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Stomata-characteristics and Responses to Soil Drought Indicating a Cultivar Specific Drought Stress Susceptibility in Asparagus

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Summary

Plant water relations, gas exchange parameters and stomata morphology of potted white asparagus (*Asparagus officinalis* L.) plants were investigated to evaluate the degree of their drought resistance. Two cultivars, 'Gijnlim' and 'Grolim', with high commercial relevance in Germany and with contrasting yield and drought susceptibility were examined. Exclusively the pore length of the kidney-shaped stomata differed but stomata density was similar between the two lines. Stomatal pore length measured on 'Grolim' plants was nearly twice that of 'Gijnlim'. Drought stress was gradually induced by withholding irrigation from one-year old fully developed asparagus plants grown under well-watered conditions in 15 L pots in a greenhouse. During desiccation, predawn water potential of plant (ψ_{PD}) and soil (ψ_{soil}), and midday gas exchange of cladophylls were determined. Under well-watered conditions ($\psi_{soil} > -8$ kPa), plant of 'Grolim' showed higher net assimilation rates (A) and leaf conductance (g_{H2O}) than 'Gijnlim' plants. Intrinsic water use efficiency (WUE_{int}) of unstressed plants did not differ between the cultivars. Only 'Grolim' plants reduced g_{H2O} in response to increased vapour pressure deficit between leaf and air (VPD_{al}), under well-watered conditions. Water shortage rather than VPD_{al} triggered stomatal control. Under severe soil drought conditions ($\psi_{soil} < -50$ kPa), WUE_{int} of 'Grolim' was higher than that of 'Gijnlim' and g_{H2O} was lower. Under air and soil drought stress, the more sensitive stomatal regulation of 'Grolim' indicates a more efficient protective mechanism against detrimental water loss. These results suggest that 'Gijnlim' employs a strategy of drought tolerance whereas 'Grolim' employs one of drought avoidance. For productivity purposes, water management of 'Grolim' is advantageous due to lower susceptibility to drought stress and higher net photosynthesis under optimal conditions.

Key words. water stress – plant water relations – drought resistance – soil water deficit – gas exchange – anisohydric – water potential

Introduction

Asparagus is the most widely grown vegetable crop per area in Germany. It is cultivated on 21.693 ha in 2007, occupying ca. 20 % of the land used for vegetable production (Behr 2008). Its high commercial importance demonstrates the need for optimal cultivation, particularly with regard to water management. Cultivation on sandy soil with low water retention capacity requires optimal water supply as spear yield in the following season is highly correlated with irrigation in the previous year (Takatori et al. 1970; Kaufmann 1977; Hartmann 1989).

Photosynthesis is highly sensitive to environmental stresses via the stomatal closure and is thus in danger of being limited by drought stress (Hsiao et al. 1976; Schulze 1986). Photosynthesis is a highly relevant mechanism through which to investigate the impact of drought stress, because it is the primary process for plant biomass production. Annual spear yield of asparagus is closely and positively associated with net photosynthetic rate (A) and photosynthetic activity differs between asparagus cultivars (Bai and Kelly 1999; Faville et al. 1999; Guo et al. 2002).

Several studies have focused on the long-term effect of contrasting water deficit treatments on the physiology and morphology of asparagus (Wilcox-Lee 1987; Hartmann 1989; Drost and Wilcox-Lee 1990, 1997; Paschold and Mayer 2005, 2006; Mayer and Paschold 2007). These studies exclusively reflect the impact of long-termed drought stress on yield, and root and shoot growth. Drought stress thresholds of hydro-physiological processes have not been reported for asparagus so far. Hence, little is known about the responses of asparagus water relations to water deficits and the factors that control these responses. This paper shall address this paucity of information.

'Gijnlim' and 'Grolim', cultivars with high commercial relevance in Germany, possess a different yield potential with regard to quality and quantity (Paschold et al. 2005). This feature is attributed to morphological differences between these genotypes (Ziegler 2002). Differences in stomata morphology may strongly influence transpiration and net CO_2 uptake. Cultivar specific features of stomata have not yet been investigated for asparagus but have been reported for other species (Borel et al. 1997; Gucci et al. 2002).

Stomatal architecture may provide an indication of the strategies used by plants to cope with drought stress (Olyslaegers et al. 2002). For example, small pore size is associated with greater stomatal responsiveness to water deficits. Differences in stomatal control among cultivars may be linked to differences in drought susceptibility (Borel et al. 1997). 'Gijnlim' plants show a higher susceptibility to drought stress than 'Grolim' plants under field conditions (Paschold 2006), which may be caused by different stomatal features and behaviour among the cultivars.

This study addresses the hypothesis that stomata-controlled water relations of 'Gijnlim' are more susceptible to water deficiency than that of 'Grolim'.

Materials and Methods

Plant material

One-year-old dormant crowns of male F_1-hybrids of *Asparagus officinalis* L. 'Gijnlim' and 'Grolim', with similar weight and bud number, were selected at the end of April 2006. Single crowns were planted into 15 L pots in sand and arranged on tables in fully randomized blocks in a glasshouse. Maximum daytime temperature was 20–25 °C. Plants were irrigated with 1 L per pot water when soil matrix potential (ψ_{soil}) decreased to −10 kPa, and fertilised weekly from mid-May to the end of August with 6.4 g N (4.4 g NO_3-N, 2 g NH_4-N), 2.4 g P_2O_5 and 10.4 g K_2O per pot in total. Plants were exposed to sunlight reaching a maximum photosynthetic photon flux density (PPFD) of 1400 µmol m^{-2} s^{-1}. For each of the six desiccation trials eight plants were selected, after cladophylls had fully developed on the first ferns. Four plants of similar size and structure were selected per cultivar. One plant per cultivar remained well-watered, as the control; three plants per cultivar had irrigation withheld in order to induce progressive drought stress. Desiccation from well-watered to severely drought-stressed plants took approximately one week. Experiments stopped when ψ_{soil} decreased below −80 kPa, the lower limit of measurable ψ_{soil} with a tensiometer. This procedure was carried out 8, 9, 13, 14, 21 and 22-weeks after crown was planted.

Soil Moisture

Soil matrix potential was measured daily at predawn and midday during the entire course of the desiccation experiments. It was monitored as suction pressure (−kPa) with tensiometers and data logged with TensioSave (Tensio-Technik, Geisenheim, Germany). Irrigation was scheduled by TensioControl-System (Knauer, Frankfurt, Germany). Predawn ψ_{soil} was compared to predawn plant water potential (ψ_{PD}), midday values were taken as reference parameter for instantaneous water supply during gas exchange measurements.

Plant Water Status

Plant water status was determined as ψ_{PD} using a Scholander-type pressure bomb (SKPM 1440, Skye Instruments Ltd., UK). Third order shoots were detached from ferns of first order, which were then chosen for gas exchange measurements at midday. Immediately after detaching, the shoot was placed in a humid plastic bag and, within 30 s, was inserted into the pressure chamber. Then pressure inside the chamber was slowly increased by $5x10^{-3}$ MPa s^{-1} (Turner et al. 1988). When xylem sap appeared on the cut surface first ψ_{PD} reading was taken, the pressure was then slowly reduced until the xylem sap disappeared from the surface. The pressure was again slowly increased until xylem sap reappeared and a second ψ_{PD} reading was taken. The pressure was raised and lowered at least twice as advised by von Willert et al. (1995).

Gas Exchange

Gas exchange measurements were made of attached and fully expanded mature cladophylls directly facing the sun. Gas exchange was measured with a portable gas exchange system (GFS-3000, WALZ, Germany). For each measurement between 20 and 30 cladophylls where inserted into the standard leaf cuvette with an 8 cm^2 surface. After each measurement the investigated twigs with cladophylls were cut from the plant and their dry mass (g) was determined after drying in an oven at 105 °C for 12 h. Contrary to appearances, cladophylls are not cylindrical but have a wavy epidermis, which enlarges the active gas exchange area (Güvenc and Koyuncu 2002). The surface area of cladophylls is therefore extremely difficult to measure. Consequently, cladophyll dry mass served as a reference parameter for net CO_2 assimilation rate (A) and leaf conductance (g_{H2O}). Air-to-leaf vapour pressure deficit (VPD_{al}) and A were calculated according to von Caemmerer and Farquhar (1981). VPD_{al} is calculated as air to leaf deficit in the mole fraction of water vapour per ambient air pressure (Pa kPa^{-1}). Water use efficiency (WUE_{int}) is defined as the ratio of A and g_{H2O}.

To provide reasonably constant environmental conditions (photosynthetic photon flux density PPFD, temperature, VPD_{al}) gas exchange was always measured in the greenhouse at midday between 11 a.m. and 1 p.m. with the PPFD controlled at 1000 µmol m^{-2} s^{-1} (LED light source 3040 L, WALZ, Germany) and the CO_2-concentration set to 466 ± 3 µL L^{-1}. On average measuring date ambient light intensity rarely exceeded 1000 µmol m^{-2} s^{-1}, which is the saturating photon flux rate of net CO_2 exchange, as determined under similar environmental conditions (data not shown). Gas exchange data were recorded when CO_2- and H_2O-concentrations in the cuvette remained stable for at least one minute. Zero difference between reference and sample infrared gas analyser was adjusted every 15 min. For each of the eight plants, measurements with twigs of three different shoots were carried out. Different ranges of VPD_{al} had to be taken into account when analysing results because of variations in atmospheric conditions (ambient temperature and air humidity) between the measurements days.

Stomatal density and stomatal pore length

For morphological analysis of stomata, the asparagus cladophyll epidermis was examined using a CCD video camera attached to a Leitz Wetzlar stereo microscope. The tissue originated from plants of the cultivars 'Gijnlim' and 'Grolim', which had been purchased as F_1-hybrid crowns from a transplant producer (LimSeeds, Nether-

lands) and grown under identical conditions as previously described (cf. plant material). Tissue samples were taken from three plants of each cultivar on stems and cladophylls on nine different positions of the fern in October 2006. Pieces of one-layer epidermis were peeled off with precision tweezers (0.34 mm tip width, Dumont, Switzerland). Stomatal density was determined and pore length of 452 stomata was measured.

Statistical analyses

Statistical analyses were performed with the open source software R (R Development Core Team 2008). Results of gas exchange measurements, ψ_{PD} and ψ_{soil} were tested for normality and homoscedasticity, non-linear and linear regression analysis and Pearson product-moment correlation coefficient (r). Kruskal-Wallis one-way analysis by ranks was employed to test for difference among the cultivars with respect to gas exchange measurements. Differences were considered significant, if $P<0.05$. ANOVA was used to test for cultivar differences in stomatal characteristics. ANCOVA was applied for cultivar differences in A referring g_{H2O} as covariate and for cultivar differences in ψ_{PD} referring ψ_{soil} as the covariate.

Results

Relationship between leaf conductance (g_{H2O}) and net assimilation rate (A)

Leaf conductance of asparagus plants directly and closely affected A over a range of soil water availability (Fig. 1). The covariance analysis, using g_{H2O} as the covariate, revealed no cultivar differences in the relationship between A and g_{H2O} ($P<0.05$).

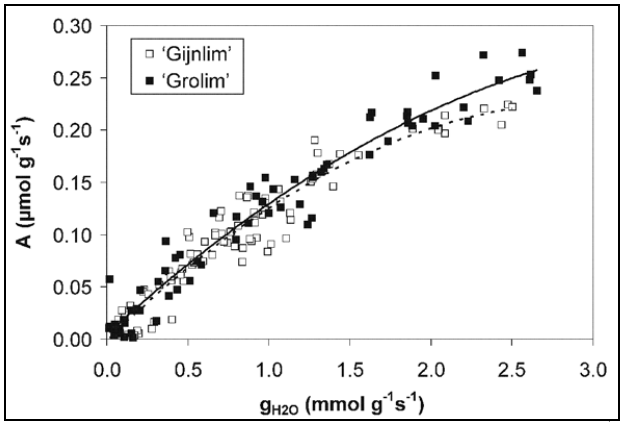


Fig. 1. Net assimilation rate (A) as a function of leaf conductance (g_{H2O}) of plants of asparagus 'Gijnlim' (dotted line; n=87) and 'Grolim' (solid line; n=60) over a range of soil water potentials. Each symbol represents the results of three shoots per plant. Equation for 'Grolim': $f(g_{H2O}) = -0.018\ g_{H2O}^2 + 0.14\ g_{H2O} + 0.0045$, $R^2 = 0.95$***; equation for 'Gijnlim' $f(g_{H2O}) = -0.025\ g_{H2O}^2 + 0.15\ g_{H2O} - 0.0015$, $R^2 = 0.92$***. The covariance analysis, using g_{H2O} as the covariate, revealed no significant differences ($P<0.05$) between the cultivars for A.

Cultivar differences in stomatal responses to evaporative demand (VPD_{al})

Evaporative demand on 24 measurement days varied within a range of 8 to 37 Pa kPa^{-1}. To qualify the influence of VPD_{al} on g_{H2O}, data is sorted into six VPD_{al} (5 ± 2.5 Pa kPa^{-1} steps) and two soil moisture classes. The relationship between VPD_{al} and g_{H2O} was examined for well-watered (ψ_{soil}> −8 kPa, Fig. 2a) and drought-stressed (ψ_{soil}<−50 kPa, Fig. 2b) plants of 'Grolim' and 'Gijnlim'. When well-watered, 'Gijnlim' plants showed no influence of VPD_{al} on g_{H2O}. Leaf conductance of 'Grolim' was significantly lower when VPD_{al} increased from 25 to 35 Pa kPa^{-1}. Non-irrigated plants of both cultivars had substantially reduced g_{H2O} compared to well-watered plants, irrespective of VPD_{al}-range. Under these conditions, 'Gijnlim' plants significantly reduced their g_{H2O} when VPD_{al} increased from 10 to 15 Pa kPa^{-1}. Any additional rise in evaporative demand had no further effect on g_{H2O} of drought-stressed plants irrespective of genotype.

Genotypic differentiation of gas exchange

Well-watered plants of 'Grolim' showed significantly higher g_{H2O} (1.50 ± 0.67 mmol g^{-1} s^{-1}) and 30 % higher

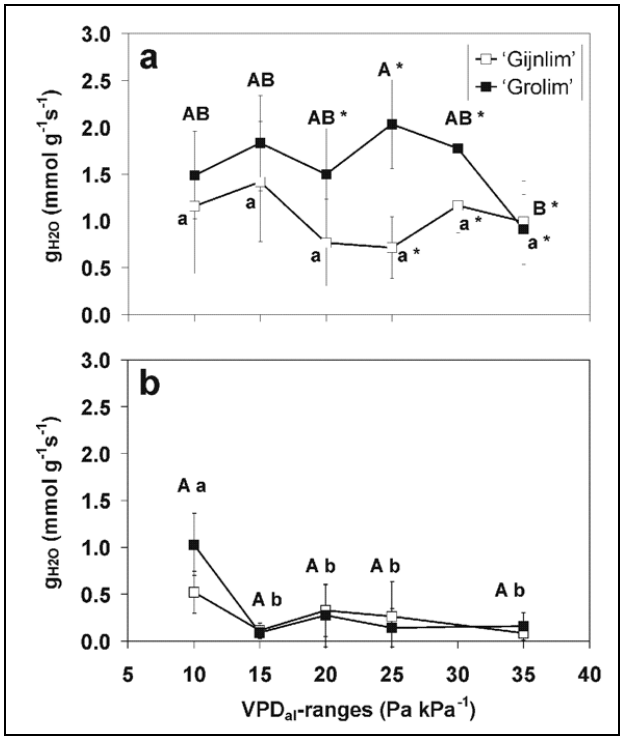


Fig. 2. Mean midday leaf conductance (g_{H2O}) at different leaf-to-air vapour pressure deficits (VPD_{al}) for plants of 'Gijnlim' (n=107) and 'Grolim' (n=90) with (a) well-watered status (soil water potential > −8 kPa), and with (b) drought-stressed status (soil water potential < −50 kPa). Each symbol represents the mean of six measurements; significant differences between g_{H2O} at the different VPD_{al}-classes are illustrated with letters ('Gijnlim': small letters, 'Grolim' capital letters). Stars mark significant cultivar difference in g_{H2O} within single VPD-classes. H-test, $P<0.05$.

A (0.17 ± 0.06 µmol m^{-2} s^{-1}) than those of 'Gijnlim' (1.05 ± 0.58 mmol g^{-1} s^{-1} and 0.12 ± 0.05 µmol g^{-1} s^{-1}), respectively (Fig. 1).

The short-term effect of decreasing soil moisture on g_{H2O} and A was analysed in order to reveal cultivar specific drought stress thresholds for ψ_{soil}. Soil matrix potential is ranked in four classes (Fig. 3) covering the increase of soil water deficit from wet (−2 to −8 kPa) to dry (−50 to −80 kPa), based on preliminary work of Mayer and Paschold (2007). Their results show differences in shoot and root growth of potted asparagus which were irrigated differently corresponding to the chosen ψ_{soil}-classes. For both cultivars, g_{H2O} tended to decrease with soil drying (Fig. 3a). Below a ψ_{soil} of −50 kPa, the decline in g_{H2O} was significant in plants of both cultivars. Significant cultivar specific differences in g_{H2O} only occurred at very high and very low soil water status. Leaf conductance of 'Grolim' plants was higher than that of 'Gijnlim', except at very low soil water availability (−50 to −80 kPa). In this case, g_{H2O} of 'Gijnlim' plants was 40 % higher with the risk of higher water loss. Net CO_2 uptake of both cultivars tended to decline with increasing soil water deficit (Fig. 3b), as expected from the dominant effect of g_{H2O} on A (Fig. 1). Differences between cultivars in A were only significant under well-watered conditions. Net CO_2 uptake of 'Grolim' plants tended to be higher than in 'Gijnlim', except at very low ψ_{soil}. 'Gijnlim' plants did not derive higher carbon gain by maintaining g_{H2O} at severe soil drought. Using the intrinsic water use efficiency (WUE_{int}), the influence of VPD_{al} on photosynthetic WUE may be neglected. Soil water potential affected WUE_{int} differently for the genotypes, however, plants of both cultivars obtained maximum WUE_{int} at the same ψ_{soil} (−30 to −50 kPa, Fig. 3c). Nevertheless, with increasing soil drought, WUE_{int} increased to a greater degree for plants of 'Grolim' than for 'Gijnlim'. Under severe water deficit (−50 to −80 kPa) plants of 'Grolim' maintained a higher WUE_{int} than those of 'Gijnlim'.

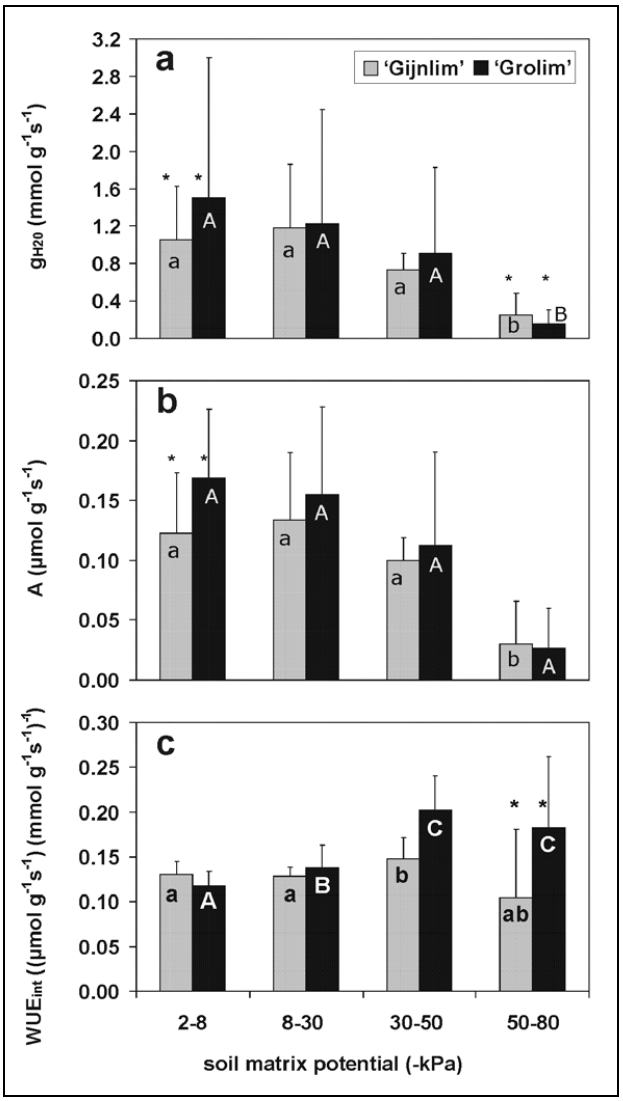


Fig. 3. (a) Leaf conductance (g_{H2O}), (b) net assimilation rate (A) and (c) intrinsic water use (WUE_{int}, c) of 'Gijnlim' (total n=86, r=3) and 'Grolim' (total n=69, r=3) plants at different soil deficit. Different letters indicate significant differences between gas exchange parameter at the various soil matrix potential classes ('Gijnlim': small letters, 'Grolim': capital letters), stars mark the variety specific differences in g_{H2O}, A and WUE_{int}. H-test. P<0.05.

Relationship between soil moisture and predawn shoot water status

Water potential of plant and soil were determined at predawn to evaluate nocturnal rehydration of plants during desiccation experiments (Fig. 4). Well-watered control plants of 'Gijnlim' and 'Grolim' in pots with ψ_{soil}> −8 kPa maintained well balanced ψ_{PD} between −0.2 and −0.3 MPa during the whole vegetation period. The ψ_{PD} in non-watered plants declined slightly without cultivar differences, with decreasing ψ_{soil}, until ψ_{soil} reached −60 kPa. The threshold, at which ψ_{PD} decreased strongly, occurred between −60 and −70 kPa corresponding for

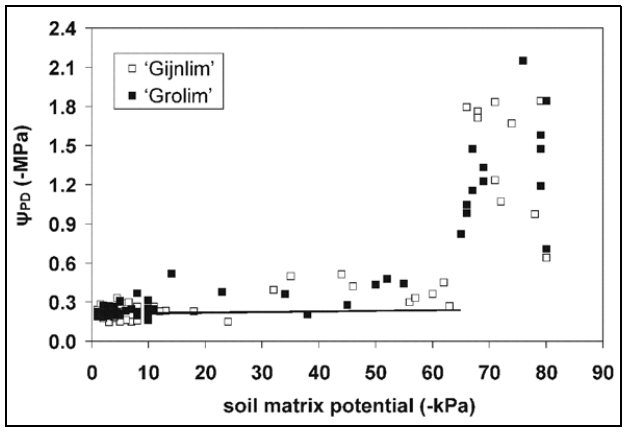


Fig. 4. Relationship between predawn shoot water potential (ψ_{PD}) and predawn soil matrix potential (ψ_{soil}) of 'Gijnlim' (n=79) and 'Grolim' (n=67); linear regression of Grolim: $f(\psi_{PD}) = 0.0006\ \psi_{soil} + 0.2053$, $R^2 = 0.0731$ and of Gijnlim: $f(\psi_{PD}) = 0.0004\ \psi_{soil} + 0.2112$, $R^2 = 0.027$; The covariance analysis, using ψ_{soil} as the covariate, revealed no significant differences (P<0.93) between the cultivars for ψ_{PD} at ψ_{soil}>65 kPa.

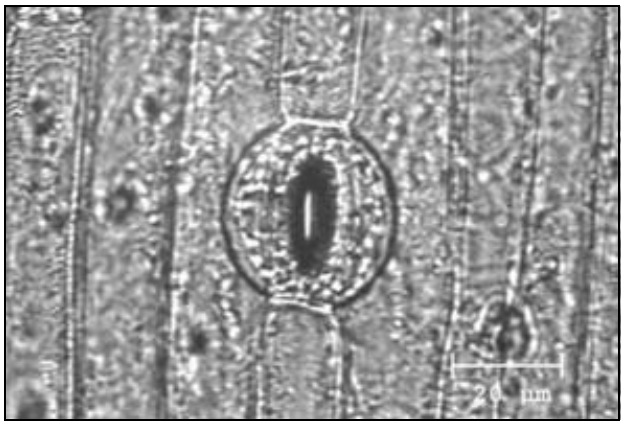


Fig. 5. Kidney-shaped stoma of liliaceae type on cladophyll of 'Grolim'.

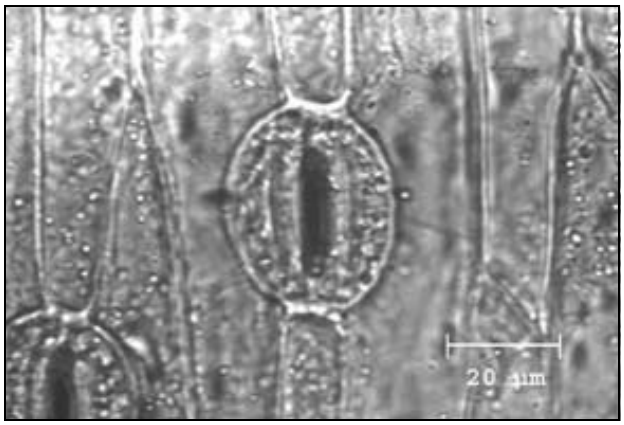


Fig. 6. Kidney-shaped stoma of liliaceae type on cladophyll of 'Gijnlim'

both cultivars. This threshold coincides with the exponential reduction of water capacity in sandy substrate (retention curve of the substrate not shown here) and suggests that water status of desiccating asparagus plants is closely related to ψ_{soil}. A regression model for the relationship was not calculated including $\psi_{soil} < -65$ kPa, because of the high statistical spread of ψ_{PD}.

Cultivar effect on stomata density and stomatal pore length

Stomata of asparagus (Fig. 5, 6) were identified as the kidney-shaped type using stomata classification of Hetherington and Woodward (2003). They are located on stems but mainly on cladophylls (Table 1). On the latter, they were found uniformly distributed. No significant cultivar specific difference exists for stomatal density. However, cladophylls of 'Grolim' had significantly and nearly two-fold longer stomatal pores than those of 'Gijnlim'.

Discussion

Photosynthesis is one of the primary processes affected by drought (Chavez et al. 2009). To evaluate the drought effect on gas exchange in this study, it is mandatory to exclude metabolic influences on net assimilation rate. On the adjusted measuring conditions, A closely correlated with g_{H2O} (Fig. 1), indicating that diffusion limitation and stomatal aperture, were the major factors influencing photosynthesis (Flexas et al. 2007). This observation permits disregarding metabolic limitation of gas exchange (Jones 1985, 1992). Stomata-controlled gas exchange defined net photosynthesis of asparagus cladophylls unspecific for genotypes. The exclusion of non-stomatal metabolic limitation of photosynthesis agrees with observations for well-watered asparagus cultivars 'UC-157', 'Jersey Giant' and 'Karapiro' under similar conditions (Faville et al. 1999).

The effects of VPD_{al} on gas exchange parameters have to be clearly characterised in order to relate explicitly the drought stress reactions of the investigated shoots to soil water deficit. The close examination of the effects of VPD_{al} on gas exchange parameters led to the classification of the single shoot measurements into VPD_{al}-classes according to Turner et al. (1985). Using this approach, the analysis of our results did not give any indication of a direct stomatal response to increasing VPD_{al} in well-watered plants of 'Gijnlim' plants, but of 'Grolim' at high evaporative demand. According to Passioura (2002) and Chavez et al. (2009) the reaction of 'Grolim' stomata to low air humidity may be explained by feed forward response and suggests the genotype could employ a strategy of drought avoidance. Drost and Wilcox-Lee (1990) described nearly constant g_{H2O} of asparagus 'Jersey Giant' with diurnal variation in VPD_{al}, whereas Bai and Kelly (1999) found a negative correlation between VPD_{al} and g_{H2O} of asparagus 'Franklim'. As it is reported for many herbaceous spe-

Table 1. Stomatal characteristics of two *Asparagus officinalis* L. 'Gijnlim' and 'Grolim'. All cladophylls were sampled in October 2006 for epidermical investigations. Stomatal density and pore length of 452 stomata were statistically compared using ANOVA and Tukey-test. Each data presents the mean ± standard deviation.

Cultivar	Stomatal density of cladophylls (number mm^{-2})	Stomatal pore length of cladophylls (µm)	Stomatal density of stems (number mm^{-2})	Stomatal pore length of stems (µm)
'Gijnlim'	98.13 ± 25.6	27.12 ± 2.2	69.77 ± 29.4	33.14 ± 4.3
'Grolim'	94.98 ± 32.2	52.43 ± 34.3	67.10 ± 26.8	43.00 ±18.4
HSD	n.s.	9.8***	n.s.	25.3***

***, n.s.: Significant at P<0.001, or not significant at P<0.05, respectively

cies, naturally growing in sunny habitats (LARCHER 1994) the authors conclude that asparagus 'Grolim' behaves anisohydrically. Anisohydric species accept the risk of substantial diurnal turgor loss in leaves and do not control stomatal with increasing VPD_{al}. Hence, asparagus 'Grolim' closely reflects the gas exchange characteristics found in other anisohydric herbaceous species like sunflower, wheat and barley (TARDIEU and SIMMONEAU 1998).

Photosynthetic susceptibility to soil drought specific for a genotype can be evaluated by examining g_{H2O} responses, since non-metabolic limitation of stomatal response was excluded (JONES 1985; SCHULTZ 1996; FAVILLE et al. 1999).

The higher A of plants of 'Grolim' plants, under high soil water supply (−2 to −8 kPa) compared to that of 'Gijnlim' plants, is consistent with previously reported genetic variation in maximum A among asparagus genotypes (BAI and KELLY 1999; FAVILLE et al. 1999; GUO et al. 2002). In plants of both cultivars, g_{H2O} and A tended to decrease from the beginning of soil drying. This is accordant with the general suggestion that soil drought is among the primary factors to cause stomatal limitation (HSIAO et al. 1976; CHAVEZ et al. 2009). Genotypic differences in A and g_{H2O} of well-watered and stressed plants characterise plants of 'Grolim' to operate with high photosynthetic efficiency at high soil water availability but to minimize A due to low g_{H2O} at severe soil water deficit.

This positive feature of plants of 'Grolim' may be due to higher stomatal sensitivity for changes in air and soil water conditions than that of 'Gijnlim' plants. Detected cultivar differences suggest a more distinctive drought avoidance strategy of 'Grolim' than 'Gijnlim', in accordance with the hypothesis of 'drought avoidance' by LEVITT (1980). Drought avoidance by 'Grolim' plants may delay soil water depletion and consequently the onset of plant water deficit (LUDLOW and MUCHOW 1990).

Examination of WUE_{int} results leads to similar conclusions. While WUE_{int} depends on prevailing environmental conditions, it provides an evaluation of the photosynthetic activity (JONES 1992). WUE_{int} of both cultivars altered with changes in soil water deficit. 'Grolim' plants tended to have higher WUE_{int} at the beginning soil drought ($\psi_{soil} < -50$ kPa) and maintained twice higher WUE_{int} than plants of 'Gijnlim' at soil drought ($\psi_{soil} < -80$ kPa). According to JONES (1992), plants with higher WUE_{int} under soil drought, like those of 'Grolim', feature drought stress avoidance. This observation agrees with characteristics of 'Grolim' plants rather than of 'Gijnlim' plants.

Analogous to the asparagus cultivars, grapevine cultivars had similar WUE_{int} at non-stress level and differed incrementally during progressive soil drying SCHULTZ (1996). Comparing the observed stomatal behaviour of asparagus cultivars under drought conditions with those of the reported grapevine genotypes, 'Grolim' would be characterized as drought avoidant and 'Gijnlim' as drought tolerant (SCHULTZ 1996).

Stomata morphology was analysed in plants of 'Grolim' and 'Gijnlim' as possible indicators of stress resistance (HETHERINGTON and WOODWARD 2003). Both cultivars had a similar stomatal density. Therefore, the significantly greater stomatal pore length of 'Grolim' may be the main reason for the significantly higher maximum g_{H2O} and consequently, higher A of well-watered plants. This positive relationship between stomatal pore length and g_{H2O} has been reported, cultivar-specifically, for barley (BOREL et al. 1997). However, stomatal characteristics were not consistent with drought avoidance stomatal behaviour, as assumed by HETHERINGTON and WOODWARD (2003) and AASAMA et al. (2001). HETHERINGTON and WOODWARD (2003) attributed stomata with greater pore size, as examined for 'Grolim', to drought insensitive, since they close more slowly and having hydraulic dysfunctions more often under drought than small pore length.

Observations by SPENCE et al. (1986) and MEIDNER and MANSFIELD (1968), that small pore length, as determined for 'Gijnlim', is advantageous for gas exchange under drought, contradict our results. On the other hand, stomatal dimensions and behaviour under drought conditions of different genotypes of olives are consistent with the results of this study (GUCCI et al. 2002). Larger pore length is associated with high water conductivity and high gas exchange rate (SACK et al. 2003). This characterises drought avoidant genotypes (OLYSLAEGERS et al. 2002). Both higher water conductivity and higher gas exchange rates are attributes of 'Grolim'. This may point to a higher drought avoidance of this genotype.

Values of predawn plant water status became highly variable for both cultivars when ψ_{soil} fell between −70 and −80 kPa. The reason for this increased variability is most likely imprecise measurements of ψ_{soil} due to the tensiometers approaching the lower limit of their metering range at −80 kPa (Tensio-Technik, Germany). Above this range, the relationship between ψ_{soil} and ψ_{PD} under non-transpiring conditions reflects the balanced water status of potted asparagus after nocturnal rehydration. According to the air-seeding hypothesis (ZIMMERMANN 1983), the exponential decrease of ψ_{PD}, when ψ_{soil} fell below −60 kPa, is caused by the change from cavitated to embolised water conduits, which impairs xylem function severely (SALLEO and LO GULLO 1989, SPERRY et al. 1993). Therefore, the observed critical ψ_{soil} interval represents the threshold for potentially irreversible drought stress response of the species without cultivar distinction. Similar observations, of almost unchanged ψ_{PD} during reduction of soil water content followed by an abrupt decrease in ψ_{PD} at threshold of soil water content, exist for anisohydric barley lines (BOREL et al. 1997). Like the anisohydric asparagus genotypes in this study, no varietal differences in the reaction of ψ_{PD} to ψ_{soil} are reported for the above mentioned barley varieties. Changes in plant water potential did not aid differentiation of genotypic genotypic responses to soil drought in the anisohydric asparagus cultivars.

Conclusions

Methods applied to investigate stomata dimensions and drought responses seem appropriate to reveal genotypic differences in drought susceptibility. 'Grolim' plants showed higher photosynthetic activity and lower susceptibility of water relations to air and soil drought than 'Gijnlim' plants. These results endorse the hypothesis of genotype specific drought vulnerability.

Future examination of plant water relations under field conditions by measurement of gas exchange, hydraulic conductivity and water potential are required to evaluate drought vulnerability under practical conditions.

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