transpiration rates. Canopy
transpiration was calculated as previously described (Chapter 2).

A weighted mean transpiration rate $\bar{E}$ ($\text{mmol m}^{-2} \text{s}^{-1}$) for the whole experiments,
consisting of several weeks were calculated for each genotype and water treatment to
take into account the development of leaf area and transpiration rate in time:

$$E = \frac{1}{n} \cdot \sum_{i=1}^{n} \left( \frac{LA_i}{LA_n} \cdot E_i \right)$$

where $LA$ and $E$ is the leaf area and transpiration rate of week $i$. The weight for each
weekly mean transpiration rate was the ratio of the leaf area of the respective week to
the leaf area at the end of the experimental period.

3.3.2 Analysis of Mineral Elements

Pot experiments

Ca, Cu, Fe, K, Mg, Mn, Na, P, Si and Zn concentrations were measured with an
Inductively Coupled Plasma Atomic Emission Spectrometer, ICP-AES, (Liberty 200,
Varian Australia Pty. Ltd., Mulgrave, Victoria, Australia) after wet-ashing with a modified
two-stage microwave digestion procedure (Fridlund et al. 1994). Leaf and stem
biomasses were dried at 65 °C and finely ground. A 250 mg sample was placed into a
HTFE-vessel. The organic matter was destroyed with 2 ml of concentrated $\text{HNO}_3$ and
6 ml of 30% $\text{H}_2\text{O}_2$ solution in a microwave system (MLS 1200 Mega, MLS GmbH,
Leutkirch, Germany). These samples were allowed to cool down and in a second
microwave digestion step silica was dissolved with 0.375 ml of 40% $\text{HF}$. The samples
were diluted to a final volume of 50 ml using a 2000 ppm boron solution to neutralize
excess HF.

Field experiments

Leaf and shoot dry matter were analyzed separately for B, Ca, Cu, Fe, K, Mg, Mn,
Na, P, Si, Zn and HCl non-soluble ash. The leaves of plants from the late stress
experiment were divided into the six youngest leaves, assumed to be mainly grown
under limited water supply, and older leaves. The samples were dried at 70 °C for 36
h and finely ground. To avoid problems of dust contamination in the samples, a
different analysis of mineral elements was conducted for field-grown plants. A 500 mg
dry matter sample was placed into a platin combustion vessel and ashed at 540 °C for
6 h in a muffle oven. The ash was dissolved in 10 ml $\text{H}_2\text{O}$ and 10 ml concentrated HCl,
placed on a hot water bath, until the solution had completely evaporated. The residue
was dissolved in 5 ml concentrated HCl and 25 ml $\text{H}_2\text{O}$ and filtrated through an ashfree
filter. The filter and the combustion vessel were rinsed with water and the filtrate was filled up to final volume of 100 ml. The filter was ashed in a muffle oven for 2 h at 540 °C and the residue was determined gravimetrically as HCl non-soluble ash (nHCl-ash). B, Ca, Cu, Fe, K, Mg, Mn, Na, P and Zn concentrations were measured by ICP-AES in the HCl-extract. To discriminate between endogenous amorphous or polymerized silica and contaminations with dust in form of quartz, nHCl-ash was dissolved in hot, concentrated Na₂CO₃ solution (10 g per 40 ml water) in a water bath. The solution was filtrated, the vessel and the filter rinsed and the filtrate was filled up to 100 ml after adding 0.4 ml 10% NaOH. The Si concentration of this filtrate was measured by ICP-AES. Contamination was determined as the residual between Si in the nHCl-ash, and Si measured in the Na₂CO₃-extract, and was found to vary between 0.1 and 0.7% of the dry weight (DW). However, a strong correlation for gravimetrically determined nHCl-ash and Si, measured in the Na₂CO₃-extract, provides a cheap and simple way of estimating the concentration of Si in maize dry matter samples (Fig. 3.1).

![Graph](image.png)

Fig. 3.1 Relation between gravimetrically determined HCl-non-soluble ash and Si concentration measured in a hot Na₂CO₃-extract of field-grown maize leaves.

3.4 Results

3.4.1 Pot Experiments

For potted plants correlations of mineral element uptake, as the total amount in the above-ground biomass, and mineral element concentration with transpiration and transpiration efficiency were significant for several macro- and micronutrients (Table 3.1). Since none of the micronutrients except B were related to transpiration in the field experiment, the correlations for micronutrients are not shown. The highest correlations to transpiration were found for Si and K. The linear relation with a non-significant
intercept between transpiration and total Si in the plant, indicated that Si was mainly taken up passively with the transpiration stream (Fig. 3.2). Si concentration was positively correlated with transpiration (Table 3.1). In contrast, the positive and significant intercept in the relation between transpiration and K uptake indicates that mass flow cannot describe the uptake of K sufficiently (Fig. 3.2). The same was found for Ca and P (data not shown).

**Table 3.1** Correlation coefficients of transpiration and transpiration efficiency with uptake (total amount) and concentration of mineral elements in the above ground biomass and the ratio of Si and K concentration (Si/K) for potted maize plants grown under different temperature, humidity, light and water supply (n=30, level of significance is given in brackets).

<table>
<thead>
<tr>
<th></th>
<th>Ca</th>
<th>K</th>
<th>Mg</th>
<th>P</th>
<th>Si</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Transpiration</strong></td>
<td>0.33</td>
<td>0.73</td>
<td>0.23</td>
<td>0.77</td>
<td>0.89</td>
</tr>
<tr>
<td>(0.075)</td>
<td>(0.0001)</td>
<td>(0.212)</td>
<td>(0.0001)</td>
<td>(0.0001)</td>
<td></td>
</tr>
<tr>
<td><strong>Transpiration efficiency</strong></td>
<td>0.08</td>
<td>-0.11</td>
<td>0.14</td>
<td>-0.19</td>
<td>-0.35</td>
</tr>
<tr>
<td>(0.87)</td>
<td>(0.57)</td>
<td>(0.46)</td>
<td>(0.31)</td>
<td>(0.058)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Ca</th>
<th>K</th>
<th>Mg</th>
<th>P</th>
<th>Si</th>
<th>Si/K</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Transpiration</strong></td>
<td>-0.66</td>
<td>-0.83</td>
<td>-0.76</td>
<td>0.13</td>
<td>0.68</td>
<td>0.86</td>
</tr>
<tr>
<td>(0.0001)</td>
<td>(0.0001)</td>
<td>(0.0001)</td>
<td>(0.0001)</td>
<td>(0.0001)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Transpiration efficiency</strong></td>
<td>0.56</td>
<td>0.57</td>
<td>0.50</td>
<td>0.15</td>
<td>-0.31</td>
<td>-0.48</td>
</tr>
<tr>
<td>(0.001)</td>
<td>(0.001)</td>
<td>(0.001)</td>
<td>(0.001)</td>
<td>(0.01)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 3.2** K and Si content in the shoots of potted maize plants in relation to cumulated whole-plant transpiration.
The concentration of K in the plant decreased with increasing transpiration. Negative correlations between mineral element concentration and transpiration were also found for Ca and Mg. Only weak correlations were found between transpiration efficiency and the concentration of mineral elements. Except for Si and the ratio of Si to K, all correlations were positive. This result is contradictory to the theoretical expectation.

3.4.2 Field Experiments

For the field experiments, only the concentration of mineral elements at the end of the experimental period and not the uptake during the experimental period was evaluated. Leaf mineral element concentrations were in general more closely related to transpiration than mineral element concentrations of stem dry matter. For the late stress experiment correlations between transpiration and mineral concentrations were higher if only the upper leaves were considered, those which were assumed to be mainly grown under limited water supply (data not shown). Correlation coefficients, based on plot means of mineral concentration in young leaves and water use parameters for the late stress experiment, are given in Table 3.2. The highest, and non-linear, correlation was obtained for nHCl-ash and transpiration rate (Fig. 3.3). The correlations of K and Ca to transpiration were weak, if all plots were compared, but were comparable to the correlations for nHCl-ash when correlations were calculated separately for each genotype (averaged for the four genotypes r = -0.85 for K and r =

![Graph](image)

**Fig. 3.3** Concentration of HCl non-soluble ash, K and Ca (in % of dry weight) of the six youngest leaves of four field-grown maize genotypes at flowering stage in relation to the weighted mean transpiration rate (in mmol m⁻² s⁻¹) for a previous period with differing water supply.
Significant genotypic differences were found for the concentration of the macronutrients Ca, K, Mg and P in the analysis of variance when cumulated transpiration was included as a covariate, whereas no genotypic differences were indicated for nHCl-ash or Si in this covariance analysis. Hence the transpiration in different water supply treatments for a single genotype might be described by the concentration of K and Ca, but across different genotypes differences in mineral element concentration cannot solely be attributed to differences in transpiration. The concentration of K tends to decrease with increasing transpiration, while Ca, B and P concentrations were increased. Significant correlations were also found for B and P. No significant correlation to transpiration rate was found for Cu, Fe, Mg, Mn, Na and Zn. No significant correlations between any of the investigated elements and transpiration efficiency were obtained in these calculations based on plot means.

Table 3.2 Coefficients of correlation of transpiration, weighted mean transpiration rate, and transpiration efficiency to mineral element concentrations and the ratio of nHCl-ash and K concentration of the six youngest leaves of field grown maize (n=32, four genotypes, two irrigation regimes, four replications).

<table>
<thead>
<tr>
<th></th>
<th>B</th>
<th>Ca</th>
<th>K</th>
<th>Mg</th>
<th>P</th>
<th>nHCl-ash</th>
<th>nHCl-ash/K</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transpiration</td>
<td>0.47</td>
<td>0.14</td>
<td>-0.72</td>
<td>-0.26</td>
<td>0.46</td>
<td>0.75</td>
<td>0.76</td>
</tr>
<tr>
<td></td>
<td>(0.007)</td>
<td>(0.44)</td>
<td>(0.001)</td>
<td>(0.146)</td>
<td>(0.013)</td>
<td>(0.0001)</td>
<td>(0.0001)</td>
</tr>
<tr>
<td>Weighted mean</td>
<td>0.38</td>
<td>0.31</td>
<td>-0.67</td>
<td>-0.05</td>
<td>0.43</td>
<td>0.83</td>
<td>0.83</td>
</tr>
<tr>
<td>transpiration rate</td>
<td>(0.033)</td>
<td>(0.085)</td>
<td>(0.001)</td>
<td>(0.77)</td>
<td>(0.014)</td>
<td>(0.0001)</td>
<td>(0.0001)</td>
</tr>
<tr>
<td>Transpiration</td>
<td>-0.03</td>
<td>-0.15</td>
<td>0.27</td>
<td>-0.18</td>
<td>-0.12</td>
<td>-0.18</td>
<td>-0.19</td>
</tr>
<tr>
<td>efficiency</td>
<td>(0.88)</td>
<td>(0.46)</td>
<td>(0.15)</td>
<td>(0.32)</td>
<td>(0.53)</td>
<td>(0.32)</td>
<td>(0.32)</td>
</tr>
</tbody>
</table>

The relation between transpiration efficiency and nHCl-ash, based on genotype means for well-watered and water stressed plants, is depicted in Fig. 3.4. No significant genotypic variation was found for transpiration efficiency and nHCl-ash (see also Chapter 4). However, a negative relation between transpiration efficiency and concentration of nHCl-ash was indicated for the limited number of genotypes and within the limited variation in transpiration efficiency. The CV for porometrically estimated transpiration efficiency and nHCl-ash was comparable (29 and 28%, respectively) in water stressed plants, but was considerably smaller for nHCl-ash (23 and 14%, respectively) in well-watered plants.

Higher variation in transpiration efficiency occurred, when not genotype means but the means of experimental replications and different treatments of both experiments were compared (Fig. 3.5). In this case high correlations were found for the nHCl-ash concentration and weighted mean of transpiration. Transpiration efficiency showed a significant, negative correlation to nHCl-ash concentration. Especially for the ratio of nHCl-ash to K unique relations to the weighted mean transpiration rates for both experiments were found.
Fig. 3.4. Genotypic means of transpiration efficiency and concentration of HCl non-soluble ash of all maize leaves for the early (left graph) and the six youngest maize leaves for the late stress experiment (right graph). Error bars indicate standard errors of mean (n=4).

Fig. 3.5 Relations between weighted mean transpiration rate and transpiration efficiency of water stressed and well-watered field-grown maize plants and leaf concentration of HCl non-soluble ash and the ratio of HCl non-soluble ash and K concentration.
3.5 Discussion

**Can mineral element concentrations reflect transpiration efficiency?**

A negative linear correlation between concentration of mineral elements or the sum of mineral concentrations and transpiration ratio (ratio of water transpired to dry matter, hence the reciprocal of transpiration efficiency) has been suggested (Masle et al. 1992), with the ratio of mineral uptake per unit of transpiration being the factor of proportionality. However, constant ratios of uptake per unit of transpiration can not generally be assumed for mineral nutrient elements. This prerequisite is especially questionable when water supply is limited, the environment where transpiration efficiency is of particular interest. The relations between mineral element concentrations and transpiration efficiency have not yet been investigated under field conditions with limited water supply.

We obtained for none of the macronutrient elements the hypothesized relation in maize, comparable to results of Masle et al. (1992) for sunflower, sorghum and wheat, whereas for nHCl-ash concentration, implicitly Si, some evidence for a relation to transpiration efficiency was found in the present experiments. For several reasons the concentration of macronutrients in the shoot is not a suitable indicator of transpiration.

**Transport and uptake of nutrient mineral elements in soil and plant**

Mass flow can account for nutrient transport to the place of uptake at the cell plasma membrane and for the transport via xylem into the shoot but not for the uptake into the symplast. Mass flow is the main transport mechanism to the root for Ca and Mg (Barber 1995), as was evidenced by the positive, linear correlation between Ca concentration and transpiration in our experiment. K and P are mostly transported to the root by diffusion (Barber 1995). Decreasing water content in the soil decreases the effective diffusion coefficient by reducing the cross sectional area of water filled soil pores for ion diffusion. The transport of K and P is then not likely to be limited due to decreased transpiration rate but rather due to a slower diffusion in the soil. Moreover, the uptake of K is closely coupled with metabolic activity and not directly influenced by water flux across the membrane (Hsiao and Läuchli 1986). Differences in the effect of water stress on K concentrations were observed for the early and late stress experiment. In the late stress experiment water stress was less severe and the higher K concentrations were found in the leaves of stressed plants. This might indicate that metabolic activity in the roots was not as severely affected as water uptake and growth. Specific and selective uptake kinetics thus hinder the use of K and P concentration as an indicator of transpiration efficiency as evidenced by the insignificant or even positive correlations with transpiration efficiency. K is, for a range of species, the major mineral
element accounting for up to 50% of the sum of mineral element content. In our experiments, the correlations for ash concentration and transpiration or transpiration efficiency were very similar to the correlations with K (data not shown). Therefore, we may have to exclude ash content as an possible indicator of transpiration and transpiration efficiency likewise.

Except B, Ca and Si, all mineral nutrients are known to be retranslocated in the plant and the concentration in the roots is believed to be a signal of shoot nutrient status and may thereby regulate ion uptake of roots. This cycling of nutrients decouples concentrations in the shoot from transpiration and uptake rate. Translocations will certainly be of major importance for determination of transpiration efficiency in mature plants or at the reproductive stage. The concentrations of mineral elements in kernels have therefore been correlated to carbon isotope discrimination, as an indicator of transpiration efficiency in barley (Febrero et al. 1994). However, expectations of correlations are not straightforward since the pathway of translocation to the kernels is in the phloem and hence not dependent on transpiration.

**Distribution of mineral elements and water uptake in the soil**

The concentrations of the mineral nutrients in the soil solution varies because they depend on factors like soil moisture, soil depth, pH, cation-exchange capacity, redox potential, quantity and distribution of organic matter, microbial activity and fertilizer application (Asher 1978). For a field, nearby the experimental site, high gradients in pH, clay content and nutrient concentrations (42, 8 and 8 mg kg$^{-1}$ P; 98, 35 and 23 mg kg$^{-1}$ K; 180, 126 and 98 mg kg$^{-1}$ Mg; 42,16, and 11 mg kg$^{-1}$ Fe and 0.27, 0.17, 0.09 mg kg$^{-1}$ B in 5, 30 and 60 cm soil depth) were observed (Neidhart 1994). In drying soils the water is successively taken up from deeper layers (see Chapter 4, Fig. 4.10), therefore, the uptake of nutrients per unit of water will decrease and introduce a bias in the estimation of transpiration based on mineral concentrations. A negative correlation between ash and water use efficiency was reported by Mayland et al. (1993) for a field experiment with crested wheat grass, where clones were planted outdoors in pots to allow gravimetric determination of transpiration. If this relation would have been found with the roots exposed to the naturally occurring gradients in the soil might therefore be questionable.

Since the mineral concentration in the shoot is to be used for the screening of genotypes with respect to transpiration and transpiration efficiency, the genotypic differences in the relations of Ca and K concentration and transpiration are of particular relevance. Estimates of transpiration based on concentrations of Ca and K are likely to be confounded with genotypic differences in nutrient uptake, which are not related to transpiration. In contrast, concentration of nHCl-ash did not vary between genotypes when differences in transpiration was accounted for.
Silicon as an indicator of transpiration

Due to the uptake of Si and B as uncharged molecules (monosilic acid, boric acid) both are thought to move passively across biological membranes. The accumulation of Si is greatly influenced by transpiration rate (Raven 1983). In contrast to most mineral elements remobilization of Si is very restricted (Samuels et al. 1991) and deposition of Si predominantly occurs at the sites of high transpiration, namely in the leaf epidermal cells (Raven 1983).

Si uptake and concentration varies among plant species (Jones and Handreck 1967, Takahashi and Miyake 1977, van der Vorm 1980). Within the *graminae*, strong accumulators have been found with Si concentrations of more than 5% in the dry matter (e.g. rice and sugarcane), whereas for oat Si uptake was perfectly related to the expected uptake as was calculated based on transpiration (Jones and Handreck 1965). Takahashi and Miyake (1977) found an average of 1.96% Si in the dry matter of 34 Si-accumulator species. In our experiments the maize leaves had Si concentrations of 1.48 and 1.04% under well-watered and water stressed conditions. Maize might therefore be described as a weak Si accumulator.

Correlations between Si uptake and transpiration have been found in field experiments with barley (Walker and Lance 1991), wheat (Hutton and Norrish 1974, Schultz and French 1976) and crested wheatgrass (Mayland et al. 1993). However, contradictory results have also been reported for wheat (Mayland et al. 1991) and other species mostly in greenhouse and pot studies (Masle et al. 1992, Mayland et al. 1993). Considerable scatter in the relation between the porometrically determined transpiration and transpiration, estimated from the soil water balance, was evidenced in our experiments (see Chapter 2) and resulted in lower correlations between nHCl-ash and transpiration, when transpiration was assessed by the soil water balance compared to the porometrically derived estimate. These methodological limitations may also have contributed to the weak correlations between transpiration efficiency and Si concentration found for wheat in the field experiments of Schultz and French (1976). Precise determination of transpiration efficiency in the field is difficult to achieve, however pot studies cannot simulate the field situation, especially under drought when genotypes vary in root proliferation and uptake of water and minerals.

Silicon uptake

Mayland et al. (1993) concluded high correlations between transpiration and Si uptake to be associated with a 1:1 ratio of Si concentration in soil solution and xylem sap. However, the necessary conditions for a correlation between transpiration efficiency and Si concentration are that both the uptake ratio, as the ratio of uptake expected from massflow and actual uptake, and the concentration in the soil solution are constant. For barley a shift from active to passive uptake of Si with increasing
transpiration rate caused by decreasing atmospheric humidity was found by Barber and Shone (1966) in solution culture experiments and similarly evidenced for soil-grown plants by Walker and Lance (1991). This shift in the uptake ratio might be valid also for maize, since in our pot study with rather low evaporative demand the correlation for Si concentration and transpiration was weak in comparison to the field, indicating that Si accumulation was less dependent on transpiration. The low or insignificant correlations between Si concentration and transpiration ratio reported by Masle et al. (1992) were also derived from greenhouse experiments with presumably rather low water vapour pressure deficits. The impact of transpiration rate might be explained by a feedback control of Si uptake. Low concentration of Si in the plant, when Si uptake is low due to low transpiration, might induce an active uptake. For rice, Jones and Handreck (1967) suggested that rather the Si transport to the shoot is dependent on transpiration rate than the uptake. However, for the target environment in the field we will most often find the required conditions of high evaporative demand.

Silicon in the soil solution

With increasing Si concentration in the solution the ratio of actual to massflow-expected uptake decreases in a hyperbolic fashion (Jones and Handreck 1969, van der Vorm 1980). A strong decrease for the uptake ratio was found with low concentrations, but with concentrations of more than 50 ppm Si(OH)₄ in the solution the contribution of mass-flow uptake was rather constant for all species except the strong accumulator rice. Due to its abundance in the soil the concentration of monosilic acid in the soil solution is presumably less variable than for other nutrient elements. Relatively small variation in Si concentration of the soil solution at eight different field sites was also assumed by Hutton and Norrish (1974), who found for all sites a unique relation of the Si concentration in wheat husks to transpiration. While the solubility of amorphous Si in water is unaffected in the range of 2 to 9 pH, concentrations in soil solutions are largely controlled by pH dependent adsorption reactions (Jones and Handreck 1967). Hence, variability in the Si concentration can not be excluded. No measurements of Si concentrations in the soil solution were made in our study. Depending on the soil type the concentration of Si(OH)₄ was found to vary between 3 and 70 ppm in soil solutions for the range of 5 to 9 pH (Scheffer and Schachtschabel 1992). Higher uptake of Si per unit of transpiration with increasing soil water supply was found by Mayland et al. (1991) and explained with higher Si concentration in the soil solution taken up by plants of the wettest treatment. The overproportional rise in Si concentration with increasing transpiration rate might also be explained as the result of a lower transpiration efficiency for well-watered plants, compared to water stressed plants, as was shown in Chapter 4. If the specific leaf weight and ratio of leaf to stem dry weight are considered constant we may express the transpiration rate as the transpiration divided by dry

37
weight, which is the transpiration ratio. A rising transpiration ratio, hence decreasing transpiration efficiency with increasing transpiration rate will result in an overproportional rise in cumulated transpiration and Si uptake per unit of dry weight and hence overproportional rise in the Si concentration. The variation in Si concentration in plants grown with different nitrogen or phosphorus supply were likewise explained by the effects of nutrient supply on transpiration efficiency (Jones and Handreck 1967).

Both, the non-linear relation between nHCl-ash concentration and transpiration rate (Fig. 3.3), and the significant correlations between transpiration efficiency and nHCl-ash concentration of different treatments (Fig. 3.5) indicate, that for maize Si concentrations may provide a means of estimating transpiration efficiency. No genotypic variation was found neither for porometrically determined transpiration efficiency nor for the Si concentration in leaves. Further evaluation of the method is needed, especially with respect to Si concentrations in the soil solution and uptake kinetics in maize before transpiration efficiency can be evaluated for a broader range of genotypes via an analysis of Si content.
Growth, Stomatal Conductance, Transpiration and Transpiration Efficiency of Tropical Maize Cultivars and Responses to Drought Stress at Vegetative Stages

4.1 Abstract

Stomatal conductance, canopy transpiration and transpiration efficiency, as the ratio of above-ground dry matter accumulation and transpiration, were investigated for eight tropical maize cultivars under field conditions during the dry season in Thailand. Responses to water stress in either of these traits were analyzed with respect to the potential for improving drought tolerance in maize. Field experiments were conducted in two years, where water stress was imposed either at the early or at the late vegetative stage by withholding the weekly furrow irrigation for a period of four weeks. Stomatal conductance was measured frequently with a porometer and used to calculate the canopy transpiration. Transpiration efficiency during the vegetative growth was calculated after determination of dry matter accumulation. Additionally, the soil water depletion was monitored at 20, 40, 60, 80 and 100 cm soil depth with neutron probe measurements.

Water stress reduced above-ground dry matter yield at the end of the stress period to 28 and 41% of the control in the early and to 70 and 82% of the control in the late vegetative stage. Grain yield was less reduced by water stress at the late vegetative stage, what was concluded rather to be the result of lower stress intensity than lower sensitivity to water stress in late vegetative stage compared to early vegetative stage. Harvest index was increased by stress at the early vegetative stage and slightly decreased by water stress at the late vegetative stage. Genotypic differences were significant for dry matter accumulation and grain yield but no genotype by water supply interaction. Stomatal conductance was strongly depressed within one week after water was withheld. The initial decline in stomatal conductance was highly correlated to the soil matric potential at 20 cm depth for the early stress experiments, and to the soil matric potential in deeper layers for the late vegetative stage.

Despite of generally high error variances genotypical differences in stomatal conductance were indicated in both irrigation treatments and growth stages. Stomatal conductance was generally low in KTX3101, sensitive to drought in KS6 and the least affected by drought in DK888. The maintenance of high stomatal conductance was beneficial for growth under water stress. Cumulated transpiration was to a very large extent determined by the differences in leaf area and only slightly affected by genotypic differences in stomatal conductances within the irrigation treatments. With increasing
stress intensity soil water was taken up successively from deeper layers and genotypic differences were indicated for soil water uptake along the profile. The genotypic shift in soil water uptake to deeper layers under drought corresponded with the maintenance of stomatal conductance and transpiration under water stress. Transpiration efficiency was increased under water stress with the increase being related to stress intensity. The highest transpiration efficiency was found when transpiration was decreased to about 50% of the control. No significant genotypic differences were found in transpiration efficiency. However, under well-watered conditions, DK888 showed consistently the highest transpiration efficiency. Correlations of genotype means between transpiration efficiency and dry matter accumulation were insignificant within the water stressed treatment and positive within the control. Therefore, not transpiration efficiency but rather the cumulated transpiration, determined by the ability to explore the water from a greater soil volume was the major characteristic of drought tolerance in maize. However, for well-watered conditions we do not have any evidence, that high transpiration efficiency has any negative effects on growth.

4.2 Introduction

Insufficient water supply is one of the major limitations to maize production. For many farmers the use of drought tolerant cultivars is the only affordable option in drought-prone areas. Tolerance to drought events is governed by several interacting physiological traits and the contribution of specific traits has to be considered with respect to the specific drought scenario. While for a mediterranean climate drought occurs mostly at the end of the growing season, spells of drought are frequent in the semiarid subtropical region throughout or particular at the beginning of the growing season. Sensitivity of grain yield to limited water supply in maize is correlated to the growth stage, with drought at or near anthesis being the most sensitive stage. Westgate and Boyer (1986) concluded that physiological processes associated with anthesis and early grain development are especially vulnerable to water deficit. However, the sensitivity of this stage might also be explained by the coincidence of the pre-anthesis growth stage with the stage of maximum biomass accumulation and water use, as was concluded by Sinclair et al. (1990). Biomass accumulation during periods of water shortage might be less reduced in varieties which exhibit high efficiency of water-use or are able to increase the efficiency of water-use with limited water supply. Genotypic variability for water-use efficiency of maize plants was shown in greenhouse experiments based on gas-exchange measurements (Sobrado 1990b) or dry matter accumulation (Sin 1993). The latter found for twelve tropical maize cultivars a strong positive relation between water-use efficiency and dry matter production under limited water supply.

Due to methodological problems in evaluating transpiration efficiency in the field, little is known about the contribution of transpiration efficiency to drought tolerance of
varieties and the relation between transpiration-efficiency and growth parameters under field conditions. Stomata have a key function in regulating the relation between growth and water consumption. According to their stomatal behaviour, i.e. the sensitivity of responses to soil water limitation in the soil, plants may be characterized as water spenders or water savers. In the context of the relation between ABA concentration and drought tolerance some authors suggested a sensitive stomata regulation to be a major characteristic of drought resistant genotypes (Pekic and Quarrie 1987). The inevitable cost of partial stomatal closure will be lower carbon assimilation and hence it is questionable if a sensitive response of stomata, implying a water saving characteristic, is advantageous for agronomic crops. Stomatal conductance is likely to influence water-use efficiency. The expected effect on transpiration efficiency is contested and depends on the ratio of stomatal and non-stomatal limitations of photosynthesis (Sinclair et al. 1975, Nobel 1983, Krieg 1983a, Sinclair et al. 1984, Ludlow and Muchow 1990).

The objectives of this study were (i) to determine stomatal conductance and transpiration rates of tropical maize cultivars during vegetative growth under sufficient and limited water supply, (ii) to estimate transpiration efficiency of field-grown maize plants with sufficient and limited water supply, (iii) to investigate genotypic variability in stomatal behaviour and transpiration efficiency, (iv) to describe the relation between stomatal behaviour, transpiration efficiency and growth parameters and the impact of drought on these relations. These informations should allow one to evaluate the potential of improving drought tolerance by selection for a specific stomatal behaviour or improved transpiration efficiency.

4.3 Materials and Methods

4.3.1 Layout of the Experiments

Four experiments were conducted during the dry season 1993/94 and 1994/95 with four replications including eight tropical maize cultivars and two water regimes arranged in a split-plot design. In all experiments water stress was induced by withholding water for approximately four weeks at the early vegetative stage (1993/94 from days after emergence (DAE) 5 to 33 and 1994/95 from DAE 9 to 37) or at the late vegetative stage (1993/94 from DAE 33 to 61 and 1994/95 from DAE 37 to 61) later referred to as "early stress" and "late stress" experiments. All plots received two (1993/94) or three (1994/95) sprinkler irrigations after planting until withholding water for the drought treatment. The treatments in 1994/95 were delayed for a few days to provide better seedling establishment. The late stress experiment 1994/95 was shortened to 17 days to prevent influences of water stress at flowering. The control plots received weekly furrow irrigation of about 70 mm. The plots included eight rows (1993/94) and six rows (1994/95) which were 0.75 m apart. The plot size was 36 m². All measurements were restricted to the inner six or four rows, respectively.
4.3.2 Genotypes

The experiments included eight tropical maize cultivars (open pollinated varieties: two originating from the Kasetsart University (KU), Thailand KS 6 and Suwan 3 and two from CIMMYT, Mexico: *Tuxpeno* seq. C0 and *Tuxpeno* seq. Cg; four modern hybrids frequently used in Thailand: *Cargill 922, DeKalb 888, Hercules 40 (Ciba-Geigy G5440)* and KU hybrid *KTX 3101*.

4.3.3 Soil

The field experiments were conducted at the National Corn and Sorghum Research Center, Farm Suwan, Kasetsart University in Thailand (14.5 °N lat.). The soil was an ustic, isohyperthermic, kaolinitic oxisol. It is characterized by high clay content (56, 85 and 86% in 5, 30 and 60 cm, respectively) and a gradient in pH values (7.5, 6.8 and 5.3 in 5, 30 and 60 cm, respectively), probably due to 20 year long irrigation with calcium carbonate enriched ground water (Neidhart 1994). Bulk soil density was 1.27, 1.23, 1.17 g cm⁻³ in 5, 30 and 60 cm soil depth for the field used in 1993/94 and 1.29, 1.25 and 1.23 g cm⁻³ in 20, 40 and 60 cm soil depth for the field used in the experiments 1994/95.

4.3.4 Meteorological Data

The experimental site has a semi-arid climate with low long-term average rainfall from November to March. Minimum and noon temperatures, humidity and vapour pressure deficit at noon for the experimental periods are given in Table 4.1. Rainfall of 12mm and 3mm occurred on DAE 53 and DAE 55 in 1994/95.

| Table 4.1 Meteorological conditions for the experimental periods in both years. |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| 1993/94 | DAE 12 - 19 | 20 - 26 | 27-33 | 40 - 47 | 48 - 54 | 55 - 61 |
| Rel. humidity at noon (%) | 41 | 34 | 49 | 40 | 30 | 36 |
| Air temperature at noon (°C) | 30.8 | 32.5 | 32.5 | 34.8 | 34.9 | 34.7 |
| Vapour pressure deficit at noon (kPa) | 2.63 | 3.23 | 2.49 | 3.36 | 3.93 | 3.56 |
| Min. temperature (°C) | 15.5 | 15.0 | 19.7 | 21.1 | 21.3 | 19.3 |
| 1994/95 | DAE 16 - 23 | 24 - 30 | 31 - 37 | 38 - 44 | 45 - 51 | 52 - 61 |
| Rel. humidity at noon (%) | 39 | 50 | 39 | 36 | 38 | 45 |
| Air temperature at noon (°C) | 32.6 | 31.1 | 28.1 | 33.4 | 34.6 | 34 |
| Vapour pressure deficit at noon (kPa) | 2.99 | 2.46 | 2.33 | 3.26 | 3.45 | 2.99 |
| Min. temperature (°C) | 15.5 | 18.0 | 12.2 | 15.7 | 16.2 | 18.0 |
4.3.5 Cultural Practices

Agricultural practices were according to the local standard. After ploughing, 60 kg N as ammonium sulfate, \(\text{P}_2\text{O}_5\) and \(\text{K}_2\text{O}\) per ha were broadcasted with a mixed fertilizer (15-15-15) and incorporated into the soil. In 1993/94 all plots of the late stress experiment received 50 kg N ha\(^{-1}\) in form of urea during the irrigation at 33 DAE. In 1994/95 residual N-content in the soil, three weeks after emergence, was high (120 kg N) and no additional fertilizer was applied. Plots were prepared in the ridge and furrow system with ridges being 0.75 m apart and 0.25 m high. Two seeds were sown manually per seed position, the distance between two seed positions was 0.2 m in 1993/94 and 0.25 m in 1994/95. After thinning to one plant per seed position the plant density was 6.6 (1993/94) and 5.3 plants per square meter (1994/95). Chemical weed control was applied with pre-emergence herbicides, atrazin and alachlor, at the rates of 3 kg ha\(^{-1}\) and 3 L ha\(^{-1}\), respectively. Thereafter weed control was carried out manually. Monocrotophos (750 ml as 60 % solution) was applied against European corn borer (\textit{Ostrinia nubalis}) and frit fly (\textit{Oscinella frit}) at the three-leaf stage.

4.3.6 Soil Water Parameters

Water retention curves were determined on 100 cm\(^3\) soil cores in a Richards pressure plate apparatus (Fig. 4.1). The fraction of plant available water was found to be low (12 to 15 % in the range of soil matric potential from -5 to -1400 kPa). Saturated hydraulic conductivity was measured with the constant head method (Klute and Dirksen 1986) and the unsaturated hydraulic conductivity was estimated using the Van Genuchten - Mualem model (Van Genuchten 1980), based on one-step pressure outflow experiments. The model parameters saturated \(\theta_s\), the residual water content \(\theta_r\), and the empirical fitting parameters \(\alpha\) and \(n\) were estimated using the computer

![Fig. 4.1 Water retention curves for different soil depths of the experimental fields E9 in 1993/94 and G11 in 1994/95.](image)
program SFiT (Kool and Parker 1987). The relative hydraulic conductivity was expressed as a function of the soil matric potential. Saturated hydraulic conductivity was $-10$ cm day$^{-1}$ and relative unsaturated conductivity was characterized by a sharp decrease at high soil matric potentials (Fig. 4.2). Soil matric potential during the experimental periods in the field was monitored with tensiometers at four depths (20, 40, 60 and 80 cm) except in early stress 1993/94, where no measurements were taken at 80 cm soil depth. Soil water depletion could well be observed with tensiometers since more than two thirds of the total plant available water was used before the soil matric potential exceeded $-80$ kPa.

![Fig. 4.2 Unsaturated relative hydraulic conductivity of the soil in the experimental field E9 in 1993/94 at 25 and 50 cm soil depth estimated from one-step pressure outflow experiments.](image)

- The volumetric water content was measured with a neutron probe (Model 503DR, CPN Co., Marinez, California, USA) in 20 cm increments down to 1 m soil depth on 32 plots with one access tube per plot for the late stress experiment 1994/95. Measurements were made two to three times per week, in watered plots before and one day after irrigation and additionally after the rainfall at DAE 54. Evaporation was determined gravimetrically from small lysimeters (12 cm diameter, 20 cm depth), which were watered according to the plot treatment. Deep percolation was estimated by monitoring daily soil water depletion after an irrigation on experimental plots, where plants were removed and the soil surface was covered to prevent water loss by evapotranspiration.

### 4.3.7 Plant Parameters

#### 4.3.7.1 Dry Matter Accumulation and Grain Yield

In weekly intervals till flowering (DAE 12, 19, 26, 33, 40, 47, 54, 61 in 1993/94 and DAE 16, 23, 30, 37, 44, 51 and 61 in 1994/95) 6 to 12 well-bordered plants per plot were harvested and partitioned into green leaves and stems/senescent leaves. Dry weight was determined after drying at 65 °C for 36 hours. Leaf area of green leaves

44
was measured with a portable, non destructive leaf area meter device (LI-3000 A, Li-Cor, Lincoln, Nebraska, USA) on the harvested plants in one replication. Mean specific leaf weight for this replication was calculated, separately for the water supply treatments, and used to determine green leaf area for the other replications based on the green leaf dry weight. Total shoot biomass, grain yield, harvest index and yield components (ears per plant, kernels per ear, kernel rows per ear, 100-kernel weight) were determined on twelve well-bordered plants per plot in the year 1994/95.

4.3.7.2 Stomatal Conductance and Transpiration Rates

All porometric measurements were conducted with a LI-1600 steady-state porometer (Li-Cor, Lincoln, Nebraska, USA). Measurements were made from 12:00 to 13:00 h in 1993/94 and 12:00 to 14:00 h in 1994/95. Only the abaxial transpiration rates of the youngest fully developed leaves were measured. Sampling techniques were as follows: (i) In 1993/94 eight genotypes were measured, with one replication per day at four to five days each week, in early and late stress experiments. Six measurements on randomly chosen plants were performed. (ii) In 1994/95 two genotypes (DK888 and KS6) in the early stress experiment and four genotypes (Cargill922, DK888, KS6, Tuxpeño C0) in the late stress experiment were measured with six measurements per plot, in four replications on four to six days per week. (iii) In the early stress experiment 1994/95 the remaining six genotypes were measured with two measurements per plot in three replications on four to six days per week. Due to the break-down of one porometer no measurements have been made on the four remaining genotypes in the late stress experiment 1994/95. Porometrical measurements for the water stress treatment were started after the first irrigation in the control, which means when water was withheld on stressed plots for at least one week. Leaf boundary layer resistance in the field was measured and all transpiration rates and stomatal conductances were adjusted for differences in leaf boundary layer resistance between cuvette enclosed and non-enclosed leaves as described previously in Chapter 2.

4.3.8 Statistical Analyses and Calculations

Analysis of variances and mean comparisons for above-ground dry matter yield and transpiration were carried out on log-transformed values, since variances were not homogeneous over years and treatments. Means of original values are given in the tables with results of multiple mean comparisons carried out on transformed values. The experimental periods were split into approximately weekly durations for calculation of transpiration and transpiration efficiency. Mean leaf area was determined, as the arithmetic mean of leaf area at the beginning and the end of a weekly period, and dry matter accumulation as the difference in shoot biomass between two harvests.

Analysis of variance and mean comparisons for porometric measurements of transpiration rate and stomatal conductance for each experimental period were
calculated according to a split-plot design including plot sampling in 1993/94, with water treatment as the main-plot factor and genotype as the sub-plot factor. The factor replication was replaced by a replication/day (rep/day) combination since only one replication was measured per day. In 1994/95 calculations were carried out according to a split-split-plot design including plot sampling, with water treatment as the main-plot factor, genotype as the sub-plot factor and day of measurement as the sub-sub-plot factor.

Canopy transpiration was calculated as previously described (Chapter 2), using empirically determined factors to take into account the ratio of abaxial and adaxial transpiration, the vertical distribution and the diurnal changes of transpiration rate. Some calculations were performed on weighted means \( \bar{x} \) of leaf area, stomatal conductance or transpiration rate, referring to the whole experiment consisting of several weeks in order to take into account the development of leaf area, stomatal

\[
\bar{x} = \frac{1}{n} \cdot \sum_{i=1}^{n} \left( \frac{LA_i}{LA_n} \cdot x_i \right)
\]

conductance and transpiration rate in time:

where \( LA_i \) is the leaf area and \( x_i \) the trait under consideration of week \( i \). The weight for each weekly mean was the ratio of the leaf area of the respective week to the leaf area at the end of the experiment.

Relative stomatal conductance was expressed according to the susceptibility index of Fischer and Maurer (1978), adapted for the weighted mean stomatal conductance in each experiment. The index is calculated as:

\[
S = \frac{1 - \frac{SC_{ww}}{SC_{ws}}}{1 - \frac{SC_{ws}}{SC_{ww}}}
\]

where \( SC_{ws} \), \( SC_{ww} \), \( SC_{ws} \) and \( SC_{ww} \) are the genotype mean stomatal conductance for well-watered and water stressed conditions and average stomatal conductance under well-watered and water stressed conditions, respectively.

4.4 Results
4.4.1 Total Shoot Biomass and Shoot Biomass Accumulation

The mean dry weight accumulation and transpiration for weekly periods of the four
Water stress in the early stage reduced dry mass accumulation to 28% in 1993/94 and 41% of the control in 1994/95. Shoot dry weight was significantly affected at the second harvest 10 and 16 days after withholding water in 1993/94 and 1994/95, respectively. Only small significant genotypic differences were found at the end of the early stress experiments in shoot biomass (Table 4.2). A vigorous early growth of KS6 was found in both years and treatments, while Tuxpeño C0 and Tuxpeño C8 were comparatively low in shoot dry weight production for both years. A significant genotype by water supply interaction was found only for the early stress experiment 1993/94 with KS6 and DK888 showing the lowest reduction in dry matter accumulation under water stress. For the early stress experiment the analysis of shoot dry matter increase during the experimental period gave very similar results as the analysis of shoot dry weight at the end of the early stress experiment, these data are therefore not shown.

Also for water stress in the late vegetative stage, above ground dry matter yield was significantly reduced in both years (Table 4.3). Due to rainfall and the shorter experimental period, water stress was less severe in 1994/95 and shoot biomass averaged for all genotypes was still 82% of the control, while it was 70% in the year
Table 4.2  Above ground dry matter yield of eight maize cultivars at the end of the early stress experiment under well-watered (ww) and water stressed (s1) conditions and ranks according to analysis of variance combined over years, and over years and irrigation treatments.

<table>
<thead>
<tr>
<th>Irrigation treatment</th>
<th>1993/94 DAE 33</th>
<th>1994/95 DAE 37</th>
<th>rank in combined analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ww</td>
<td>s1</td>
<td>ww</td>
</tr>
<tr>
<td>Cargill 922</td>
<td>80.4 a (4)</td>
<td>20.7 ab (5)</td>
<td>151.6 a (4)</td>
</tr>
<tr>
<td>DK 888</td>
<td>72.0 a (6)</td>
<td>24.6 ab (2)</td>
<td>160.2 a (3)</td>
</tr>
<tr>
<td>Hercules 40</td>
<td>85.2 a (3)</td>
<td>21.7 ab (3)</td>
<td>164.4 a (1)</td>
</tr>
<tr>
<td>KS 6</td>
<td>92.2 a (2)</td>
<td>32.5 a (1)</td>
<td>161.0 a (2)</td>
</tr>
<tr>
<td>KTX 3101</td>
<td>77.7 a (5)</td>
<td>20.4 ab (4)</td>
<td>125.2 a (7)</td>
</tr>
<tr>
<td>Suwan 3</td>
<td>96.3 a (1)</td>
<td>19.4 ab (6)</td>
<td>145.8 a (5)</td>
</tr>
<tr>
<td>Tuxpeño C0</td>
<td>55.3 b (7)</td>
<td>15.3 b (7)</td>
<td>114.1 a (8)</td>
</tr>
<tr>
<td>Tuxpeño C8</td>
<td>53.9 b (8)</td>
<td>14.1 b (8)</td>
<td>133.0 a (6)</td>
</tr>
<tr>
<td>Average</td>
<td>76.6 A</td>
<td>21.1 B</td>
<td>144.4 A</td>
</tr>
</tbody>
</table>

Means in a column not followed by the same letter are significantly different at the 0.1 probability level according to Student-Newman-Keuls-Test.

1 Treatment differences are indicated by different capital letters.
Table 4.3  Above ground dry matter yield of eight maize cultivars at flowering stage under well-watered (ww) and water stressed (s2) conditions and ranks according to analysis of variance combined over years, and over years and irrigation treatments.

<table>
<thead>
<tr>
<th>Days after emergence</th>
<th>1993/94 DAE 61</th>
<th>1994/95 DAE 61</th>
<th>rank in combined analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irrigation treatment</td>
<td>ww</td>
<td>s2</td>
<td>ww</td>
</tr>
<tr>
<td>----------------------</td>
<td>-----</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>Cargill 922</td>
<td>558 b (7)</td>
<td>419 a (4)</td>
<td>693 ab (2)</td>
</tr>
<tr>
<td>DK 888</td>
<td>718 a (1)</td>
<td>466 a (2)</td>
<td>725 a (1)</td>
</tr>
<tr>
<td>Hercules 40</td>
<td>603 ab (2)</td>
<td>445 a (3)</td>
<td>660 ab (3)</td>
</tr>
<tr>
<td>KS 6</td>
<td>551 b (5)</td>
<td>485 a (1)</td>
<td>650 abc (4)</td>
</tr>
<tr>
<td>KTX 3101</td>
<td>606 ab (3)</td>
<td>356 a (7)</td>
<td>622 abc (6)</td>
</tr>
<tr>
<td>Suwan 3</td>
<td>538 b (8)</td>
<td>402 a (5)</td>
<td>619 abc (5)</td>
</tr>
<tr>
<td>Tuxpño C0</td>
<td>553 b (6)</td>
<td>337 a (8)</td>
<td>534 c (8)</td>
</tr>
<tr>
<td>Tuxpño C8</td>
<td>571 b (4)</td>
<td>386 a (6)</td>
<td>577 bc (7)</td>
</tr>
<tr>
<td>Average1</td>
<td>587.6 A</td>
<td>412.4 B</td>
<td>635.1 A</td>
</tr>
</tbody>
</table>

Means in a column not followed by the same letter are significantly different at the 0.1 probability level according to Student-Newman-Keuls-Test.

1 Treatment differences are indicated by different capital letters.
before. Significant genotypic differences in dry weight at 61 DAE (flowering stage) were found in both years for well-watered and water stressed conditions. A higher shoot dry weight with high water supply was found for the hybrids (esp. DK888, Hercules40 and Cargill922) compared to open-pollinated varieties. In contrast, two open pollinated varieties had the highest shoot dry weight under limited water supply (KS6 for 1993/94 and Suwan3 in 1994/95), although these differences were not significant. No genotype by water supply interaction was significant. Combined over years and water supply, DK888 had significantly the highest, and both cycles of the Tuxpeño population the lowest, shoot dry weight at the flowering stage.

Dry matter increase during the late stress experimental period was reduced to 57% of control in 1993/94 and 68% of control in 1994/95 as a mean for all genotypes. Basically similar genotype ranking was found for dry weight at the flowering stage and dry weight increase during the late stress experiments, but less significant differences were found, again the data are not shown. KS6 showed high shoot dry weight at the flowering stage in spite of relatively low dry weight increase during the late vegetative period. In contrast we found for the improved population of Tuxpeño C8, relatively higher shoot dry weight increase than shoot dry weight.

4.4.2 Grain Yield and Harvest Index

Water stress at the early vegetative stage resulted, in 1994/95, in higher yield reduction than water stress at the late vegetative stage (Table 4.4). Plants of the early stress treatment could not compensate for the reduction in biomass accumulation during the stress period. Dry matter at flowering, four weeks after stress release was still only 52% and at maturity 63% of the control. The grain yield was 71% of the control. Harvest index was increased (Table 4.5). In the case of water stress at late vegetative stage, the reduction compared to the control was quite similar for the dry weight at the time of stress release at flowering, for the dry weight at maturity, and for the grain yield (18-20%). Harvest index was decreased by water stress in the late vegetative stage. Significant genotypic differences in grain yield existed in all irrigation treatments. Analysis of variance for grain yield did not reveal any genotypic differences in the response to water supply. The ranking of genotypes was similar for total biomass accumulation at flowering, at maturity and for grain yield.

Genotypic differences were also found for the harvest index (Table 4.5) and yield components. Especially important in our investigations is the harvest index, since cultivars which produce more grain yield per total biomass are underestimated in their performance on a biomass basis during the vegetative growth. Cargill922 and KTX3101 had a high harvest index and their ranking for grain yield is higher than for growth rates under water stress during vegetative growth and total biomass at maturity.
Table 4.4  Grain yield and ranking of eight maize cultivars in 1994/95 with weekly irrigation and with water stress imposed at early or late vegetative stage.

<table>
<thead>
<tr>
<th></th>
<th>well-watered</th>
<th>early stress</th>
<th>late stress</th>
<th>total mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>grain yield (g m$^{-2}$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cargill 922</td>
<td>350 (2) ab</td>
<td>262 (1) a</td>
<td>290 (1) a</td>
<td>(2) a</td>
</tr>
<tr>
<td>DK 888</td>
<td>366 (1) a</td>
<td>252 (2) ab</td>
<td>342 (2) ab</td>
<td>(1) a</td>
</tr>
<tr>
<td>Hercules 40</td>
<td>290 (5) abc</td>
<td>217 (4) abc</td>
<td>273 (3) abc</td>
<td>(3) b</td>
</tr>
<tr>
<td>KS 6</td>
<td>261 (6) bc</td>
<td>184 (6) bc</td>
<td>201 (6) dc</td>
<td>(6) cd</td>
</tr>
<tr>
<td>KTX 3101</td>
<td>309 (3) abc</td>
<td>198 (5) abc</td>
<td>240 (4) bcd</td>
<td>(4) bc</td>
</tr>
<tr>
<td>Suwan 3</td>
<td>303 (4) abc</td>
<td>221 (3) abc</td>
<td>221 (5) bcd</td>
<td>(5) bc</td>
</tr>
<tr>
<td>Tuxpeno C0</td>
<td>222 (8) c</td>
<td>170 (7) c</td>
<td>169 (7) d</td>
<td>(8) d</td>
</tr>
<tr>
<td>Tuxpeno C8</td>
<td>249 (7) c</td>
<td>169 (8) c</td>
<td>158 (8) d</td>
<td>(7) d</td>
</tr>
<tr>
<td><strong>Average$^1$</strong></td>
<td><strong>294 A</strong></td>
<td><strong>209 C</strong></td>
<td><strong>237 B</strong></td>
<td></td>
</tr>
</tbody>
</table>

Means in a column not followed by the same letter are significantly different at the 0.1 probability level according to Student-Newman-Keuls-Test.  

1 Treatment differences are indicated by different capital letters.

Table 4.5  Harvest index of eight tropical maize cultivars in 1994/95 and ranking with weekly irrigation and with water stress imposed at early or late vegetative stage.

<table>
<thead>
<tr>
<th></th>
<th>well-watered</th>
<th>early stress</th>
<th>late stress</th>
<th>total mean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cargill 922</td>
<td>0.520 (2) ab</td>
<td>0.601 (1) a</td>
<td>0.519 (1) a</td>
<td>0.547 (1) a</td>
</tr>
<tr>
<td>DK 888</td>
<td>0.485 (4) abc</td>
<td>0.545 (5) bc</td>
<td>0.513 (2) a</td>
<td>0.515 (3) b</td>
</tr>
<tr>
<td>Hercules 40</td>
<td>0.488 (3) abc</td>
<td>0.555 (4) bc</td>
<td>0.483 (4) a</td>
<td>0.509 (4) b</td>
</tr>
<tr>
<td>KS 6</td>
<td>0.454 (8) c</td>
<td>0.558 (3) bc</td>
<td>0.449 (5) bc</td>
<td>0.487 (5) bc</td>
</tr>
<tr>
<td>KTX 3101</td>
<td>0.534 (1) a</td>
<td>0.576 (2) ab</td>
<td>0.501 (3) a</td>
<td>0.540 (2) a</td>
</tr>
<tr>
<td>Suwan 3</td>
<td>0.462 (7) c</td>
<td>0.495 (8) d</td>
<td>0.426 (6) bc</td>
<td>0.461 (8) d</td>
</tr>
<tr>
<td>Tuxpeno C0</td>
<td>0.478 (6) bc</td>
<td>0.523 (7) cd</td>
<td>0.402 (7) c</td>
<td>0.468 (7) cd</td>
</tr>
<tr>
<td>Tuxpeno C8</td>
<td>0.484 (5) abc</td>
<td>0.536 (6) bc</td>
<td>0.394 (8) c</td>
<td>0.471 (6) cd</td>
</tr>
<tr>
<td><strong>Average$^1$</strong></td>
<td><strong>0.488 B</strong></td>
<td><strong>0.549 A</strong></td>
<td><strong>0.462 C</strong></td>
<td><strong>0.500</strong></td>
</tr>
</tbody>
</table>

Means in a column not followed by the same letter are significantly different at the 0.1 probability level according to Student-Newman-Keuls-Test.  

1 Treatment differences are indicated by different capital letters.

4.4.3  Stomatal Conductance During Vegetative Growth

4.4.3.1  Response of Stomatal Conductance to Soil Water Limitation

The soil water potential at four depths during the experimental periods is shown
in Fig. 4.4 for well-watered and water stressed plots, respectively. The later onset of water stress and lower plant density in the experiment 1994/95 led to a more gradual decrease in the soil matric potential. In later growth stages water stress developed much faster and the decline of soil matric potential was steeper. The decrease of the soil matric potential in well-watered plots was strongly dependent on the growth stage. In the late vegetative stage soil matric potential at 40 and 60 cm depth was decreased, while in the early vegetative stage only the soil matric potential of the upper soil layer was significantly affected within the weekly irrigation cycle.

![Soil matric potential measured by tensiometers in the well-watered and water stressed plots for early and late stress experiments. Dark triangles indicate days of irrigation, the arrows indicate rainfall (12 mm) on DAE 52 in 1994/95.](image)

Stomatal conductance averaged for all genotypes during the experiments is depicted in Fig. 4.5. Stomatal conductance was strongly depressed by water stress right from the beginning in all experiments, indicating that rewatering after one week of withholding water resulted in significant increases in stomatal conductance, even in the early vegetative stage, where plant water uptake is still low. Better seedling establishment in the early stress experiment 1994/95 led to a more gradual decrease in stomatal conductance throughout the experimental period compared to the first year, where stomatal conductance was strongly decreased after the first week of observation. In later growth stages water stress developed much faster and the initial decline in stomatal conductance was more drastic. In the early stress experiments decline in daily means of stomatal conductance of water stressed plants were highly correlated to the soil water potential at 20 cm depth ($r=0.89^{**}$, compared to $r=0.55^{**}$ for 40 and $r=0.50^{**}$
for 60 cm depth). When water stress occurred in the late vegetative stage, stronger correlations to the soil matric potential at deeper layers (r=0.55, r=0.79***, r=0.62** and r=0.66* for 20, 40, 60 and 80 cm soil depth, respectively) indicated that stomatal conductance was more sensitive to the soil water status at deeper layers. The estimated parameters for linear regressions are given in the appendix A1.

The soil matric potential at the end of a weekly irrigation period in well-watered plots is, especially in the late vegetative stage, reduced to levels lower than -40 kPa, which is likely to induce partial stomatal closure. In the late stress experiments, significant correlations of stomatal conductance and transpiration rates were found with comparatively still high soil matric potentials of about -41, -35, -25 and -22 kPa at 20, 40, 60 and 80 cm soil depth, respectively.

![Stomatal conductance and air humidity during the early and late stress experiments.](image)

**Fig. 4.5** Stomatal conductance and air humidity during the early and late stress experiments. The arrow indicates rainfall (12 mm) at DAE 52 in 1994/95. The experiments included eight or four genotypes.

### 4.4.3.2 Response of Stomatal Conductance to Atmospheric Humidity

Variation of stomatal conductance was affected by air humidity, especially for well-watered plants (Fig. 4.6). The late stress experiment in 1993/94 has been excluded in the analysis of the response of stomata to air humidity because the
Fig. 4.6 Relation between air humidity and stomatal conductance of well-watered (ww) and water stressed (ws) maize. Each point represents the daily means measured at noon. The experiments included eight or four genotypes.

Fig. 4.7 Relation between stomatal conductance and transpiration rate of well-watered (ww) and water stressed (ws) maize. Each point represents daily means measured at noon. The experiments included eight or four genotypes.

measurements of humidity were not made within the canopy of well-watered and water stressed plots separately and high gradients, in air humidity between both treatments, are likely for dense canopies with high differences in transpiration. Stomatal response to humidity led to a decoupling of transpiration rate from stomatal conductance. This decoupling was complete for well-watered plants (Fig. 4.7), whereas a ten percent increase in stomatal conductance due to increased humidity for a water stressed plant \((g_s = 100 \text{ mmol cm}^{-2} \text{ s}^{-1})\) resulted in a six percent increase in transpiration.

For the late stress experiment in 1994/95 all genotypes had the same principal response of stomatal conductance to water vapour pressure difference (Fig. 4.8). However, Tuxpeno C\(_0\) showed at any water vapour pressure difference a higher
stomatal conductance than the other genotypes, which showed a rather close relation.

![Graph showing stomatal conductance vs. VPD](image)

**Fig. 4.8** Relation between stomatal conductance and leaf to air water vapour pressure difference (VPD\textsubscript{leaf to air}) of four maize varieties under well-watered conditions during the late vegetative stage in 1994/1995. Each point represents daily means of measurements at noon.

### 4.4.3.3 Genotypic Variability in Stomatal Conductance

Genotypic mean stomatal conductances for the genotypes in all experimental periods are given in Tables 4.6 and 4.7 for the early stress experiments and Table 4.8 and 4.9 for the late stress experiments. In general, error variances were high and the coefficients of variation rose to more than 50% for severely water-stressed plants. However, in both irrigation treatments and especially in later growth stages, some genotypic differences were significant and consistent genotypical characteristics were detected. For Tuxpeño C\textsubscript{0}, under well-watered conditions, we found a low stomatal conductance at the early growth stage and a high stomatal conductance in the later growth stage, while stomatal conductance of Tuxpeño C\textsubscript{8} was low throughout the vegetative period. In early growth stages relatively high stomatal conductances were observed for KS6 and Hercules40. KTX3101 showed consistently over treatments, periods and years, a low stomatal conductance. For the genotypes DK888 and KS6, there was never a significant difference in stomatal conductance. However, in all weekly periods of all four experiments except for the last week in late stress 1993/94, when water stress was most severe and stomata were virtually closed (conductance of
### Table 4.6  Stomatal conductance and rankings of eight tropical maize cultivars under well-watered (ww) and water stressed (s1) conditions for the early stress experiment 1993/94.

<table>
<thead>
<tr>
<th>Irrigation treatment</th>
<th>Days after emergence</th>
<th>12 - 19 DAE</th>
<th></th>
<th>20 - 26 DAE</th>
<th></th>
<th>27 - 33 DAE</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ww</td>
<td>s1</td>
<td>ww</td>
<td>s1</td>
<td>ww</td>
<td>s1</td>
<td>mmol m⁻² s⁻¹</td>
</tr>
<tr>
<td>Cargill 922</td>
<td>227 b (8)</td>
<td>191 ab (2)</td>
<td>223 ab (3)</td>
<td>93.3 a (2)</td>
<td>368 a (1)</td>
<td>91.1 a (5)</td>
<td></td>
</tr>
<tr>
<td>DK 888</td>
<td>261 ab (3)</td>
<td>195 a (1)</td>
<td>236 a (1)</td>
<td>106 a (1)</td>
<td>338 a (6)</td>
<td>113 a (3)</td>
<td></td>
</tr>
<tr>
<td>Hercules 40</td>
<td>276 a (1)</td>
<td>146 b (8)</td>
<td>213 ab (6)</td>
<td>74.2 a (6)</td>
<td>350 a (3)</td>
<td>88.7 a (7)</td>
<td></td>
</tr>
<tr>
<td>KS 6</td>
<td>271 a (2)</td>
<td>166 ab (3)</td>
<td>210 ab (7)</td>
<td>65.5 a (8)</td>
<td>339 a (5)</td>
<td>92.0 a (4)</td>
<td></td>
</tr>
<tr>
<td>KTX 3101</td>
<td>244 ab (5)</td>
<td>157 ab (6)</td>
<td>193 b (8)</td>
<td>72.0 a (7)</td>
<td>323 a (7)</td>
<td>84.0 a (6)</td>
<td></td>
</tr>
<tr>
<td>Suwan 3</td>
<td>255 ab (4)</td>
<td>160 ab (5)</td>
<td>236 a (2)</td>
<td>74.3 a (5)</td>
<td>355 a (2)</td>
<td>90.0 a (6)</td>
<td></td>
</tr>
<tr>
<td>Tuxpeño Co</td>
<td>242 ab (6)</td>
<td>166 ab (4)</td>
<td>220 ab (4)</td>
<td>80.6 a (4)</td>
<td>340 a (4)</td>
<td>138 a (1)</td>
<td></td>
</tr>
<tr>
<td>Tuxpeño C8</td>
<td>238 ab (7)</td>
<td>157 ab (7)</td>
<td>215 ab (5)</td>
<td>86.4 a (3)</td>
<td>315 a (8)</td>
<td>119 a (2)</td>
<td></td>
</tr>
</tbody>
</table>

**Average¹**  

|  | 251.4 A | 167.9 B | 218.7 A | 81.9 B | 340.4 A | 104.1 B |

Means in a column not followed by the same letter are significantly different at the 0.1 probability level according to Student-Newman-Keuls-Test.

¹ Treatment differences are indicated by different capital letters.
Table 4.7  Stomatal conductance and rankings of eight tropical maize cultivars under well-watered (ww) and water stressed (s1) conditions for the early stress experiment 1994/95.

<table>
<thead>
<tr>
<th>Days after emergence</th>
<th>16 - 23 DAE</th>
<th>24 - 30 DAE</th>
<th>30 - 37 DAE</th>
<th>38 - 44 DAE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irrigation treatment</td>
<td>ww</td>
<td>s1</td>
<td>ww</td>
<td>s1</td>
</tr>
<tr>
<td></td>
<td>mmol m(^{-2}) s(^{-1})</td>
<td></td>
<td>mmol m(^{-2}) s(^{-1})</td>
<td></td>
</tr>
<tr>
<td>Cargill 922</td>
<td>262 a (1)</td>
<td>158 a (2)</td>
<td>236 a (5)</td>
<td>122 a (2)</td>
</tr>
<tr>
<td>DK 888</td>
<td>239 ab (5)</td>
<td>136 ab (3)</td>
<td>258 a (2)</td>
<td>120 a (3)</td>
</tr>
<tr>
<td>Hercules 40</td>
<td>249 ab (3)</td>
<td>97 b (7)</td>
<td>274 a (1)</td>
<td>111 a (5)</td>
</tr>
<tr>
<td>KS 6</td>
<td>250 ab (2)</td>
<td>128 ab (6)</td>
<td>248 a (3)</td>
<td>105 a (7)</td>
</tr>
<tr>
<td>KTX 3101</td>
<td>208 b (8)</td>
<td>90.4 b (8)</td>
<td>226 a (7)</td>
<td>109 a (6)</td>
</tr>
<tr>
<td>Suwan 3</td>
<td>240 ab (4)</td>
<td>162 a (1)</td>
<td>220 a (8)</td>
<td>96.5 a (8)</td>
</tr>
<tr>
<td>Tuxpeño C(_0)</td>
<td>231 ab (6)</td>
<td>130 ab (5)</td>
<td>244 a (4)</td>
<td>113 a (4)</td>
</tr>
<tr>
<td>Tuxpeño C(_8)</td>
<td>230 ab (7)</td>
<td>134 ab (4)</td>
<td>227 a (6)</td>
<td>140 a (1)</td>
</tr>
<tr>
<td>Average(^1)</td>
<td>241.6 A</td>
<td>131.0 B</td>
<td>246.5 A</td>
<td>113.7 B</td>
</tr>
</tbody>
</table>

Means in a column not followed by the same letter are significantly different at the 0.1 probability level according to Student-Newman-Keuls-Test.

\(^1\) Treatment differences are indicated by different capital letters.
Table 4.8  Stomatal conductance and rankings of eight tropical maize cultivars under well-watered (ww) and water stressed (s2) conditions for the late stress experiment 1993/94.

<table>
<thead>
<tr>
<th>Days after emergence</th>
<th>40 - 47 DAE</th>
<th>48 - 54 DAE</th>
<th>55 - 61 DAE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irrigation treatment</td>
<td>ww</td>
<td>s2</td>
<td>ww</td>
</tr>
<tr>
<td>Cargill 922</td>
<td>280 a (7)</td>
<td>189 bc (5)</td>
<td>218 c (8)</td>
</tr>
<tr>
<td>DKK 888</td>
<td>327 a (1)</td>
<td>251 a (1)</td>
<td>368 a (1)</td>
</tr>
<tr>
<td>Hercules 40</td>
<td>313 a (3)</td>
<td>247 a (2)</td>
<td>319 ab (3)</td>
</tr>
<tr>
<td>KS 6</td>
<td>298 a (5)</td>
<td>161 bcd (6)</td>
<td>287 abc (5)</td>
</tr>
<tr>
<td>KTX 3101</td>
<td>269 a (8)</td>
<td>124 d (8)</td>
<td>284 abc (6)</td>
</tr>
<tr>
<td>Suwan 3</td>
<td>312 a (4)</td>
<td>154 cd (7)</td>
<td>296 abc (4)</td>
</tr>
<tr>
<td>Tuxpeño C0</td>
<td>326 a (2)</td>
<td>213 ab (3)</td>
<td>332 ab (2)</td>
</tr>
<tr>
<td>Tuxpeño C8</td>
<td>287 a (6)</td>
<td>209 ab (4)</td>
<td>255 bc (7)</td>
</tr>
<tr>
<td>Average1</td>
<td>302.3 A</td>
<td>194.4 B</td>
<td>295.1 A</td>
</tr>
</tbody>
</table>

Means in a column not followed by the same letter are significantly different at the 0.1 probability level according to Student-Newman-Keuls-Test.

1 Treatment differences are indicated by different capital letters.
Table 4.9  Stomatal conductance and rankings of four tropical maize cultivars under well-watered (ww) and water stressed (s2) conditions for the late stress experiment 1994/95.

<table>
<thead>
<tr>
<th>Days after emergence</th>
<th>45 - 51 DAE</th>
<th>52 - 61 DAE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ww</td>
<td>s2</td>
</tr>
<tr>
<td>Irrigation treatment</td>
<td></td>
<td>mmol m$^{-2}$ s$^{-1}$</td>
</tr>
<tr>
<td>Cargill 922</td>
<td>207 b (4)</td>
<td>166 a (2)</td>
</tr>
<tr>
<td>DK 888</td>
<td>234 b (2)</td>
<td>181 a (1)</td>
</tr>
<tr>
<td>KS 6</td>
<td>232 b (3)</td>
<td>150 a (3)</td>
</tr>
<tr>
<td>Tuxpeño C$_0$</td>
<td>275 a (1)</td>
<td>144 a (4)</td>
</tr>
<tr>
<td>Average$^1$</td>
<td>237.2 A</td>
<td>160.2 B</td>
</tr>
</tbody>
</table>

Means in a column not followed by the same letter are significantly different at the 0.1 probability level according to Student-Newman-Keuls-Test.

$^1$ Treatment differences are indicated by different capital letters.
water stressed plants was about 20% of the control), DK888 showed a 10 to 30% higher stomatal conductance than KS6. This may indicate that there is a higher stimulus for stomatal closure by soil dryness in KS6 and a tendency to maintain relatively high stomatal conductance under water stress for DK888. For the experimental period when water stress was most severe (last week in late stress 1994/95), the highest conductance was found for KS6 and KTX3101, which might be a result of water savings by early stomatal closure during the development of water stress.

Sensitivity of stomata to water stress might be expressed in terms of relative stomatal conductance compared to the well-watered control. A genotypic susceptibility index (see eq. 4.2), adapted from Fischer and Maurer (1978) for the weighted mean stomatal conductance, is given in Table 4.10. An index higher than 1 indicates a higher reduction of stomatal conductance than the average of all genotypes, whereas an index lower than 1 indicates a relatively small reduction.

Table 4.10 Susceptibility index according to Fischer and Maurer (1978) for the weighted mean stomatal conductance for each experiments.

<table>
<thead>
<tr>
<th></th>
<th>early stress experiment</th>
<th>late stress experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cargill 922</td>
<td>1.02</td>
<td>0.91</td>
</tr>
<tr>
<td>DK888</td>
<td>0.66</td>
<td>0.88</td>
</tr>
<tr>
<td>Hercules 40</td>
<td>1.27</td>
<td>1.22</td>
</tr>
<tr>
<td>KS 6</td>
<td>1.08</td>
<td>1.17</td>
</tr>
<tr>
<td>KTX 3101</td>
<td>1.03</td>
<td>1.14</td>
</tr>
<tr>
<td>Suwan</td>
<td>1.11</td>
<td>0.99</td>
</tr>
<tr>
<td>Tuxpeno C0</td>
<td>0.93</td>
<td>0.93</td>
</tr>
<tr>
<td>Tuxpeno C8</td>
<td>0.92</td>
<td>0.76</td>
</tr>
</tbody>
</table>

Within a single experiment the differences between genotypes in the susceptibility index was never significant, however in all experiments we found for the genotype DK888 comparatively low and KTX3101 high reduction of stomatal conductance under stress. High tendency for stomatal closure was observed for Hercules 40 and KS6 in both years at the early vegetative stage. No consistent classification is suggested for the other varieties since the reaction varied from experiment to experiment.

4.4.4 Canopy Transpiration

Canopy transpirations for the early and late stress experiments, based on mean transpiration rates calculated from the measurements of stomatal conductance and leaf
Table 4.11  Transpiration of eight maize cultivars under well-watered (ww) and water stressed (s1) conditions during the early stress experiments and ranks according to analysis of variance combined over years.

<table>
<thead>
<tr>
<th>Days after emergence</th>
<th>1993/94 DAE 12 - 33</th>
<th>1994/95 DAE 16 - 37</th>
<th>rank in combined analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ww</td>
<td>s1</td>
<td>ww</td>
</tr>
<tr>
<td>Irrigation treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>kg water m⁻²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cargill 922</td>
<td>34.3 bc (5)</td>
<td>7.6 ab (3)</td>
<td>47.6 a (4)</td>
</tr>
<tr>
<td>DK 888</td>
<td>30.2 cd (6)</td>
<td>8.5 ab (2)</td>
<td>48.1 a (3)</td>
</tr>
<tr>
<td>Hercules 40</td>
<td>40.9 ab (2)</td>
<td>7.4 ab (4)</td>
<td>57.5 a (1)</td>
</tr>
<tr>
<td>KS 6</td>
<td>42.2 a (1)</td>
<td>9.6 a (1)</td>
<td>50.8 a (2)</td>
</tr>
<tr>
<td>KTX 3101</td>
<td>35.3 bc (4)</td>
<td>6.6 b (6)</td>
<td>40.9 a (6)</td>
</tr>
<tr>
<td>Suwan 3</td>
<td>39.6 ab (3)</td>
<td>7.1 ab (5)</td>
<td>42.5 a (5)</td>
</tr>
<tr>
<td>Tuxpeño C₀</td>
<td>27.7 d (7)</td>
<td>6.3 b (7)</td>
<td>40.4 a (7)</td>
</tr>
<tr>
<td>Tuxpeño C₈</td>
<td>25.8 d (8)</td>
<td>5.9 b (8)</td>
<td>40.1 a (8)</td>
</tr>
<tr>
<td>Average¹</td>
<td>34.5 A</td>
<td>7.4 B</td>
<td>46.0 A</td>
</tr>
</tbody>
</table>

Means in a column not followed by the same letter are significantly different at the 0.1 probability level according to Student-Newman-Keuls-Test.

¹ Treatment differences are indicated by different capital letters.
**Table 4.12** Transpiration of eight maize cultivars under well-watered (ww) and water stressed (s2) conditions during the late stress experiments and ranks according to analysis of variance combined over years, and over years and irrigation treatments.

<table>
<thead>
<tr>
<th>Days after emergence</th>
<th>1993/94 DAE 40 - 61</th>
<th>1994/95 DAE 44 - 61</th>
<th>rank in combined analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irrigation treatment</td>
<td>ww</td>
<td>s2</td>
<td>ww</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cargill 922</td>
<td>167.2 ab (4)</td>
<td>74.2 b (4)</td>
<td>123.3 a (1)</td>
</tr>
<tr>
<td>DK 888</td>
<td>185.8 a (1)</td>
<td>90.9 a (1)</td>
<td>118.8 a (3)</td>
</tr>
<tr>
<td>Hercules 40</td>
<td>165.8 ab (5)</td>
<td>79.1 ab (3)</td>
<td></td>
</tr>
<tr>
<td>KS 6</td>
<td>158.2 ab (6)</td>
<td>79.2 ab (2)</td>
<td>119.9 a (2)</td>
</tr>
<tr>
<td>KTX 3101</td>
<td>168.6 ab (3)</td>
<td>48.3 c (8)</td>
<td></td>
</tr>
<tr>
<td>Suwan 3</td>
<td>169.3 ab (2)</td>
<td>69.9 b (5)</td>
<td></td>
</tr>
<tr>
<td>Tuxpeño C&lt;sub&gt;0&lt;/sub&gt;</td>
<td>149.3 b (7)</td>
<td>67.5 b (6)</td>
<td>101.6 b (4)</td>
</tr>
<tr>
<td>Tuxpeño C&lt;sub&gt;8&lt;/sub&gt;</td>
<td>142.5 b (8)</td>
<td>64.8 b (7)</td>
<td></td>
</tr>
<tr>
<td><strong>Average&lt;sup&gt;1&lt;/sup&gt;</strong></td>
<td><strong>163.3 A</strong></td>
<td><strong>71.7 B</strong></td>
<td><strong>115.9 A</strong></td>
</tr>
</tbody>
</table>

Means in a column not followed by the same letter are significantly different at the 0.1 probability level according to Student-Newman-Keuls-Test.  
<sup>1</sup> Treatment differences are indicated by different capital letters.
area determinations (see Chapter 2), are shown in Tables 4.11 and 4.12, respectively. Significant genotypic differences were found in all experiments except for the early stress experiment in 1994/95. Genotypic ranking for transpiration was similar to the ranking for dry matter accumulation.

Cumulated transpiration within the irrigation treatment was to a very large extent explained by the leaf area. Modifications due to differences in stomatal conductance and transpiration rates within the water supply treatments were only small. For the late vegetative stage, when a high leaf area had been developed before water was withheld, cumulated transpiration was more closely correlated with transpiration rate than with leaf area, as is shown by a comparison of the correlations of leaf area and transpiration rates with cumulated canopy transpiration (Table 4.13). To account for the development in time of leaf area and transpiration rate the correlations were calculated on weighted means of either trait. The weight for each weekly mean was the ratio of actual leaf area to the maximum leaf area of the experimental period (see eq. 4.1).

Table 4.13 Correlations of genotype means between cumulated transpiration and (a) weighted mean leaf area and (b) weighted mean transpiration rate and the autocorrelation between both latter parameters (n=8, except late vegetative stage 1994/95, where n=4).

<table>
<thead>
<tr>
<th>vegetative stage</th>
<th>early</th>
<th>late</th>
</tr>
</thead>
<tbody>
<tr>
<td>year</td>
<td>1993/94</td>
<td>1994/95</td>
</tr>
<tr>
<td>irrigation</td>
<td>ww</td>
<td>s1</td>
</tr>
<tr>
<td>(a)</td>
<td>0.98</td>
<td>0.85</td>
</tr>
<tr>
<td>(b)</td>
<td>0.08</td>
<td>0.04</td>
</tr>
<tr>
<td>autocorrelation</td>
<td>-0.10</td>
<td>-0.49</td>
</tr>
</tbody>
</table>

* significant with $\alpha < 0.05$
** significant with $\alpha < 0.01$
*** significant with $\alpha < 0.001$
NS not significant

Genotypic differences in soil water uptake for the late vegetative stage were also found with the soil water balance based on neutron probe measurements of soil water content. The zone of maximum water uptake shifted down the soil profile with increasing stress intensity (Fig. 4.9). For well-watered plants most water was taken up from the most upper layer. Maximum rate of water uptake was found at 40 to 50 cm soil depth for the first week of water stress and 60 to 80 cm soil depth in the second period of water stress. The profiles of soil water uptake showed genotypic differences in the depth of maximum water uptake under water stress. While Cargill922 showed the highest uptake from 60 to 80 cm soil depth with considerable uptake from 100 cm,
Fig. 4.9 Profile of cumulated soil water depletion for four varieties under well-watered and water stressed conditions after 8 days (dotted line) and after 17 days (straight line) in the late stress experiment 1994/95. Error bars denote one standard error of mean (n=4).
maximum uptake was restricted to 40 to 60 cm soil depth for Tuxpeño C0. DK888 and KS6 showed maximum uptake also from 60 to 80 cm, but took up less water than Cargill922 at 100 cm soil depth.

4.4.5 Transpiration Efficiency

4.4.5.1 Transpiration Efficiency and Water Limitation

Transpiration efficiency was increased in all experiments under water stress conditions (Tables 4.14 and 4.15). The higher transpiration efficiency, seems however to be of minor importance with respect to the differences in total transpiration and dry matter accumulation between well-watered and water stressed treatments (Fig. 4.10).

![Graph](image)

The change in transpiration efficiency with limited water supply was related to stress intensity, e.g. expressed as the transpiration relative to the control. Transpiration efficiency was similar compared to the control for moderate and severe stress levels, but increased nearly 40% for intermediate levels of stress intensity, when transpiration was decreased by about 40-50% (Fig. 4.11A). A significant contribution of increased transpiration efficiency to dry matter production for water stressed plants is then shown by the relation between relative dry matter accumulation and relative transpiration (Fig. 4.11B). For maximized transpiration efficiency, dry matter accumulation was still about 65% of the control despite a relative transpiration of about 50%.
4.4.5.2 Genotypic Variability for Transpiration Efficiency

For the early stress experiments genotypic differences were found under well-watered conditions (Table 4.14). DK888 showed the highest and Tuxpeño C₀ the lowest transpiration efficiency. In case of water limitation no consistent genotypic differences were found. KS6, which used the water most efficient in 1993/94, showed the lowest ranking for transpiration efficiency in 1994/95. The low amount of water transpired in the early vegetative stage compared to later stages clearly emphasizes the higher importance of transpiration efficiency in later vegetative stages.

No significant genotypic differences for transpiration efficiency were found in the late vegetative stage under both well-watered and water stressed conditions (Table 4.15). However, for well-watered conditions DK888 revealed the highest transpiration efficiency (13% higher than the mean of the well-watered treatment), confirming the results from early stress and first year experiments. With limited water supply the results are contradictory. Tuxpeño C₀, combining low ranking in growth and low transpiration efficiency in the year 1993/94, was superior in transpiration efficiency under water stress in 1994/95.
### Table 4.14 Transpiration efficiency of eight maize cultivars under well-watered (ww) and water stressed (s1) treatments during the early stress experiments and ranks according to the analysis of variance combined over years, and over years and irrigation treatments.

<table>
<thead>
<tr>
<th>Days after emergence</th>
<th>1993/94 DAE 12 - 33</th>
<th>1994/95 DAE 16 - 37</th>
<th>rank in combined analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irrigation treatment</td>
<td>ww</td>
<td>s1</td>
<td>ww</td>
</tr>
<tr>
<td></td>
<td>g dry matter (kg water) $^1$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cargill 922</td>
<td>2.23 a (3)</td>
<td>2.31 ab (6)</td>
<td>3.30 a (4)</td>
</tr>
<tr>
<td>DKK 888</td>
<td>2.32 a (2)</td>
<td>2.54 ab (3)</td>
<td>3.21 a (3)</td>
</tr>
<tr>
<td>Hercules 40</td>
<td>2.00 a (6)</td>
<td>2.44 ab (4)</td>
<td>3.01 a (7)</td>
</tr>
<tr>
<td>KS 6</td>
<td>2.10 a (4)</td>
<td>3.02 a (1)</td>
<td>3.08 a (6)</td>
</tr>
<tr>
<td>KTX 3101</td>
<td>2.10 a (5)</td>
<td>2.72 ab (2)</td>
<td>3.51 a (1)</td>
</tr>
<tr>
<td>Suwan 3</td>
<td>2.34 a (1)</td>
<td>2.33 ab (5)</td>
<td>3.30 a (5)</td>
</tr>
<tr>
<td>Tuxpeño $C_0$</td>
<td>1.91 a (8)</td>
<td>2.10 b (7)</td>
<td>2.91 a (8)</td>
</tr>
<tr>
<td>Tuxpeño $C_3$</td>
<td>1.98 a (7)</td>
<td>2.03 b (8)</td>
<td>3.41 a (2)</td>
</tr>
<tr>
<td>Average $^1$</td>
<td>2.12 A</td>
<td>2.43 B</td>
<td>3.22 A</td>
</tr>
</tbody>
</table>

Means in a column not followed by the same letter are significantly different at the 0.1 probability level according to Student-Newman-Keuls-Test.

$^1$ Treatment differences are indicated by different capital letters.
<table>
<thead>
<tr>
<th>Days after emergence</th>
<th>1993/94 DAE 40 - 61</th>
<th>1994/95 DAE 44 - 61</th>
<th>rank in combined analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irrigation treatment</td>
<td>ww</td>
<td>s2</td>
<td>ww</td>
</tr>
<tr>
<td>Cargill 922</td>
<td>2.31 a (7)</td>
<td>3.43 a (4)</td>
<td>3.11 a (2)</td>
</tr>
<tr>
<td>DK 888</td>
<td>3.03 a (2)</td>
<td>3.31 a (5)</td>
<td>3.49 a (1)</td>
</tr>
<tr>
<td>Hercules 40</td>
<td>2.68 a (4)</td>
<td>3.20 a (7)</td>
<td>2.67 a (4)</td>
</tr>
<tr>
<td>KS 6</td>
<td>2.45 a (6)</td>
<td>3.66 a (3)</td>
<td>3.02 a (4)</td>
</tr>
<tr>
<td>KTX 3101</td>
<td>2.57 a (5)</td>
<td>4.42 a (1)</td>
<td>2.88 a (2)</td>
</tr>
<tr>
<td>Suwan 3</td>
<td>2.25 a (8)</td>
<td>3.29 a (6)</td>
<td>2.89 a (2)</td>
</tr>
<tr>
<td>Tuxpño C₀</td>
<td>2.94 a (3)</td>
<td>2.60 a (8)</td>
<td>3.95 a (1)</td>
</tr>
<tr>
<td>Tuxpño C₈</td>
<td>3.09 a (1)</td>
<td>3.96 a (2)</td>
<td>2.88 A</td>
</tr>
<tr>
<td>Average¹</td>
<td>2.67 B</td>
<td>3.48 A</td>
<td>3.08 A</td>
</tr>
</tbody>
</table>

Means in a column not followed by the same letter are significantly different at the 0.1 probability level according to Student-Newman-Keuls-Test.

¹ Treatment differences are indicated by different capital letters.
4.4.6 Relations between Transpiration Efficiency, Stomatal Conductance and Yield Parameters

Correlations between growth and transpiration efficiency

Transpiration efficiency was negatively correlated with growth traits when genotype means of both water supply treatments were considered, since transpiration efficiency tends to increase while growth was depressed under limited water supply (compare Table 4.2, 4.3, 4.14 and 4.15). Within either irrigation treatment correlations between transpiration efficiency and dry matter accumulation, based on genotype means, were mostly positive (Table 4.16), especially for well-watered conditions. Positive and partially high correlations between transpiration efficiency and growth were found in both water supply treatments, when correlations were based on plot means in either water supply treatment.

Table 4.16 Correlations between transpiration efficiency and dry matter accumulation based on genotypic means (n=8, except for late vegetative stage 1994/95, where n=4) and plot means (n=32, except for late vegetative stage 1994/95, where n=16).

<table>
<thead>
<tr>
<th>vegetative stage</th>
<th>early</th>
<th>late</th>
</tr>
</thead>
<tbody>
<tr>
<td>year</td>
<td>1993/94</td>
<td>1994/95</td>
</tr>
<tr>
<td>irrigation</td>
<td>ww</td>
<td>s1</td>
</tr>
<tr>
<td>genotypes</td>
<td>0.59NS</td>
<td>0.91***</td>
</tr>
<tr>
<td>plot</td>
<td>0.87***</td>
<td>0.90***</td>
</tr>
</tbody>
</table>

* significant with α < 0.05
** significant with α < 0.01
*** significant with α < 0.001
NS not significant

Negative, but not significant correlations were found between the susceptibility index for stomatal conductance and dry matter accumulation under water stress in absolute values or as percentage of the control treatment (data not shown). The rather similar ranking in biomass accumulation of DK888 and Hercules40 or KS6 for the early vegetative stage (Table 4.2) and the rather contrasting rankings in relative stomatal conductance (Table 4.10) indicated, that the sensitivity of stomata was not a major characteristic for the growth of the varieties under drought.

Correlations with grain yield

High correlations of genotype means between shoot biomass at flowering and grain yield were found for the well-watered (r=0.89***, n=8) and early stress treatment (r=0.90***, n=8), whereas the correlation was less strong for the late stress treatment (r=0.57 ns, n=8). Positive, but only for the well-watered treatment in the late stress experiment significant correlations were found for dry matter accumulation during the experimental period and final grain yield (Table 4.17). Both dry weight accumulation
and dry weight at flowering were only weakly correlated to grain yield for water stress at late vegetative stage. This may stress the relevance of the genotypic ability to recover after water stress for the final grain yield.

Table 4.17 Correlations of genotype means between grain yield and dry weight accumulation during the experimental period (n=8) and transpiration efficiency during the experimental period (n=4).

<table>
<thead>
<tr>
<th></th>
<th>well-watered</th>
<th></th>
<th>water stressed</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>early</td>
<td>late</td>
<td>early</td>
<td>late</td>
</tr>
<tr>
<td>dry weight accumulation for experimental period</td>
<td>0.53 NS</td>
<td>0.81*</td>
<td>0.60 NS</td>
<td>0.19 NS</td>
</tr>
<tr>
<td>transpiration efficiency for experimental period</td>
<td>0.47 NS</td>
<td>0.89 NS</td>
<td>0.36 NS</td>
<td>-0.38 NS</td>
</tr>
</tbody>
</table>

* significant with α < 0.05
NS not significant

4.5 Discussion
4.5.1 Shoot Biomass and Grain Yield
Response to pre-anthesis drought

Anthesis and the early grain filling period are the most vulnerable stages to drought stress, presumably due to the high sensitivity of reproducing tissues with respect to water and carbohydrate status (Westgate and Grant 1989, Westgate and Boyer 1985). Therefore, most investigations considered the effects of water deficits during anthesis or grain filling period. Little attention in research was given so far to water stress during vegetative growth, although maize grain yield can be severely affected when water deficits occur during vegetative growth (Muchow 1989, Weerathaworn et al. 1992, Neidhart 1994). Sobrado (1990a) reported grain yield decreases of 10 to 25% and 50 to 70% after withholding water for 25 and 40 days before flowering, compared to a yield reduction of 29 and 20% after 28 and 23 days without irrigation during early and late vegetative stage in our experiments.

Sinclair et al. (1990) found a close relation between biomass at maturity and grain yield when water stress was applied for different periods during anthesis. Experiments of Sobrado (1990a) along with our results showed a close relation for dry weight or leaf area at flowering and grain yield in case of pre-anthesis drought events. In the study of Sobrado water stress was released a few days before anthesis. Correlations of genotype means between vegetative growth until flowering and grain yield were low within stress treatments, as found by Sobrado (1990a) and in our late stress experiment. Higher correlations were revealed, when a period of recovery after drought was included (compare the correlations for the early stress treatment). This finding indicates that the recovery after water stress and the genotypic variability for this ability is probably as important as actual growth under limited water supply in determining grain yield.
Developmental stage

Increased resistance to water stress of maize with ageing of the crop was previously reported. When water was withheld either in the vegetative stage or at tassel initiation and grain filling period, the leaf water potential, where assimilation and stomatal conductance declined to zero was higher for the earlier growth stage (Human et al. 1990, Ackerson 1983). For both periods of pre-anthesis drought in our experiments no change in the sensitivity of vegetative growth to water stress was found, since reduction in dry matter accumulation could be described as a function of the stress intensity, measured as reduction in transpiration. The higher grain yield reduction in 1994/95 after water stress in the early vegetative stage was most probably due to the higher stress intensity in terms of transpiration deficits.

By water stress at the early vegetative stage the harvest index was considerably increased. Increase of the harvest index after a pre-anthesis water stress was also found by Siri (1993) and Neidhart (1994), whereas a stable or decreasing harvest index is reported from other investigations, when water stress was applied either at tasselling or in later growth stages (Denmead and Shaw 1960, McPherson and Boyer 1977, Bolaños and Edmeades 1993, Crauford and Peacock 1993). Depression of early vegetative growth probably caused a bigger proportion of assimilation actually taking place, after reproductive tissue was generated. A smaller proportion of the total assimilates finally found in the grain needed therefore to be translocated from the reserves already present at anthesis.

Selection for drought tolerance

The growth of an open-pollinated variety (KS6) was comparatively vigorous under severe water stress. In 1994/95 the high ranking in biomass after release of early water stress, was maintained in comparison to the hybrids until the final grain harvest. A relatively high grain yield of an open-pollinated variety under severe water stress was reported previously and seemed to be related to high water-use efficiency (Sobrado 1990ab). KS6, too, showed high water-use efficiency for the severe stress situations in the early and late stress experiment in 1993/94. However, no significant genotype by water treatment interaction occurred, therefore a specific drought tolerance was not revealed in our experiments, neither for vegetative growth under water stress nor for grain yield. Similar results were reported for five maize hybrids with highly diverse genetics background (Undersander 1987). Bolaños and Edmeades (1993) could not find a significant cultivar by water supply interaction for the yield of different cycles of the Tuxpeno population, where significant improvement of drought tolerance had been achieved. It seems therefore, that selection for drought tolerant cultivars must not necessarily be undertaken in drought environments. It was in the latter study however concluded, that the selection under water stressed conditions is advantageous for the tolerance to drought events at the flowering stage, since for the anthesis-silking
interval a significant interaction between the gain of selection and selection environment (well-watered or water stressed) was found (Byrne et al. 1995, Bolaños and Edmeades 1993).

4.5.2 Stomatal Conductance

The importance of the investigation of the stomatal behaviour during vegetative growth stages is emphasized by Ackerson and Krieg (1977), who observed only limited stomatal regulation of water loss at the reproductive stage. Reported values of maximum stomatal conductances of 0.6 to 0.8 cm s⁻¹ (Körner et al. 1979, Ackerson and Krieg 1977, Ackerson 1983) agree reasonably well with our measurements in well-watered plants, although accurate comparisons of conductances in velocity units require knowledge about leaf and air temperatures.

Response to soil water status

Response in stomatal conductance to water stress depends like other physiological effects very much on intensity and history of the stress event. Comparisons of genotypes are possibly confounded by different stress intensities, for example induced by faster growth and consequently a faster development of water deficit. Therefore, parameters are needed to describe the water stress status of plants. Differences in leaf water potential between well-watered and water stressed plants do not necessarily occur in field experiments. Differences in stomatal conductance can often not be accounted for by daytime or pre-dawn leaf water potentials and are rather explained by a chemical root signal like ABA or other phytohormones (Gollan et al. 1986, Tardieu et al. 1991, see also Chapter 5). Soil water status might be used as an independent descriptor of stress intensity. Due to problems in estimating soil water retention, transmission properties, and spatial gradients in soil water potential, the water available to plants is not always reflected accurately in soil-based measurements. However, the initial decline in stomatal conductance of water stressed plants was in all experiments strongly correlated with the bulk soil matric potential, as measured by tensiometers. After the initial decrease, stomatal conductance varied from day to day but did not show anymore a decline along with further soil water depletion. Stomatal conductance was at a minimum, before the bulk soil matric potential reached -80 kPa. This is a rather high soil matric potential compared to a permanent wilting point of about -1200 to -1500 kPa. Sharp and Davies (1985) showed, that the rate of root proliferation and water uptake decreased, when the bulk soil matric potential fell below -500 kPa. The water availability in the investigated field site is most probably not primarily limited by the bulk soil matric potential, but by the extremely decreased hydraulic conductivity with decreasing soil water content and insufficient replenishment of water to the soil near to the root surface. The effective soil matric potentials close to the roots are probably much lower than indicated by tensiometers.
Response to air humidity

Partial stomatal closure with decreasing humidity or increasing leaf to air water vapour pressure difference has extensively been studied in many crops in the context of stomatal and environmental control of transpiration (Lange et al. 1971, Lösch and Tenhunen 1981). It is supposed, that transpiration rate or epidermal water loss is sensed and exhibits control of stomatal guard cell behaviour, rather than humidity at the leaf surface or water vapour pressure difference between leaf and air is sensed directly (Bunce 1985, Mott and Parkhurst 1991). Adaptation of stomatal conductance to humidity in a seasonal or daily time scale can enhance the average transpiration efficiency because assimilation is shifted to periods of lower vapour pressure deficit (Sinclair et al. 1984). Partial stomatal closure has been observed in several crops for the time of highest evaporative demand at noon (Farquhar and Sharkey 1982, Ferreira and Katerji 1992). This stomatal regulation is believed to be a mechanism to optimize transpiration efficiency since it prevents excessive transpiration with increased water vapour pressure difference (Cowan and Farquhar 1977, Farquhar et al. 1980). The parallel decrease in conductance with increasing water vapour pressure deficit for all genotypes in Fig. 4.8 indicates little genotypic variation in the adaptation to diurnal variation in water vapour pressure deficits. With limited water supply, there was little or no correlation between stomatal conductance and water vapour pressure deficit, and the genotypic differences diminished. Therefore, the chances to improve transpiration efficiency by adaptation of stomatal response to canopy air humidity are low, especially for conditions of limited water supply, where high transpiration efficiency should be of particular relevance. The low transpiration efficiency of Tuxpeño C₀ under well-watered conditions compared to the other genotypes was indeed a result of higher stomatal conductance at any water vapour pressure difference.

Genotypic variation

The susceptibility index for stomatal conductance can be used to describe genotypes with respect to the sensitivity of stomatal closure. There was no consistent relation to growth under water stress, but low sensitivity was found for the high yielding hybrids DK888 and Cargill922. In other investigations limited to two genotypes, the drought tolerant variety was found to maintain higher stomatal conductance under water stress (Ackerson 1983, Lorens et al. 1987a, O'Regan et al. 1993). It seems therefore, that sensitive stomatal closure is not necessarily a trait characterizing drought tolerance of cultivars selected for high yield instead of survival. It might well be important for drought events occurring under a mediterranean climate, where reduced transpiration in vegetative stage may save water for post-anthesis growth with beneficial effect on grain yield and harvest index, as was proposed for wheat (Passioura 1977).
4.5.3 Canopy Transpiration

Genotypic differences in transpiration and positive correlations between transpiration and growth under limited water supply were found. The profiles of soil water uptake showed differences in the depth of maximum water uptake between well-watered and water stressed plants as well as between genotypes within the water stressed treatment. Sharp and Davies (1985) showed that soil water depletion in deeper soil layers corresponded with both root density and the rate of water uptake per unit root length. Lorens et al. (1987b) showed higher root length densities and O'Regan et al. (1993) a higher percentage of root dry weight in deeper soil layers in the more drought tolerant hybrid. Similar results were observed in sorghum (Wright and Smith 1983). The genotypical ability to penetrate into deeper soil layers and to explore a greater soil volume significantly determines transpiration and growth under limited water supply. The genotypical shift in soil water uptake to deeper layers under drought corresponded well with relative stomatal conductance. This indicates that water uptake enables the plant to maintain higher stomatal conductance as was claimed by O'Regan et al. (1993). On the other hand, high stomatal conductance is a prerequisite for assimilation and hence enables the plant to invest assimilates in the rooting system.

4.5.4 Transpiration Efficiency under Drought

Transpiration and transpiration efficiency in field experiments are mostly estimated either based on a soil water balance or gas-exchange measurements. Our estimates of transpiration efficiency are composed of measurements of leaf transpiration rates and dry matter accumulation. The upscaling of leaf transpiration measurements was described and shown to represent soil water depletion (see Chapter 2). Gas-exchange measurements of assimilation rates or instantaneous water-use efficiencies are difficult to extrapolate to the agronomical meaningful water-use efficiency in terms of shoot biomass or grain yield per unit of water transpired because of respiratory losses and partitioning of gained assimilates between shoot and roots (Martin and Thorstenson 1988). However, most investigations, of genotypic differences in transpiration efficiency, consider the instantaneous gas-exchange water-use efficiency, because of the methodological problems in estimating canopy transpiration on a plot level.

Comparison to gas-exchange transpiration efficiency

The theory of gas-exchange in species with C4 assimilation pathway predicts either increased or decreased instantaneous transpiration-efficiency with partial stomatal closure due to water stress, depending on the relation between stomatal and mesophyll resistance and their changes under water stress (Krieg 1983a, Nobel 1983, Sinclair et al. 1975). Under controlled environmental conditions, a constant ratio of assimilation and stomatal conductance was reported, hence leading to a constant transpiration efficiency even with decreasing stomatal conductance (Wong et al. 1979,
Wong et al. 1985, Takeda et al. 1978), but also increased transpiration efficiencies were found in droughted maize (Evéquoz 1993) and sorghum plants (Premachandra et al. 1994). Moreover, Sobrado (1990b) measured genotypically varying response of transpiration efficiency to decreased leaf water potentials in maize. Pearcy (1983) pointed out, that pretreatment and velocity of stress development determines the contribution of stomatal and non-stomatal limitations of photosynthesis. For slowly imposed water stress and cycles of water stress, the non-stomatal inhibition is assumed to be small and higher dehydration is needed to cause a rise in the mesophyll resistance. The rather slow development of water stress in the field situation, compared to pot studies, might explain the increased transpiration efficiency in our field experiments. Increased values of transpiration efficiency under water stress, based on biomass accumulation and soil water depletion in the field have recently been reported with values of 4.2 - 6 g kg\(^{-1}\) for well-watered and 6.5 - 8.3 g kg\(^{-1}\) for water stressed maize (Otegui et al. 1995). In Chapter 2, we stated an overestimation of transpiration with the porometer. This overestimation might also explain the lower values of transpiration efficiency obtained in our experiments compared to the results of Otegui et al. (1995).

The enhancement of transpiration efficiency was strongly depending on stress intensity. For severely stressed plants negative effects on transpiration efficiency are partly the result of increases in water vapour pressure gradients from leaf to air when decreased transpiration prohibits sufficient cooling of the leaf since transpiration efficiency is negatively associated with the water vapour pressure gradient between leaf and air (Sinclair et al. 1984, Jones 1985). The effect of an increased water vapour pressure gradient due to higher leaf temperature is pronounced in the field with high radiation compared to greenhouse and growth chamber experiments. For severely stressed plants water loss via the cuticula, assumed to be negligible for well-watered plants, makes up a higher proportion of total water loss. Estimates of cuticular conductance for water vapour range from 1 to 20 mmol m\(^{-2}\) s\(^{-1}\) (Schönherr 1982). The upper bound estimate is about half of the stomatal conductance of severely stressed maize plants in our experiment. The increased fraction of epidermal water loss may then contribute to a decreased transpiration efficiency with severe water stress, because it has no link to \(\text{CO}_2\) transport to the site of carboxylation.

**Partitioning of assimilates**

Differences in the partitioning of assimilates in response to water limitations might also result in lower transpiration efficiency defined on above-ground biomass. Enhanced root growth and increased root-shoot ratio under limited water supply has early been reported (Müller-Thurgau 1875). No attempt has been made to estimate produced root dry matter in the field study. Close values for water-use efficiency, based on leaf gas-exchange and measured shoot biomass and water use of well-watered field-grown sorghum plants, indicated little deviation in the carbon balance due to root biomass and respirational losses in sorghum (Peng and Krieg 1992). Ludlow and
Muchow (1990) concluded the carbon costs of an increased rooting system under drought to be of minor importance with respect to the small ratio of root and shoot biomass of plants in later growth stages. However, due to the decay of root biomass, this ratio is likely to underestimate the carbon cost for the rooting system. The pronounced transfer of assimilates into root growth as an early reaction of water stress and the negative effect on transpiration efficiency is also evidenced by the genotypic profiles of soil water uptake. Transpiration efficiency was not increased under the moderate stress intensity in the late stress experiment 1994/95 for the varieties Cargill922, DK888 and KS6; varieties, which all showed strong response to water stress in water depletion of deeper soil layers and therefore presumably strong response in root growth. The high transpiration efficiency of Tuxpeño C0, which did not show a deep root proliferation, could hence partly be a consequence of a shoot pronounced partitioning of assimilates.

**Prospects for the breeding of drought tolerant maize**

In the late vegetative stage the total amount of transpired water is high and the efficient use of water should be especially important for plants under drought conditions. However, for this period no significant genotypic correlations were found between transpiration efficiency and growth parameters under limited water supply, indicating that transpiration efficiency was probably not the most important trait for growth under water stress. The high positive correlation between water-use efficiency and growth under limited water supply, found in the greenhouse study of Siri (1993), could not be observed for drought tolerance in the field. In the greenhouse study limitations for growth under stress may indeed exist in the efficiency of water-use since in small pots genotypic ability for soil water extraction from deeper layers is not essential. In the field, the total amount of transpiration, and hence the efficiency of the rooting system in extracting the soil water, is more limiting. Transpiration efficiency, however, was positively correlated with growth under well-watered conditions. Therefore we do not have any evidence, that by selecting for high transpiration efficiency, we may select for genotypes with a water saving strategy and negative effect on growth, as was assumed by Jones (1993). Also in wheat it has been shown, that transpiration efficiency was positively correlated with growth and yield traits (Siddique et al. 1990, López-Casteñada and Richards 1994). The positive association might be especially valid for early vegetative growth, since in fast developing canopies the average boundary layer resistance of the canopy is higher with beneficial effect on transpiration efficiency via a lower canopy temperature and water vapour pressure deficit. The maintenance of stomatal conductance is hence not only of relevance for assimilation but will certainly help in the efficient use of the sparse water in water stressed canopies.
Diurnal and Seasonal Changes of Leaf Water Status and Relations to Stomatal Conductance

5.1 Abstract

The maintenance of stomatal conductance at a low leaf water status may enable drought resistant genotypes to maintain assimilation and water uptake to higher soil water deficits. Therefore, the relation between leaf water status and stomatal conductance of tropical maize varieties has been investigated. In field experiments during the dry season in Thailand seasonal and diurnal courses of leaf water potential and components, stomatal conductance and transpiration rate have been measured during the period of vegetative growth. Water stress was applied either at the early or at the late vegetative stage. The early afternoon leaf water potential declined throughout the vegetative growth and was linearly correlated with the leaf to air water vapour pressure gradient and the stomatal conductance. Sensitivity of the leaf water potential to changes in either of these parameters decreased at the late vegetative stage, but was not affected by the water supply treatment. After onset of water stress the leaf water potential declined, but was later stabilized due to stomatal closure. No significant difference in the leaf osmotic potential between well-watered and water stressed plants was found. Diurnal courses of leaf water potential revealed that differences between the water supply treatments could either be found early in the morning or during the afternoon and night, but diminished for the hours of maximum transpiration at noon. Maximum stomatal conductance was found early in the morning and did not change substantially for the first hours despite of a steep drop in leaf water potential. The hysteresis in the relation between leaf water potential and stomatal conductance could partly be accounted for by differences in temperature and water vapour pressure deficit, but might additionally be caused by an "afternoon fatigue" in soil water supply to the root, particularly in soils with low unsaturated hydraulic conductivity. One variety (KS6) showed consistently a lower leaf water potential, which was not associated with lower stomatal conductance. The lower water potential might enable this genotype to maintain water uptake to the lower soil water potential.

5.2 Introduction

Stomatal conductance and transpiration rate of tropical maize varieties under different water supply were previously evaluated with respect to the effects on transpiration efficiency and growth parameters (Chapter 4). A substantial effect of the changing stomatal conductance with increasing stress intensity was observed. Transpiration efficiency was initially increased, until under severe water stress non-stomatal limitations of photosynthesis and/or increased water vapour pressure
gradients between leaf and air, caused a decrease in transpiration efficiency. Two major feedback loops are involved in stomatal regulation. By the CO₂ loop, stomatal aperture is regulated by the internal CO₂ concentration as the result of CO₂ influx through the stomata and the photosynthetic capacity of the mesophyll (Willmer and Fricker 1996). Leaf tissue water potential as the result of water lost by transpiration and the availability of water from other plant tissue or the soil, is the central part in the second feedback loop. Physical (hydropassive) and/or chemical (hydroactive) signals transmit leaf water status to the guard cells, which actually exhibit control of stomatal aperture.

In many drought prone regions deficits in the soil water supply occur coincidentally with dry and hot atmospheric conditions. A plant has then to adapt to both low soil water potential and high water vapour saturation deficits in the air. For a range of species including maize, stomatal conductance is assumed to be largely independent of leaf water potential until a critical level is reached. When leaf water potentials drops below this threshold, which is modified by environmental factors and stress history stomatal closure is observed (Hsiao and Bradford 1983).

Osmotic adjustment, as the net accumulation of solutes in response to water deficits, has been shown in a range of species. It is considered to maintain stomatal opening, photosynthesis and the growth of roots and leaves to lower soil and leaf water potential (Turner 1986). Adaptations in the interdependencies between leaf water relations and stomatal conductance under water stress may so provide a means of discrimination between drought sensitive and drought susceptible varieties. Information about the relation between leaf water potential and stomatal conductance is of further interest since the leaf water potential is frequently included in the modelling of stomatal conductance (Lösch et al. 1992, Rochette et al. 1991).

Therefore, we investigated in this study the relations between leaf water status and stomatal conductance as influenced by water stress and high evaporative demand in the field.

5.3 Materials and Methods

5.3.1 Layout of the Experiments

Four experiments were conducted during the dry season 1993/94 and 1994/95 with four replications including eight tropical maize cultivars and two water regimes arranged in a split-plot design. In all experiments water stress was induced by withholding water for approximately four weeks at the early vegetative stage (1993/94 from 5 to 33 days after emergence (DAE) and 1994/95 from 9 to 37 DAE) or at the late vegetative stage (1993/94 from 33 to 61 DAE and 1994/95 from 37 to 61 DAE) later called "early stress" and "late stress" experiments. All plots received two (1993/94) or three (1994/95) sprinkler irrigations after planting until the withholding of water for the
drought treatment. The control plots received weekly furrow irrigation of about 70 mm. The plots included eight (1993/94) or six rows (1994/95) which were 0.75 m apart and the plot size was 36 m². All measurements were restricted to the inner six or four rows. Cultural practices and a detailed description of soil water parameters is given in Chapter 4.

5.3.2 Genotypes

The experiments included eight tropical maize cultivars (open pollinated varieties: two originating from Kasetsart University (KU), Thailand: KS 6 and Suwan 3 and two from CIMMYT, Mexico: Tuxpeno seq. C₀ and Tuxpeno seq. C₇) four hybrids frequently used in Thailand: Cargill 922, DeKalb 888, Hercules 40 (Ciba-Geigy G5440) and KU hybrid KTX 3101.

5.3.3 Leaf water parameters

Porometric measurements were conducted with a LI-1600 steady-state porometer (Li-Cor, Lincoln, Neb. USA). Leaf water potentials were measured with a portable pressure chamber (PMS 1002, PMS Inc., Corvallis, Oregon, USA). The distal part (approximately one third) of the youngest fully developed leaf was cut and immediately inserted into the pressure chamber. For older leaves with prominent midribs the leaf was cut so that about 2 cm of the leaf blade without midrib could be placed into the rubber stopper of the pressure chamber top. After the measurement, the leaf was immediately sealed into a plastic bag and placed on ice in the field until all measurements were finished. Afterwards the samples were stored in a deep freezer. That part of the leaf not used for the measurement of the osmotic potential was sealed into a plastic bag for determination of the water content. Leaves were thawed for about 1-2 hours before cell sap was expressed and osmotic potentials were measured with a vapour pressure osmometer (Wescor 5500, Wescor Inc., Logan, Utah, USA). Leaf osmotic potential has been corrected for the dilution by apoplastic water which occurs when sap is expressed from leaf tissue. The apoplastic water fraction was assumed to be 15%, according to estimates based on concomitant measurements of the osmotic potential by vapour pressure osmometry of mixed sap, and by modified pressure-volume curve measurements (Wenkert 1980). Leaves were not rehydrated. Thus, reported values represent the leaf osmotic potential at the actual relative water content in the field for both pre-dawn and daytime measurements.

Seasonal time courses of stomatal conductance, transpiration rate, leaf water potential and components were established by porometer measurements from 12:00 to 13:00 h and leaf water status measurements from 13:15 to 14:30 h throughout the experiments in the year 1993/94. All porometric measurements were carried out on the abaxial surface of the youngest fully developed leaf. Only non-shaded leaves were used. On each of four to five days per week one replication was measured with six
measurements on randomly chosen plants per plot. The results of the analysis of variance represents weekly means. In the year 1994/95 we measured pre-dawn leaf water potentials and components at the end of the stress periods in all replications with three leaves per plot.

Diurnal changes of stomatal conductance, transpiration rate, leaf water potential and components were measured approximately once a week on one or two genotypes. Each point represents the mean of four to six leaves per time of day, genotype and treatment.

5.4 Results

5.4.1 Seasonal Course of the Water Status of Transpiring Leaves

In Fig. 5.1 the transpiration rate, water potential, osmotic potential and turgor potential of the youngest fully developed leaf are depicted for the course of both experiments in 1993/94. For the well-watered plants leaf water potential declined from -0.8 to -1.3 MPa with ageing of the plant. Turgor potential decreased concomitantly, while leaf osmotic potential showed variation from day to day, but did not exhibit a significant seasonal trend.

The leaf water potential in water stressed plants declined to -1.5 MPa at the end of the late stress experiment. The decrease of leaf water potential occurred mainly at the beginning of the stress period, and was later on stabilized by stomatal closure and low transpiration rates. Except for two days in the early stress experiment the difference in leaf osmotic potential between the well-watered and water stressed plants was low. During the late stress experiment positive turgor potential was not maintained in the leaves of water stressed plants.

The decline of leaf water potentials throughout the experiments was at least partly explained by the higher water vapour pressure deficit during the late stress experiment, since leaf water potentials decreased with increasing water vapour pressure difference between leaf and air (Fig. 5.2). With increasing age the sensitivity for changes in humidity decreased. The developmental stage affected also the relation between leaf water potential and stomatal conductance (Fig. 5.3). Leaf water potential decreased with decreasing stomatal conductance and the leaf water status of plants in the late vegetative stage was significantly lower than in the early vegetative stage for similar values of stomatal conductance. Extrapolations of the regressions suggest a complete stomatal closure with a leaf water potential of about -1.5 MPa, independent of the developmental stage and water treatment. Water stress did not alter the relation between water vapour pressure gradient, respectively stomatal conductance, and leaf water potential significantly, therefore, regressions were calculated for both developmental stages. The effect of developmental stage was similarly evidenced in the relation of leaf water potential and transpiration rate (data not shown). The difference in the leaf water potential between early and late vegetative stage for similar
Fig. 5.1 Early afternoon water potential ($\Psi_L$), osmotic ($\Psi_o$), turgor potential ($\Psi_p$) and noon transpiration rates of well-watered and water stressed maize leaves and noon water vapour pressure difference. The experiments were conducted during vegetative growth and included eight genotypes. The total means of water supply treatments are presented with error bars indicating one standard error of mean (n=32).
Fig. 5.2 Relation between early afternoon leaf water potential and leaf to air water vapour pressure difference (VPD_{leaf to air}) for well-watered (ww) and water stressed (ws) maize at the early (y= -0.17-0.33x) and late (y=-0.96-0.11x) vegetative stage. Each point represents a mean of eight genotypes. Regressions are calculated for each developmental stage and include both water supply treatments.

Fig. 5.3 Relation between leaf water potential and stomatal conductance for well-watered (ww) and water stressed (ws) maize at the early and late vegetative stage. Each point represents a mean of eight genotypes. Regressions are calculated for each developmental stage and include both water supply treatments.

values of stomatal conductance was therefore not solely due to the increased water vapour pressure difference in later vegetative stages.

### 5.4.2 Genotypic Variability in Leaf Water Relations

Genotypic means of water potential, osmotic and turgor potential of leaves, measured, in the early afternoon, during the late stress experiment 1993/94 are given in Table 5.1. Some genotypic differences were found for the leaf water potential and osmotic potential but not for the turgor potential. KS6 showed consistently the lowest water and osmotic potential. The difference in osmotic potential between well-watered
and water stressed plants was very small and not significant, except for the intermediate period of the early stress experiment.

The pre-dawn leaf water potential, measured in the experiments 1993/94, was significantly higher for the well-watered treatment (shown for the end of the late stress period in Table 5.2). Osmotic potential and turgor potential of leaves were only insignificantly decreased in water stressed plants. Apparent osmotic adjustment, as the difference in osmotic potential in well-watered and water stressed plants at maximum rehydration was about 0.08 MPa and, in accordance with the results of daytime measurements, not significant. No significant genotypic variation for the apparent osmotic adjustment was found.

Leaf water potential and stomatal conductance of the means of genotypes were negatively correlated (Fig. 5.4). KS6 showed the lower leaf water potential despite similar values of stomatal conductance in comparison to all other genotypes in both water supply treatments. This potentially different behaviour of KS6 was further investigated in the year 1994/95 by measuring stomatal conductance, transpiration rate and leaf water status in response to the diurnal course of radiation, humidity and temperature.

Table 5.2  Pre-dawn leaf water potential ($\psi_L$), osmotic ($\psi_H$) and turgor potential ($\psi_T$) at the end of the late stress experiment 1994/95 (DAE 60) for four tropical maize cultivars under well-watered (ww) and water stressed (s2) conditions (in MPa).

<table>
<thead>
<tr>
<th>Water Supply</th>
<th>WW</th>
<th>S2</th>
<th>WW</th>
<th>S2</th>
<th>WW</th>
<th>S2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\psi_L$</td>
<td>$\psi_H$</td>
<td>$\psi_T$</td>
<td>$\psi_L$</td>
<td>$\psi_H$</td>
<td>$\psi_T$</td>
</tr>
<tr>
<td>Cargill 922</td>
<td>-0.06a</td>
<td>-0.16a</td>
<td>-1.05a</td>
<td>-1.12a</td>
<td>1.00a</td>
<td>0.96a</td>
</tr>
<tr>
<td>DKK 888</td>
<td>-0.07a</td>
<td>-0.27a</td>
<td>-1.06a</td>
<td>-1.14a</td>
<td>1.00a</td>
<td>0.87a</td>
</tr>
<tr>
<td>KS 6</td>
<td>-0.05a</td>
<td>-0.21a</td>
<td>-1.13a</td>
<td>-1.18a</td>
<td>1.08a</td>
<td>0.88a</td>
</tr>
<tr>
<td>Tuxpeño C0</td>
<td>-0.10a</td>
<td>-0.21a</td>
<td>-1.06a</td>
<td>-1.18a</td>
<td>0.96a</td>
<td>0.97a</td>
</tr>
<tr>
<td>Average 1</td>
<td>A</td>
<td>B</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
</tbody>
</table>

Means in a column not followed by the same letter are significantly different at the 0.1 probability level according to Student-Newman-Keuls-Test.

1 Treatment differences are indicated by different capital letters.
Table 5.1 Leaf water potential ($\psi_L$), osmotic potential ($\psi_o$), and turgor potential ($\psi_P$) at noon and rankings of eight tropical maize cultivars under well-watered (ww) and water stressed (s2) conditions during the late stress experiment 1993/94 (in MPa).

<table>
<thead>
<tr>
<th>Water supply</th>
<th>40 - 47 DAE</th>
<th>48 - 54 DAE</th>
<th>55 - 61 DAE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\psi_L$</td>
<td>$\psi_o$</td>
<td>$\psi_P$</td>
</tr>
<tr>
<td>Cargill 922</td>
<td>-1.27 a (7)</td>
<td>-1.39 a (6)</td>
<td>-1.40 ab (6)</td>
</tr>
<tr>
<td>DKK 888</td>
<td>-1.17 a (2)</td>
<td>-1.34 ab (2)</td>
<td>-1.42 ab (6)</td>
</tr>
<tr>
<td>Hercules 40</td>
<td>-1.12 a (1)</td>
<td>-1.21 a (1)</td>
<td>-1.38 ab (2)</td>
</tr>
<tr>
<td>KS 6</td>
<td>-1.36 a (8)</td>
<td>-1.53 b (8)</td>
<td>-1.41 ab (5)</td>
</tr>
<tr>
<td>KTX 3101</td>
<td>-1.25 a (5)</td>
<td>-1.43 ab (7)</td>
<td>-1.32 a (1)</td>
</tr>
<tr>
<td>Suwan 3</td>
<td>-1.21 a (3)</td>
<td>-1.40 a (3)</td>
<td>-1.40 ab (5)</td>
</tr>
<tr>
<td>Tuxpeno C0</td>
<td>-1.21 a (4)</td>
<td>-1.35 ab (4)</td>
<td>-1.41 a (4)</td>
</tr>
<tr>
<td>Tuxpeno C8</td>
<td>-1.27 a (6)</td>
<td>-1.44 a (8)</td>
<td>-1.56 b (8)</td>
</tr>
</tbody>
</table>

Average

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>A</th>
<th>A</th>
<th>A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cargill 922</td>
<td>-1.38 b (7)</td>
<td>-1.36 a (1)</td>
<td>-1.48 a (8)</td>
<td>-1.38 a (3)</td>
<td>0.10 a (4)</td>
</tr>
<tr>
<td>DKK 888</td>
<td>-1.09 a (1)</td>
<td>-1.14 a (3)</td>
<td>-1.34 a (3)</td>
<td>-1.36 a (2)</td>
<td>0.25 a (1)</td>
</tr>
<tr>
<td>Hercules 40</td>
<td>-1.20 ab (2)</td>
<td>-1.22 a (1)</td>
<td>-1.40 a (4)</td>
<td>0.02 a (6)</td>
<td></td>
</tr>
<tr>
<td>KS 6</td>
<td>-1.39 b (8)</td>
<td>-1.38 a (6)</td>
<td>-1.57 b (8)</td>
<td>-0.01 a (7)</td>
<td></td>
</tr>
<tr>
<td>KTX 3101</td>
<td>-1.30 ab (5)</td>
<td>-1.36 a (4)</td>
<td>-1.33 a (1)</td>
<td>0.06 a (5)</td>
<td></td>
</tr>
<tr>
<td>Suwan 3</td>
<td>-1.35 b (6)</td>
<td>-1.29 a (2)</td>
<td>-1.42 a (5)</td>
<td>-0.05 a (8)</td>
<td></td>
</tr>
<tr>
<td>Tuxpeno C0</td>
<td>-1.21 ab (3)</td>
<td>-1.39 a (2)</td>
<td>-1.42 a (6)</td>
<td>0.16 a (2)</td>
<td></td>
</tr>
<tr>
<td>Tuxpeno C8</td>
<td>-1.22 ab (4)</td>
<td>-1.43 a (4)</td>
<td>-1.37 a (5)</td>
<td>-1.46 a (7)</td>
<td></td>
</tr>
</tbody>
</table>

Average

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>A</th>
<th>A</th>
<th>A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cargill 922</td>
<td>-1.31 a (6)</td>
<td>-1.46 a (4)</td>
<td>-1.53 a (6)</td>
<td>-1.27 a (1)</td>
<td>0.22 a (3)</td>
</tr>
<tr>
<td>DKK 888</td>
<td>-1.29 a (5)</td>
<td>-1.49 a (6)</td>
<td>-1.41 a (4)</td>
<td>-1.36 a (3)</td>
<td>0.12 a (7)</td>
</tr>
<tr>
<td>Hercules 40</td>
<td>-1.29 a (4)</td>
<td>-1.50 a (7)</td>
<td>-1.39 a (3)</td>
<td>-1.42 a (6)</td>
<td>0.10 a (8)</td>
</tr>
<tr>
<td>KS 6</td>
<td>-1.35 a (8)</td>
<td>-1.58 a (8)</td>
<td>-1.53 a (7)</td>
<td>0.23 a (2)</td>
<td></td>
</tr>
<tr>
<td>KTX 3101</td>
<td>-1.19 a (2)</td>
<td>-1.33 a (1)</td>
<td>-1.42 a (5)</td>
<td>0.13 a (6)</td>
<td></td>
</tr>
<tr>
<td>Suwan 3</td>
<td>-1.17 a (1)</td>
<td>-1.33 a (2)</td>
<td>-1.36 a (2)</td>
<td>0.17 a (5)</td>
<td></td>
</tr>
<tr>
<td>Tuxpeno C0</td>
<td>-1.23 a (3)</td>
<td>-1.44 a (5)</td>
<td>-1.38 a (4)</td>
<td>0.21 a (4)</td>
<td></td>
</tr>
<tr>
<td>Tuxpeno C8</td>
<td>-1.33 a (7)</td>
<td>-1.45 a (3)</td>
<td>-1.56 a (7)</td>
<td>-1.53 a (8)</td>
<td></td>
</tr>
</tbody>
</table>

Means in a column not followed by the same letter are significantly different at the 0.1 probability level according to Student-Newman-Keuls-Test. Treatment differences are indicated by different capital letters.

84
5.4.3 Diurnal Course of Leaf Water Relations, Stomatal Conductance and Transpiration Rate

Typical diurnal courses of water potential, osmotic and turgor potential, stomatal conductance and transpiration rate of leaves, are shown in Fig. 5.5 for the genotypes KS6 and DK888 at the end of the late stress experiment 1995 (DAE 60), along with the diurnal courses of leaf and air temperatures, photosynthetic photon flux density PPFD and water vapour pressure deficit in the air VPD. For the hours of maximum transpiration the leaf water potential was not different in water stressed and well-watered plants. The recovery of plant water status throughout the afternoon and the night was significantly delayed in water stressed plants and leaf water potential at pre-dawn were significantly lower. Osmotic potential decreased in the course of the day from about -1 MPa at pre-dawn to -1.5 MPa at the early afternoon with no significant differences between the water supply treatments. Measurements of water content indicated, that the concentration of solutes due to dehydration can not account for the decreased osmotic potential during the day as a consequence of dehydration (data not shown). The turgor potential declined from about 1 MPa at pre-dawn to zero at the early
Fig. 5.5 Diurnal course for leaf water status (left graphs), stomatal conductance, transpiration rate, leaf temperature for two genotypes at the end of the late stress period along with air temperature, water vapour pressure deficit VPD and photosynthetic photon flux density PPFD (right graphs). Each point represents the mean of four to six measurements.
afternoon for both well-watered and water stressed plants. Significantly higher leaf turgor potentials were observed in well-watered plants during the late afternoon and the night.

Stomatal conductance in well-watered plants was high in the morning when both VPD and PPFD were low and declined throughout the afternoon when PPFD decreased but VPD was still high. Transpiration rate showed a more symmetrical pattern throughout the day and was maximum at noon or early afternoon. The time of maximum stomatal conductance in water stressed plants was dependent on stress intensity, but was generally reached earlier in the morning and declined throughout the day. Leaf temperatures were significantly higher than ambient for water-stressed plants and lower for well-watered plants during the hours of transpiration. These differences in leaf temperatures for the different water supply treatments increased significantly the leaf to air water vapour pressure gradient and consequently transpiration rate (compare Chapter 2).

KS6 showed typically for the hours of maximum transpiration a slightly lower leaf water and leaf osmotic potential compared to DK888, what confirms the results of the seasonal measurements in the preceding year. In contrast, diurnal courses of transpiration rate, stomatal conductance and leaf temperatures did at none of the investigated days indicate any significant genotypic differences.

In the diurnal course of the relation between stomatal conductance or transpiration rate and leaf water potential a strong hysteresis effect was found (Fig. 5.6). For the morning hours stomatal conductance did not change substantially despite of the drop in leaf water potential, and for the transpiration rate a significantly higher leaf water potential was observed in the morning compared to the afternoon. These relations were shifted to lower values of leaf water potential and stomatal conductance under water stress but the pattern of hysteresis was maintained. From six diurnal courses, three from the late stress experiment in the year 1993/95 and three from the late stress experiment in the year 1994/95, a data set was extracted with observations of leaf water potential, stomatal conductance and air temperature, humidity and water vapour pressure gradient measured at similar PPFD (1200 to 1700 μmol m⁻² s⁻¹) at about 10:00 and 14:30 h in both the well-watered and the water stressed treatment. Simple and multiple regressions were calculated to reveal if the change in temperature and air humidity can account for the hysteresis in the relation of leaf water potential and stomatal conductance.

Coefficients of determination for the different models are given in Table 5.3, parameter estimates for the models on the whole data set are listed in the appendix A2. About half of the variation in stomatal conductance for the whole data set could be explained by the parameters leaf water potential and temperature. The same degree of determination was found for the stomatal conductance of water stressed plants with the leaf water potential alone. Atmospheric conditions, and especially temperature,
Table 5.3 Coefficients of determination ($r^2$) for simple and multiple regressions of leaf water potential ($\psi_L$), water vapour pressure deficit in the air (VPD) and air temperature (T) on stomatal conductance. Data sets included either all observations (all), or only observations in the morning (AM), or afternoon (PM), or only on well-watered (ww) or on water stressed (s2) plants. Levels of significance are given in brackets, n is the number of observations.

<table>
<thead>
<tr>
<th>data set</th>
<th>n</th>
<th>model $\psi_L$</th>
<th>$\psi_L$ and VPD</th>
<th>$\psi_L$ and T</th>
</tr>
</thead>
<tbody>
<tr>
<td>all</td>
<td>22</td>
<td>0.29 (0.009)</td>
<td>0.32 (0.034)</td>
<td>0.47 (0.003)</td>
</tr>
<tr>
<td>AM</td>
<td>12</td>
<td>0.20 (0.146)</td>
<td>0.36 (0.134)</td>
<td>0.47 (0.057)</td>
</tr>
<tr>
<td>PM</td>
<td>10</td>
<td>0.33 (0.082)</td>
<td>0.33 (0.242)</td>
<td>0.39 (0.180)</td>
</tr>
<tr>
<td>ww</td>
<td>11</td>
<td>0.25 (0.120)</td>
<td>0.25 (0.327)</td>
<td>0.50 (0.063)</td>
</tr>
<tr>
<td>s2</td>
<td>11</td>
<td>0.52 (0.012)</td>
<td>0.53 (0.049)</td>
<td>0.65 (0.015)</td>
</tr>
</tbody>
</table>

could at least partly account for the hysteresis since the coefficients of determinations increased substantially for all data sets, except for the observations in the afternoon.
For this time of the day, stomatal conductance can not sufficiently be explained by leaf water potential and meteorological parameters, indicating that the hysteresis in the relation between stomatal conductance and leaf water potential is not solely due to the change of meteorological parameters. In general, the coefficients of determination were rather low. By including the bulk soil water potential (averaged over all depths) into the model with leaf water potential and temperature, the determination was significantly increased for water stressed plants ($r^2 = 0.84, p=0.004$), and for observations in the morning ($r^2 = 0.60, p=0.050$), but not for the other data sets.

Radiation did not significantly affect stomatal conductance in the selected data set. Also Bethenod et al. (1990, in Tardieu and Davies 1993) reported the maximum stomatal conductance of field-grown maize being reached with less than 1000 μmol m$^{-2}$ s$^{-1}$, moreover, stomatal conductance was usually maximum in the morning and started to decrease despite that PPFD was still rising.

5.5 Discussion

Leaf water potential and stomatal conductance on a seasonal time scale

The higher sensitivity of leaf water potentials to humidity were found in the early vegetative stage, compared to the later vegetative stage, what might be the result of a higher boundary layer resistance in the later vegetative stage (Chapter 2, Fig. 2.2) and higher degree of decoupling the leaf water relations from atmospheric conditions. Throughout the vegetative development leaf water potentials at noon decreased for both well-watered and water stressed maize plants without a consistent decrease in leaf osmotic potentials. However, the leaf water potential, where stomatal conductance declined to zero, was not significantly reduced in the late vegetative stage compared to the early vegetative stage. In other investigations, the leaf water potential, where stomatal conductance or gas-exchange zeroed, was lower at anthesis or grainfilling compared to the vegetative stage. This indicates a higher resistance to water stress in these later stages for maize (Human et al. 1990, Ackerson 1983, McPherson and Boyer 1977), wheat (Teare et al. 1982), or pearl millet (Henson et al. 1983). Decrease in leaf water potentials with ageing of the crop was also found in other field experiments with maize (Fereres et al. 1978, Wolfe et al. 1988). The lowering of the leaf water status in a growing maize canopy may indicate that root proliferation into deeper soil layers and the increase of water uptake does not sufficiently compensate for the higher transpirational water loss of a fully developed maize canopy. This is supported by the finding of a decreasing root-shoot ratio for field-grown maize between 20 to 100 days after emergence (Foth 1962). Bolaños et al. (1993) observed an decrease of leaf water potential from 50 to 100 days after emergence. Maximum leaf area was presumably reached earlier and they postulated an increased resistance to water uptake with ageing of the crop. Stomatal closure at the threshold leaf water potential is assumed to be a mechanism to prevent cavitations in the xylem (Saliendra et al. 1995).
crops might then be less susceptible to xylem cavitations at later growth stages. The lower minimum leaf water potentials may likewise indicate a high priority of maintaining assimilation at reproductive stage, even with the risk of decreased conductivity in the xylem, and uptake of water and nutrients from the soil.

The response in leaf water potential to differences in VPD was not changed by the water supply treatment. The same was found for the relation between leaf water potential and stomatal conductance, both measured in the early afternoon, suggesting a role of the leaf water relations in regulating stomatal conductance independent of the bulk soil water potential. Stomatal responses to atmospheric humidity do, however, not necessarily need a change in the bulk or mesophyll leaf water potential, but are probably induced by cuticular transpiration of the guard cells and neighbouring cells, so called peri-stomatal transpiration, which mainly affects epidermal water potential (Lange et al. 1971, Hsiao and Bradford 1983).

**Leaf water potential and stomatal conductance on a daily time scale**

On a seasonal time scale the values of stomatal conductance and leaf water potential measured at the early afternoon were highly correlated, whereas on a diurnal time scale leaf water potential can by far not account for the change of stomatal conductance. In contrast to growth chamber experiments, where lower leaf water potentials in water stressed maize plants mirrors the lower availability of soil water also throughout the day, this is no longer valid under high evaporative demand in the field. Here the threshold leaf water potential, where stomatal closure occurs, is reached during the hours of high evaporative demand in both well-watered and water stressed plants. Already early in the morning the difference in stomatal conductance and transpiration between well-watered and water stressed plants cannot be explained by differences in leaf water potential, or requires a pre-conditioning of stomatal conductance by the difference in pre-dawn leaf water potential.

Low correspondence between leaf water potential and stomatal conductance has frequently been shown (Bates and Hall 1981, Gollan et al. 1986, Henson et al. 1989ab, Tardieu et al. 1991). ABA synthesis in dehydrating roots and its transport to the shoot via the xylem is believed to override the leaf water status in regulating stomatal conductance (Walton et al. 1976, Davies and Zhang 1991, Tardieu and Davies 1993). Kramer (1988) considered the experimental approaches, where part of the roots or the whole root system was exposed to dry soil, when experimentally a high shoot water status was maintained, as being little related to the field situation. Here water deficits may occur in leaves as a consequence of transpiration despite a rather high bulk soil matric potential. Our field experiments evidenced the regulation of stomatal conductance without changes in leaf water potentials even for a rather high evaporative demand. A constant leaf water status with decreasing stomatal conductance requires a feedforward regulation of stomatal conductance so that the reduced transpiration then
allows the partial recovery of leaf water status. Besides the dehydration of roots in dry soil, changes in the hydraulic resistance in the root have been suggested to be involved in the stomatal control of transpiration (Saliendra and Meinzer 1989). For maize and sugarcane the hydraulic resistance in the root was found to increase gradually with decreasing soil water potentials (Saliendra and Meinzer 1989, Schmidhalter 1995).

The diurnal variation in stomatal conductance may be influenced by a decrease of the soil water potential, near to the root, affecting root water status or root hydraulic conductance, as a consequence of the water uptake during the day, which can not sufficiently be replenished. Diurnal changes of the soil water potential, near to the root, might be particularly high in this soil with low unsaturated hydraulic conductivity. The pattern of hysteresis in the relation between leaf water potential and stomatal conductance was observed for both well-watered and water stressed plants, but the diurnal range of stomatal conductance decreased with decreasing soil water status. No data about diurnal decrease in soil/root water potential for the observed field situation are available, but with the higher rate of water uptake the diurnal drop in soil water status near to the root might be higher for the well-watered plant and cause the greater decline of stomatal conductance in well-watered plants.

The diurnal hysteresis in the relation of leaf water potential and stomatal conductance due to an "afternoon fatigue" in soil water supply may also be valid for other sites, depending on soil hydraulic parameters, and can then provide an explanation for a lack of agreement between modelled and measured values of diurnal courses of stomatal conductance, based on leaf water potential, radiation and meteorological parameters, e.g. the overestimation of stomatal conductance in the afternoon, as observed by Lösch et al. (1992).

**Osmotic adjustment**

A decrease of osmotic potential was observed during the day, but not on a seasonal time scale, neither with measurements at noon nor with pre-dawn measurements. The measurements of osmotic potentials at noon are likely to be confounded by the concentration of solutes due to a decrease in water content, cellular shrinkage, a change in leaf tissue elasticity, or relative partitioning between symplastic and apoplastic water fractions (Girma and Krieg 1992a). At pre-dawn the leaf tissue experiences maximum rehydration and the leaf water potential is assumed to be in equilibrium with the soil water potential in the wettest part of the soil (Schmidhalter 1994). Seasonal changes of osmotic adjustment should rather be elaborated from pressure-volume curves, where information about relative water content, leaf tissue elasticity, and partitioning between apoplastic and symplastic fraction all are available. However, since the relation between leaf water potential and stomatal conductance has not been modified by water stress, a significant effect of osmotic adjustment on
stomatal behaviour was not evidenced in our experiments.

The radiation dependent build-up of solutes induces the decrease of leaf osmotic potentials during the morning hours of the day (Turner and Begg 1973, Acevedo et al. 1979, Wenkert 1981). In contrast to our results, Turner (1974) reported a significantly higher diurnal change of osmotic potentials for maize plants at lower soil matric potentials. The influence of the velocity of water stress development was emphasized by Turner and Jones (1980) and the rather fast development of water stress, particularly for the late stress experiment with the transpiration rate decreasing more than 50% within 10 days, might then explain the lack of osmotic adjustment. However, Premachandra et al. (1989) reported a substantial decrease of osmotic potential (-0.35 and -0.38 MPa) already ten and twenty days after withholding water. The time of sampling in the latter study ranged from 10:00 to 13:00 h. Therefore, the increase might partly be confounded with diurnal adjustment. In accordance with our results Fereres et al. (1978) reported osmotic adjustment only for moderate stress intensity and the early vegetative stage. Limited capacity and genetic variability for osmotic adjustment in tropical germplasm was also reported from other experiments including a broad range of cultivars and populations (Bolaños and Edmeades 1991, Bolaños et al. 1993, Guei and Wassom 1993). The osmotic adjustment observed in sorghum was not associated with the maintenance of stomatal conductance and assimilation rate under water stress (Girma and Krieg 1992b, Flower et al. 1990).

We only focused on the impact of osmotic adjustment on the maintenance of stomatal conductance. Osmotic adjustment certainly affects growth under water stress via other mechanisms, e.g. effects of maintained turgor on cell expansion. For droughted plants the solutes accounting for the decreased osmotic potential are mainly organic (Evequoz 1993). The value of osmotic adjustment for drought tolerance, in the agronomic meaning of higher growth under drought, has then been questioned, since the solutes accounting for it have to be diverted from other processes like protein and cell wall synthesis (Munns 1988). In conclusion, osmotic adjustment seems not to be a substantial tool for increasing drought tolerance in maize.

Genotypic variability in the interdependencies of plant water relations and stomatal conductance

Some genotypic variation for leaf water and leaf osmotic potential was evidenced. Particularly for the water stressed treatment and in tendency also for the well-watered treatment, KS6 had the lower leaf water potential and lower osmotic potential compared to the other varieties but did not deviate in stomatal conductance. In Chapter 4 it was shown, that KS6 showed an early stomatal closure after onset of water stress, but had relatively high stomatal conductance with severe water stress. Comparatively high growth under water stress was found for this variety in the early vegetative stage, which is, with respect to total dry matter accumulation, less important than the late vegetative
stage. A somewhat lower water potential might enable KS6 to maintain water uptake to the lower soil water potential. McGowan et al. (1984) estimated an increase in cumulated water uptake by about 25 mm for wheat, as a consequence of osmotic adjustment and decreased leaf water potentials by about 0.5 MPa. KS6 differed by about 0.15 MPa from the other varieties. For our field experiments, water uptake to a soil water potential of about 0.15 MPa below the average, would increase water uptake by about 2 mm (ΔΘ = Θ (-1.1 MPa) and Θ (-1.15 MPa), 1m depth). In relation to the total water uptake for the stress period, this can be an advantage for the early vegetative stage, but is certainly of minor relevance for the late vegetative stage with a water consumption of up to 50 mm per week (see Chapter 4, Fig. 4.3) In comparison, additionally 15 mm water might be gained by increasing the depth of water uptake by 10 cm (15% plant available water, water uptake of 1 m depth). Both theoretical considerations will hold, for severe and prolonged periods of water stress, and have restricted meaning for spells of drought, where extraction of plant available water is frequently not complete. However, it may indicate some limits for improving water consumption and consequently growth under water stress with selection for the lower leaf water potential. The lower leaf water potential can additionally be advantageous due to the higher uptake of water from the upper soil layers with higher concentrations of mineral nutrients. Besides the amount of total extractable water the increased gradient between soil and plant water potential will also increase the water uptake rate.

In comparisons of drought tolerant and drought susceptible genotypes, the maintenance of higher stomatal conductance to the lower leaf water potential was found for the drought resistant genotype by Skretkowicz and Thurtell (1983). The latter relation was also found to be associated with higher drought resistance in sorghum (Wright et al. 1983, Premachandra et al. 1994). Lorens et al. (1987b) reported the higher leaf water potential along with higher stomatal conductance for the resistant genotype. The common attribute of the drought tolerant variety was the higher root length density deeper in the soil profile for maize and sorghum (Lorens et al. 1987b, Wright and Smith 1983). Wright and Smith (1983) found for the drought resistant genotype in sorghum 65 mm higher soil water depletion at the end of the season due to both more extensive utilization of stored water and the higher uptake in deeper soil layers. The beneficial effect of an enhanced soil water exploration in deeper soil layers for maintenance of growth and stomatal conductance was also emphasized in our experiments (see Chapter 4), but the combination of both traits was not evidenced in the investigated varieties.

The lower leaf water potential observed for KS6 was not associated with the higher plant water stress intensity, since stomatal conductance was maintained and therefore assimilation was at least not prevented by stomatal closure. Hence, the leaf water potential was in this investigation a less suitable tool to monitor water stress intensity for hours of maximum transpiration. A difference in the leaf water potential between well-watered and water stressed plants could either be found at pre-dawn or
in the late afternoon. Therefore, if we are interested in even smaller genotypic differences, we may rather have to measure during the afternoon and at the phase of recovery than during the time of maximum transpiration. Obviously, with respect to the rather steep rise in leaf water potential in the afternoon this is impractical for a higher number of entries. The information about the plant water stress status was in our experiments rather provided by the measurement of stomatal conductance.
In the Chapter 2 and 3 different methods have been evaluated to estimate transpiration and transpiration efficiency in field experiments (porometric determination, soil water balance, mineral element content). With neither of these methods significant genotypic differences in transpiration efficiency were indicated, particularly for water stressed conditions. There are other methods, which were not considered in this study. Meteorological parameters can be used to calculate canopy transpiration according to the Monteith combination equation. Common plot size in field experiments mostly does not fulfill requirements for representative meteorological measurements, however with respect to the uncertainty in the soil water balance it may serve as the reference method in the field and allows furthermore short term measurements on a diurnal time scale (Rochette et al. 1991, Lösch et al. 1992, Jensen et al. 1993). Other ready available systems monitor either the transduction of heat pulses in the stem, or the heat balance for a stem segment, as estimates of sap flow velocity. These sap flow measurements revealed in different woody and herbaceous species a high precision for single plant transpiration rates (Cohen et al. 1990; Steinberg et al. 1989, Ishida et al. 1991). For maize however, severe limitations were found. For the heat balance method the heterogeneous distribution of conducting vessels in the stem leads to poor representation of temperature changes in the stem cross-sectional area because only the stem surface temperature is measured (Cohen and Fuchs 1989, Cohen et al. 1993). For the heat pulse method the low sap flow rates, especially for water-stressed plants, caused poor accuracy. Moreover, this approach is rather appropriate for precise measurements in a few plants than for long term observations in field experiments, since for every plant to measure well-fitting gauges have to be installed. The measurement of assimilation and instantaneous gas-exchange transpiration efficiency does not necessarily represent the transpiration efficiency in an agronomical meaning of above-ground dry matter yield per unit of transpiration. The porometric method has been questioned because of possible artifacts due to the cuvette enclosure of the leaf, but also because of the limited stomatal control of transpiration in a dense crop stand with low boundary layer conductance (Jones 1987). However, reasonable agreement was found for porometrically determined transpiration and transpiration assessed by the soil water balance in this study. Moreover, transpiration rate of a well-watered crop
stand was indeed not strongly dependent on stomatal conductance, whereas for water stressed plants stomatal conductance was a major determinant of transpiration (Fig 4.7). In conclusion, the choice of method is unlikely to be responsible for the insignificant genotypic variation for transpiration efficiency in the field. It may either not exist at all, or at least not in a magnitude, which is significant in field experiments and plays a major role for the dry matter accumulation of genotypes under spells of drought during vegetative growth.

The above methods for determination of transpiration (porometry, gas-exchange measurements, soil water balance) have, however, potential in evaluating traits, which are related to drought tolerance. The impact of water stress on transpiration efficiency is still under debate (see contrasting results of gas-exchange measurements). The measurement of gas-exchange can show whether the limitation of assimilation in water stressed plants is mainly stomatal, as we concluded from the increased transpiration efficiency, or is rather located in the photosynthetic capacity in water stressed plants. Moreover, the relation between carbon assimilation and above-ground biomass may provide information about the root-shoot partitioning of assimilates of different genotypes. Peng and Krieg (1992) assumed that the assimilated carbon, which is not found in shoot biomass has been translocated to the root system. The neutron probe measurements of soil water depletion were found to describe a very decisive trait for the performance under drought. The profile of water uptake indicates the desired trait of water uptake and not the root growth per se, which was speculated to be potentially higher than optimum (Passioura 1983). The genotypic correspondence of growth under drought and soil water extraction at deeper soil layers suggests that the investment into the root system does not imply deficits in shoot growth. Fischer et al. (1983) suggested in view of the inherent difficulties in measuring root growth and water uptake that the selection for an improved leaf water status would result in an improved root system. The higher root length density and higher leaf water potential was found for the drought resistant genotype by Lorens et al. (1987b). However, the higher leaf water status can be misleading in maize, considering our results where differences in the leaf water status did not have a meaning for stomatal conductance under water stress. Extraction of soil water in deeper soil layers was rather mirrored in a maintained stomatal conductance. The investigation of soil water uptake was in this study limited to four genotypes. Since relative stomatal conductance corresponded with the genotypic profiles of soil water uptake further studies are required to manifest the potential screening of rooting characteristics by this measurement on the easy accessible part of the plant.
Genotypic variability for stomatal response to water stress has previously been shown in maize (Ackerson et al. 1980, Skretkowicz and Thurtell 1983, Lorens et al. 1987b, Human et al. 1990, O'Regan et al. 1993). These experiments were mostly done with potted plants and were all limited to two genotypes. In this study the limited applicability of stomatal conductance measurements in a range of genotypes under field conditions was evidenced with the insufficient precision and thus impractical high number of measurements which are required. The high variability in stomatal conductance with changing environmental conditions has not been sufficiently considered and is often likely to be responsible for the low correlations with more integrative traits of performance under drought (see e.g. Tuberosa et al. 1994). For selection the variability does not only restrict the gain of selection but may also increase the risk for discarding potentially good lines. With respect to the high variability of stomatal conductance we may therefore look for other traits characterising a specific stomatal behaviour with less environmentally mediated variation. The concentration of ABA in leaf tissue or xylem sap is such a potential trait, although ABA is known to induce many changes in the physiology and development of crops (see e.g. Quarrie 1991). Sensitive stomatal closure was correlated with high ABA concentration and suggested to be associated with drought tolerance (Pekic and Quarrie 1987, Lebreton et al. 1994). Our results of stomatal behaviour and performance under drought suggest that rather the maintenance of stomatal conductance is a trait characterising drought tolerance than a sensitive stomatal behaviour. Landi et al. (1995) found delayed pollen shed, reduced plant height and higher grain yield reduction under water stress in hybrids with the higher ABA concentration in leaves and concluded the concentration of ABA rather to be an indicator of plant water stress than a causal agent capable of limiting yield reduction under drought stress. Thus, it is not yet consistently shown which designation of stomatal behaviour and ABA concentration is characterising drought tolerance in an agronomical meaning of higher yield under drought, and is certainly dependent on the drought scenario studied. It might also be possible that the drought tolerance enhancing effect of ABA is not primarily linked to the stomatal behaviour, but to the enhancement of root growth with consequences for the total water uptake. When genotypic variation in transpiration efficiency does not exist the amount of total water uptake determines dry matter yields under limited water supply.
Summary

Spells of drought during the growing season are major limitations to maize production in semi-arid regions. In the range of physiological traits related to drought tolerance, transpiration efficiency, as the ratio of dry matter produced per unit of transpiration, has received early scientific interest. However, and at least partly due to methodological problems, little information is currently available about transpiration efficiency of field-grown maize plants and its potential for improving drought tolerance.

Therefore, we investigated (i) methodological approaches to determine transpiration efficiency and (ii) genotypic variability in transpiration, transpiration efficiency and the related traits stomatal behaviour and leaf water relations. Responses to water stress in either of these traits were analyzed with respect to the potential for improving drought tolerance in maize.

In growth chamber and field experiments during the dry season in Thailand, coefficients have been determined to measure plant and canopy transpiration based on porometric assessment of stomatal conductance. Gravimetric determination of water loss from potted plants and a soil water balance, based on neutron probe measurements, served as the reference methods. Above-ground dry matter accumulation, stomatal conductance, canopy transpiration and transpiration efficiency, and leaf water relations were investigated for eight tropical maize cultivars during the vegetative growth. The field experiments were conducted for two years during the dry season in Thailand. Water stress was imposed either at the early or at the late vegetative stage by withholding the weekly furrow irrigation for a period of four weeks. Additionally, the soil water depletion was monitored at 20, 40, 60, 80 and 100 cm soil depth with neutron probe measurements.

Transpiration of a maize canopy could be described by the stomatal conductance at a defined leaf position and the whole plant leaf area. Some limitations of the method caused an overestimation of transpiration with porometry for field conditions, but relative comparisons were reliable. Statistical analysis of the variability of transpiration rates in the field revealed that porometric measurements may allow detection of genotypic differences for well-watered plants, but with water stressed plants differences must exceed 20%.

Another more integrative approach for the determination of transpiration efficiency has been investigated. For mineral elements, taken up by mass-flow, the total mineral
element content in the plant should be correlated with cumulated transpiration, and consequently, the concentration of the mineral element to the transpiration efficiency. Maize plants were grown, either in pots or in the field, under different water supply. The concentration of mineral elements and hydrochloric acid non-soluble ash (nHCl-ash) as an indicator of Si in the plant tissue was measured. In the pot experiments the uptake of Si, could be described assuming mass-flow. In the field a high, non-linear correlation was found for the concentration of nHCl-ash in the leaves and transpiration rate, averaged for the preceding period. In accordance with the porometric result there was no significant genotypic differences in transpiration efficiency indicated by the concentration of nHCl-ash. When variation in transpiration efficiency was increased by considering treatment means significant correlations were obtained. The concentration of other mineral elements was not suitable for evaluating transpiration and transpiration efficiency in maize.

Water stress reduced dry matter accumulation, stomatal conductance and transpiration. With increasing stress intensity water was taken up successively from deeper soil layers. Transpiration efficiency increased initially under water stress to about 140% of the control, and decreased with higher stress intensity. Diurnal courses of leaf water potential revealed that differences in leaf water potential between water supply treatments can either be found early in the morning or during the afternoon and night, but diminished for the hours of maximum transpiration at noon.

Genotypic differences were significant for dry matter accumulation and transpiration but not for the response to water stress. Despite generally high error variances genotypical differences in stomatal conductance and in the response to water stress were indicated. Differences in cumulated transpiration were to a very large extent determined by the leaf area and only slightly affected by genotypic differences in stomatal conductances within the irrigation treatment. The genotypical shift in soil water uptake to deeper soil layers under drought corresponded with the maintenance of high stomatal conductance under water stress. One variety showed consistently a lower leaf water potential in both water supply treatments. The lower leaf water potential was not associated with higher plant stress, since stomatal conductance was maintained. Genotype means of dry matter accumulation were positively correlated with transpiration efficiency in well-watered plants, but were insignificantly correlated within the water stressed treatment.

No significant genotypic differences were found for transpiration efficiency in eight tropical maize cultivars. Therefore, not the transpiration efficiency but rather the cumulated transpiration, determined by the ability to explore water from a greater soil volume was the major characteristic of drought tolerance in maize.
References


KRIEG, D. R. 1983b. Whole-plant response to water deficits: Carbon assimilation and utilization. Pp. 319-329 in Limitations to efficient water use in crop...


VAN DER VORM, P. D. J. 1980. Uptake of Si by five plant species, as influenced by variation in Si-supply. Plant Soil 56, 153-156.


**Appendix**

**Table A1.** Estimated parameters for linear regression models of soil matric potential in the range of -5 to -80 kPa as measured by tensiometers, on stomatal conductance (in mmol m\(^{-2}\) s\(^{-1}\)). Data from Fig. 4.4 and 4.5.

<table>
<thead>
<tr>
<th>Soil matric potential at</th>
<th>n</th>
<th>Intercept ± S.E.</th>
<th>Regression coefficient ± S.E.</th>
<th>(r^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 cm depth</td>
<td>36</td>
<td>262*** ± 7.47</td>
<td>2.58*** ± 0.25</td>
<td>0.75***</td>
</tr>
<tr>
<td>40 cm depth</td>
<td>47</td>
<td>240*** ± 10.0</td>
<td>3.84*** ± 0.48</td>
<td>0.58***</td>
</tr>
<tr>
<td>60 cm depth</td>
<td>50</td>
<td>272*** ± 11.3</td>
<td>8.76*** ± 0.88</td>
<td>0.67***</td>
</tr>
<tr>
<td>80 cm depth*</td>
<td>24</td>
<td>308*** ± 19.2</td>
<td>13.6*** ± 1.80</td>
<td>0.70***</td>
</tr>
</tbody>
</table>

* significant with \(\alpha < 0.001\)

# only for one year (1994/95) and one field

**Table A2.** Estimated parameters and coefficients of determination \((r^2)\) for simple and multiple regressions of leaf water potential \((\psi_L\text{, MPa})\), water vapour pressure deficit in the air \((VPD\text{, kPa})\) and air temperature \((T\text{, }°C)\) on stomatal conductance \((g_s\text{, mmol m}^{-2}\text{ s}^{-1})\). Data set consisted of measurements on six different days at late vegetative stage at about 10:00 and 14:30 h and similar PPFD (1200 to 1700 \(\mu\text{mol m}^{-2}\text{ s}^{-1}\)) in both well-watered and the water stressed maize plants \((n=22)\).

<table>
<thead>
<tr>
<th>(a) ± S.E.</th>
<th>(b) ± S.E.</th>
<th>(c) ± S.E.</th>
<th>(r^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>model: (g_s = a + b \psi_L)</td>
<td>421*** ± 94.2</td>
<td>206** ± 72</td>
<td>0.29**</td>
</tr>
<tr>
<td>model: (g_s = a + b \psi_L + c VPD)</td>
<td>435*** ± 100</td>
<td>252* ± 116</td>
<td>15.2NS ± 29.9</td>
</tr>
<tr>
<td>model: (g_s = a + b \psi_L + c T)</td>
<td>148NS ± 147</td>
<td>418** ± 114</td>
<td>16.9* ± 7.4</td>
</tr>
<tr>
<td>model: (g_s = b \psi_L + c T)</td>
<td>457*** ± 107</td>
<td>23.0*** ± 4.3</td>
<td>0.83***</td>
</tr>
</tbody>
</table>

*** significant with \(\alpha < 0.001\)

** significant with \(\alpha < 0.01\)

* significant with \(\alpha < 0.05\)

NS not significant
ZUSAMMENFASSUNG

Ertragsausfälle im Maisanbau werden in tropischen und subtropischen Regionen häufig durch das Ausbleiben von Niederschlägen für einige Wochen während der Vegetationsperiode verursacht. Die Transpirationseffizienz, als das Verhältnis produzierter Biomasse zur Gesamttranspiration, ist eines der möglichen physiologischen Merkmale, um die Trockenheitstoleranz verschiedener Maisorten zu beeinflussen. Dass die Transpirationseffizienz und ihre Bedeutung für die Trockenheitstoleranz verschiedener Sorten dennoch bisher selten untersucht wurde, ist im wesentlichen auf Probleme bei der präzisen Bestimmung der Transpiration unter Feldbedingungen zurückzuführen.


während der Versuchsperiode bestimmt.


Eine limitierte Wasserversorgung führte zur Reduktion der Trockenmassebildung, der stomatären Leitfähigkeit und der Transpiration; die Wasseraufnahme verlagerte sich in tiefere Bodenschichten. Die Transpirationseffizienz zeigte eine deutliche Optimumsbeziehung zur Stressintensität und war maximal um ca. 40% erhöht, wenn die kumulierte Transpiration auf ca. 50% der Kontrolle gesunken war. Infolge der limitierten Wasserversorgung sanken auch das Blattwasser- und Turgorpotential, wobei allerdings die Unterschiede zur Zeit der maximalen Transpiration am Mittag gering waren im Vergleich zu den Unterschieden, die am Morgen, am Nachmittag oder während der Nacht gemessen wurden.


Es zeigten sich keine signifikanten Unterschiede in der Transpirationseffizienz der untersuchten acht Sorten. Schlussfolgernd ist daher die Trockenheitstoleranz einer Maisorte eher charakterisiert durch die Gesamttranspiration - im wesentlichen bestimmt durch die Fähigkeit ein möglichst großes Bodenvolumen für die Wasseraufnahme zu erschliessen - als durch eine hohe Transpirationseffizienz.
Acknowledgements

I wish to thank Prof. Dr. Peter Stamp for providing me with the opportunity to work on this interesting project, and for the moral support he always gave. I especially appreciated his visits in Thailand, where I was lucky to receive unbelievable amounts of the sparse time of a professor.

I am particularly grateful to Dr. Urs Schmidhalter, the grounder of the project for his introduction into the world of 'water in plants'. In numerous discussions he contributed to many ideas, and his fruitful criticism during all stages of my study have been invaluable.

Furthermore, my sincere thanks go to Dr. Christian R. Jensen for reading the thesis as a co-examiner and for his constructive comments.

I would like to thank Mrs. Yuppapan and Prof. Dr. R. Thiraporn of the Kasetsart University in Bangkok for providing the opportunity and infrastructure for the field experiments on Farm Suwan. Many thanks go also to the staff and the workers on the farm - I will never forget the time I spent at this research station.

I am also thankful to Prof. Dr. E. Frossard who kindly permitted the use of all the facilities in the group of Plant Nutrition.

My thanks go further to Mrs. Theres Roesch for her help with the analysis of mineral elements and Michel Evéguiz and Yuncai Hu for all their adept advice. I gratefully acknowledge the help of the students Renate, Urs and Sâmi, who contributed to parts of the experimental work in the field. I wish to thank all the colleagues of the old and new group of Plant Nutrition and of the group of Crop Science in Eschikon and Zurich. Their friendship ensured an excellent working atmosphere. An unforgettable experience was furthermore the spirit of the different Sola-Teams G'stoertlis, Framps and Ackerschnecken, where, for the sake of the team, nobody failed to prevent being the last.

Finally, I wish to thank Claudia for her love, support and patience and for the distraction in times when it was needed.

This project was funded by the Swiss National Science Foundation.
Curriculum Vitae

January 13, 1964
Born to Johannes Camp and Irmgard née Gleumes

1970 - 1974
Primary school in Heiligenhaus

1974 - 1983
Grammar school in Heiligenhaus

1983 - 1985
Civil service with the Johanniter Unfallhilfe in Ratingen

1985 - 1987
Apprenticeship in farming with F. Schmitz in Duisburg and L. Weber in Essen

1987 - 1992
Student at the Faculty of Agriculture of the University of Kiel (Germany)

1992
Diploma in Plant Production

1990 - 1992
Research assistant at the Institute of Crop Science and Plant Breeding of the University in Kiel

1993 - 1996
Research assistant at the Institute of Plant Sciences of the Swiss Federal Institute of Technology (ETH) in Zürich (Switzerland)