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ORIGINAL PAPER

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# Mycorrhizal impact on drought stress tolerance of rose plants probed by chlorophyll *a* fluorescence, proline content and visual scoring

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**Abstract** Micropropagated rose plants (*Rosa hybrida* L., cv. New Dawn) were inoculated with the arbuscular mycorrhizal (AM) fungus *Glomus intraradices* (Schenk and Smith) and subjected to different drought regimens. The dual objectives of these experiments were to investigate the mechanism and the extent to which AM can prevent drought damages and whether physiological analyses reveal enhanced drought tolerance of an economically important plant such as the rose. In a long-term drought experiment with four different water regimens, visual scoring of wilt symptoms affirmed that AM in a selected host–symbiont combination increased plant performance. This effect was mostly expressed if moderate drought stress was constantly applied over a long period. In a short-term experiment in which severe drought stress was implemented and plants were allowed to recover after 4 or 9 days, no visual differences between mycorrhizal and non-mycorrhizal roses were observed. Therefore, the early physiological steps conferring drought tolerance were prone to investigation. Proline content in leaves proved to be an unsuitable marker for AM-induced drought tolerance, whereas analysis of chlorophyll *a* fluorescence using the JIP test (collecting stress-induced changes of the polyphasic O-J-I-P fluorescence kinetics in a non-destructive tissue screening) was more explanatory. Parameters derived from this test could describe the extent of foliar stress response and help to differentiate physiological mechanisms of stress tolerance. AM led to a more intense electron flow and a higher productive photosynthetic activity at several sites of the photosynthetic electron transport chain. A K step, known as a stress indicator of general character, appeared in the fluorescence transient only in drought-stressed non-mycorrhizal plants; conversely, the data elucidate a stabilising effect of AM on the oxygen-evolving complex at the donor site of photosystem (PS) II and at the electron-transport chain between PS II and PS I. If drought stress intensity was reduced by a prolonged and milder drying phase, these significant tolerance features were less pronounced or missing, indicating a possible threshold level for mycorrhizal tolerance induction.

**Keywords** Arbuscular mycorrhiza · Chlorophyll fluorescence · Drought stress tolerance · *Glomus intraradices* · *Rosa hybrida*

**Abbreviations** ABS: absorption · AM: arbuscular mycorrhiza · AMF: arbuscular mycorrhizal fungi · Chl *a*: chlorophyll *a* · DI: dissipation · ET: energy flux for electron transport · Fo, Fm: initial and maximum Chl *a* fluorescence · Fv/Fm: maximum quantum efficiency of primary photochemistry of photosystem II · O, K, J, I, P: intermediate steps of Chl *a* fluorescence rise between Fo and Fm · OEC: oxygen-evolving complex · PI: performance index · PS I and PS II: photosystems I and II · QA: plastoquinone · RC: reaction centre · Sm: normalized area above the Chl *a* fluorescence transient · TR: energy flux for trapping

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## Introduction

Drought leads to serious changes in plant physiology particularly in nitrogen and carbon metabolism of the root (Augé et al. 1992), often followed by a decrease of photosynthetic activity and thus reduced assimilation (Chaves et al. 2002). The ability to cope with drought stress is not only genetically dictated, but also delineated by the individual history of a plant (i.e. specific hardening conditions). Drought stress tolerance can be induced by arbuscular my-

corrhiza (AM) in several plants (Augé 2001). However, if AM fungi (AMF) are to be applied in the plant production process to achieve mycorrhiza-dependent drought-tolerance effects, an in-depth understanding of this phenomenon is essential for an early and continuous evaluation of plant vitality and tolerance against abiotic stress.

The facets of drought stress tolerance are multiple and cannot simply be attributed to the enhanced phosphorus supply of mycorrhizal plants (Davies et al. 1996; Schönbeck et al. 1994). Goicoechea et al. (1998) observed that AM enhanced the content of free polyamines in alfalfa, thus suggesting a better adaptation of mycorrhizal plants to drought. Augé et al. (1992) traced the positive effect of AM on drought tolerance in *Rosa hybrida* L. cv. Love to an increased content of free amino acids and sugars in the roots. In addition, the exploitation of an enlarged soil water volume by mycorrhizal hyphae may contribute to better plant performance (Bethlenfalvay et al. 1988). AM-induced drought stress tolerance involves several physiological processes: (1) modifications of foliar water relation parameters such as gas exchange, leaf water potential, leaf tissue elasticity and stomatal behaviour (Larcher 1994) and (2) alterations of root turgor and the root to shoot signals, e.g. in mycorrhizal cowpea plants (Duan et al. 1996).

The development of an appropriate and easily applicable methodology to evaluate the performance of stressed plants in a non-destructive manner remains an unsolved issue until now. Furthermore, for the explanation of stress tolerance effects in mycorrhizal symbiosis definite tools in plant physiology are required.

In general, stressed plants cope with suboptimal physical conditions by adjusting thermodynamical stability (Tsimilli-Michael and Strasser 2002). AMF obviously influence the initial physiological state of their host plants and/or modify the reactions towards stress parameters that enhance internal entropy. Strasser et al. (1995) detected the polyphasic character of the chlorophyll *a* (chl *a*) fluorescence curves that changes due to environmental variation and so reflects the state of the photosynthetic apparatus. In the JIP test, data points of this fluorescence curve (O, J, I, P) and derived parameters are selected for the characterization of photosynthetic activity. By means of this test, Tsimilli-Michael et al. (2000) showed that AMF increased electron transport activity in non-stressed alfalfa. Furthermore, Rivera-Becerril et al. (2002) were able to follow up how cadmium damage to pea was reduced by AM, using the same assessment procedure.

Hence, the objectives of this study were to investigate how AM can prevent drought damages in a high-value woody perennial, and whether an enhanced drought tolerance can be determined by physiological analyses of proline content, previously used as a stress marker (Rhodes et al. 1998), and analyses of the chl *a* fluorescence transient with parameters of the JIP test.

## Materials and methods

### Plant material, arbuscular mycorrhizal fungi and conditions of cultivation

Micropropagated plants of *R. hybrida* L. (Rosaceae) cv. New Dawn, a climbing rose, were provided by R. Mayer Ltd. (Strullendorf, Germany). After rooting and before acclimatisation, 6-week-old plants with one single unbranched shoot were transferred into a commercially available peat-based substrate for roses (Stangenberg Ltd., Einheitserdewerk Hameln, Germany). This substrate had been mixed with inoculum of *Glomus intraradices* Schenk and Smith (Glomeromycota) on expanded clay (5% v/v; Dehne and Backhaus 1986) for inoculation with AMF. The isolate (no. 301, collection of the Institute of Plant Diseases and Plant Protection, University of Hannover, Germany) had previously been selected subsequent to a compatibility test of rose cultivars to several AMF isolates. Plants were fertilized weekly with 30 ml per pot of a 0.5% solution of Wuxal Top N (N:P:K=12:4:6; Aglukon Ltd., Duesseldorf, Germany). After 4 weeks of growth in plug trays, the plants were transferred to pots (14 cm diameter) filled with a mixture (3:1) of rose substrate and sterilized sand. All experiments were conducted under greenhouse conditions during summer months (i.e. 20–22°C lowest and 32°C highest temperature). Additional light was provided (195 μmol $s^{-1}$ $m^{-2}$ for 16 h; Philips lamps SRG 102/400, Philips Ltd., Belgium). Water supply varied abiding by the experimental conditions (see below).

### Mycorrhizal development

Mycorrhizal development was assessed from ten randomly selected plants before the drying phase: root pieces were taken from the middle part of the root system, and 20 root sections of 1-cm length from each plant were tested in bright-field microscopy (Axiolab, Carl Zeiss Jena, Germany). The AMF colonization frequency was quantified in fixed and stained roots (Vierheilig et al. 1998) following the scale (0–3) of Backhaus (1984). Drought stress trials were conducted on a high mycorrhiza level. The frequency of mycorrhizal colonization in experiment I was 95.3± 8.8% (mean±SD). In experiment II the AM was established with colonization levels of 98.5±3.4% and 100% in part A and part B, respectively. None of the control plants (−myc) was colonized by AMF.

### Statistical analysis

Data from quantitative parameters were analysed using ANOVA (SPSS, Version 10). Significance of differences between treatments was tested after Tukey ($P<0.05$).

Experimental set-up

*Experiment I (long-term drought stress)*

Plants were cultivated under daily water supply near to full-field capacity (35% volumetric soil moisture) and then allowed to dry. Four different treatments were tested for drought tolerance, i.e. daily irrigation up to full-field capacity (treatment A), irrigation every second day up to full-field capacity (treatment B), irrigation every third to fourth day up to full-field capacity (treatment C), and daily minimal watering of 25 ml per plant (treatment D). Each treatment consisted of a total of 16 equal-sized plants: eight with mycorrhiza ('+myc') and eight without mycorrhiza ('−myc'); plant age was 5 months at the start of the experiments. During the drying period (6 weeks), additional water supply adjusted the differences in water contents between pots of the same treatment (reference: the wettest pot). To assess drought impact, visual scoring of drought damage and shoot length measures were recorded for each plant.

*Visual drought damage scoring*

The evaluation of drought damage followed a centigrade scale, ranging from 0 (totally vital and green) to 100 (dead and dried). Whole shoots were scored for drooping, wilting, yellowing, drying and dying off. This visual quantification was chosen to trace and record the long-term stress response on drought in a non-destructive manner without loss of leaf tissue or injuries that might trigger secondary plant reactions.

*Experiment II (short-term drought stress)*

The experiment was conducted twice (A and B; $n$=8). After 6 months of growth with daily irrigation up to full-field capacity (35% volumetric soil moisture), watering was stopped until severe leaf drooping was visible. Leaf drooping and shoot wilting began at the top of the plants and spread from the youngest parts of the rose plants to the basal leaves. Re-watering was implemented simultaneously for all plants at the phase when all −myc stressed plants exhibited notorious previously cited wilting symptoms.

*Soil water content*

To control the level of drought stress, soil water content was surveyed daily from the start (full water supply) to the day of most pronounced drought using a Theta Probe ML2x (Delta T Devices Ltd., Cambridge, UK), which measures the moisture content by responding to changes in the apparent dielectric constant of moist soil. Plants were cultivated under daily water supply near to full-field capacity (35% volumetric soil moisture) and then subjected to permanent drought. In experiment IIA, conducted under greenhouse conditions, the stress period lasted for 4 days to reach a key wilting matching a soil water content of 9% volumetric soil moisture. Experiment IIB diverged from IIA in that the drying period took 9 days until severe drought symptoms appeared at a level of 16% volumetric soil moisture. Daily survey and occasional adjustment of soil moisture between pots of each drought treatment led to uniform test conditions: both repetitions A and B illustrated no differences in the soil water content with non-mycorrhizal and mycorrhizal plants.

*Visual drought damage scoring*

Visual drought damage evaluation was carried out as outlined in experiment I.

*Chlorophyll a fluorescence measurements and analysis of the fluorescence transients*

Plants were adapted to the dark for 45 min before measurements. Chlorophyll *a* fluorescence kinetics was determined using the Plant Efficiency Analyzer (Handy PEA, Hansatech Ltd., King's Lynn, Norfolk, UK); data were analysed and normalized with the 'Biolyzer' software (Laboratory of Bioenergetics, University of Geneva, Switzerland). Measurements were conducted with six to eight plants on terminal leaflets of fully expanded leaves at mid-stem position. Data on chl *a* fluorescence were gathered at various steps in the course of stress development: before water shortage (−stress), during moderate drought impact, at severe drought impact (+stress) and during the recovery phase with a first measurement 2 h after re-watering (r1), followed by daily measurements (r2 and r3). Plant tissue was exposed to light excitation for 1 s, using actinic illumination with a light intensity of 2,500 μmol $m^{-2}$ $s^{-1}$. Fluorescence data were recorded stepwise: with a sampling rate of 10 μs (from 10 to 300 μs), then 100 μs (from 300 μs to 3 ms), then 1 ms (from 3 to 300 ms), and 100 ms (from 300 ms to 1 s). These data were stored with 12-bit resolution and analysed following the equations of the JIP test (Strasser et al. 2000).

The vitality state of the rose plants was characterized with the performance index PI (Strasser et al. 1999, 2000). This includes three independent parameters: (1) density of fully active reaction centres per chlorophyll (= TR/ABS), where TR is the energy flux for trapping and ABS is absorption; (2) efficiency with which a trapped exciton moves an electron into the electron transport chain further than $Q_A$ (= ET/TR), where ET is the energy flux for electron transport; and (3) the probability that an absorbed photon will be trapped by the reaction centre RC (= ET/ABS). Per definition, the PI accounts for functionality of both photosystems II and I and provides a general quantitative value of the actual state of plant vitality by combining several physiological events that favour photosynthetic

performance. Additionally, the parameter DI/RC was analysed separately as a representative of the energy dissipation (DI) per excited reaction centre.

Data of four parameters (RC/ABS, ET/ABS, TR/ABS and $S_m$) were connected in four different mathematical constellations: Stress sensitivity and stress tolerance were characterized in relation to stressed/non-stressed for +myc and −myc plants, whereas the mycorrhizal buffering capacity was demonstrated in relation to mycorrhizal/non-mycorrhizal for stressed and non-stressed plants. The parameter RC/ABS represents the ratio of chlorophyll as reaction centres and chlorophyll as antenna complexes. The parameter TR/ABS stands for the maximum quantum yield of primary photochemistry, whereas ET/ABS displays the maximum quantum yield of electron transport. The parameter $S_m$ seizes the area above the fluorescence transient [$S_m$=Area/($F_m$−$F_o$)] as an integral. Literally, it quantifies the energy needed to close all reaction centres through reduction of $Q_A$ and the following electron acceptors.

Finally, the fluorescence curves were normalized between 0.01 and 2 ms, subtracted from each other and presented on a linear time scale to investigate the shape of the curve. This first phase of the fluorescence rise corresponds mainly to single turnover events. That means events where $Q_A$ is reduced for the first time after adaptation to dark.

### *Proline content*

For determination of free proline content, leaf samples were collected during non-stressed state and during severe wilting. Proline content was assessed colorimetrically, using the protocol of Bates et al. (1973).

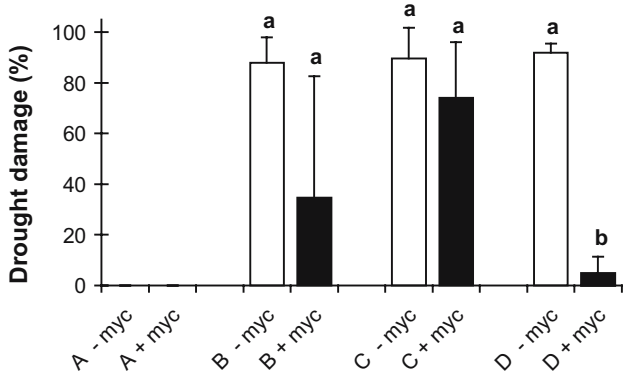


**Fig. 1** Visible drought damage scores (mean±SD) of 6.5-month-old rose plants cv. New Dawn without mycorrhiza (−myc) and inoculated with *G. intraradices* (+myc) after 6 weeks of drought impact (long-term drought experiment I): *A* daily irrigation up to full field capacity, *B* irrigation every second day up to full field capacity, *C* irrigation every third to fourth day up to full field capacity, *D* daily watering of 25 ml per plant. $n$=8. Significant differences (ANOVA, Tukey test, $P$<0.05) between non-mycorrhizal (−myc) and mycorrhizal (+myc) plants of each treatment are marked by different letters above columns

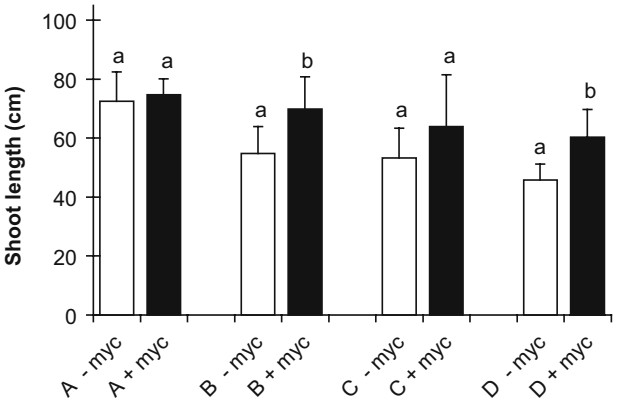


**Fig. 2** Shoot length (mean±SD) of 6.5-month-old rose plants cv. New Dawn at the end of the drought period under four different water-supply regimens (*A–D*, see Fig. 1): $n$≥6. Significant differences (ANOVA, Tukey test, $P$<0.05) between non-mycorrhizal (−myc) and mycorrhizal (+myc) plants of each regimen are marked by different letters above columns

## Results

### Experiment I (long-term drought stress)

### *Visually assessed drought damage*

After 6 weeks under daily minimal watering (treatment D), mycorrhizal plants showed significantly higher survival (Fig. 1). No significant effects of AM on drought damage were found in treatments B (irrigation every second day up to full-field capacity) and C (irrigation every third to fourth day up to full-field capacity), although the mean wilting values were reduced by 20 to 60%. The large standard deviation in treatment B may be explained by the fact that +myc plants either overcame drought (0% damage) or severely suffered (up to 85% damage).

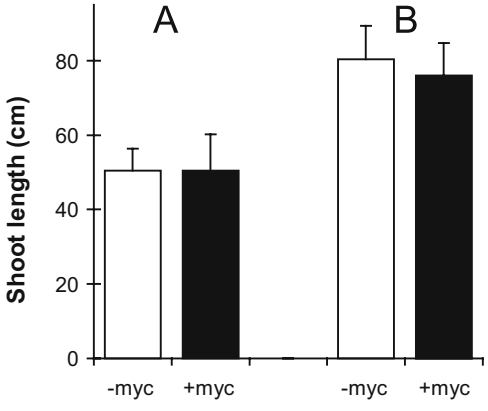


**Fig. 3** Shoot length (mean±SD) of 6-month-old rose plants cv. New Dawn at the end of short-term drought in experiment IIA and B. $n$=8. No significant differences between non-mycorrhizal (−myc) and mycorrhizal (+myc) plants of each trial (ANOVA, Tukey test, $P$<0.05)

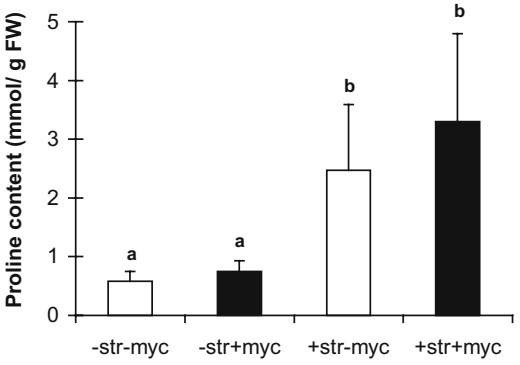


**Fig. 4** Proline content (mean±SD) of 6-month-old rose plants cv. New Dawn without mycorrhiza (−myc) and inoculated with *G. intraradices* (+myc). *n*=8; measurements before stress impact (−stress) and during severe drought stress (+stress) in experiment IIA. Mean values with same letters above columns are not significantly different (ANOVA, Tukey test, $P \leq 0.05$)

*Shoot growth*

In addition to visual scoring of wilt symptoms, the length of the shoot was measured at the end of the stress experiment (Fig. 2). The apparently more vigorous +myc plants showed a significant mycorrhiza-dependent shoot-length enhancement under irrigation every second day (treatment B) and also under daily minimal water supply (treatment D), whereas plants stressed by the longest drought intervals (treatment C) fell short of the significance level.

## Experiment II (short-term drought stress)

*Visual scoring of drought and measurements of shoot length*

In both experiments (A and B), neither necroses nor dying of shoots occurred under short-term drought stress; the

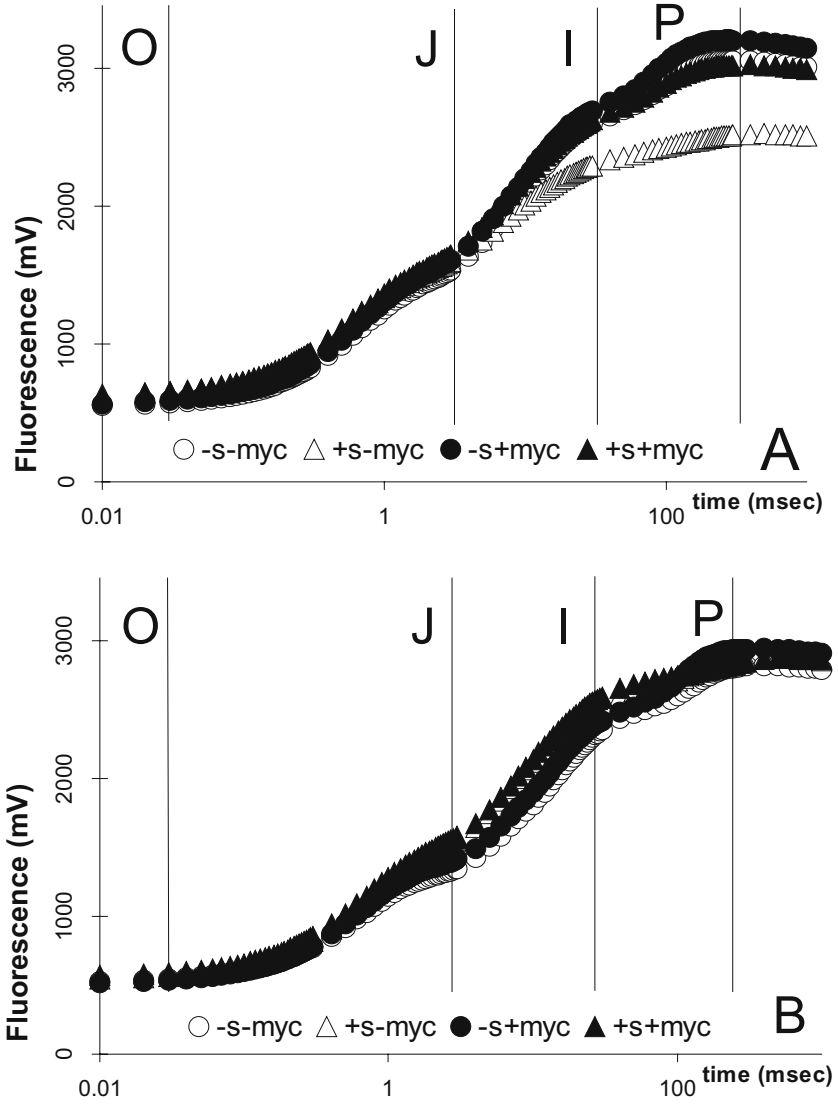


**Fig. 5** Fast chlorophyll *a* fluorescence increase of 6-month-old rose plants cv. New Dawn inoculated with *G. intraradices* (+myc) and without mycorrhiza (−myc), before stress (−s) and during severe drought stress (+s). Data are from the short-term drought experiment IIA and B, respectively, plotted on a logarithmic time scale (0.01 to 1 s)

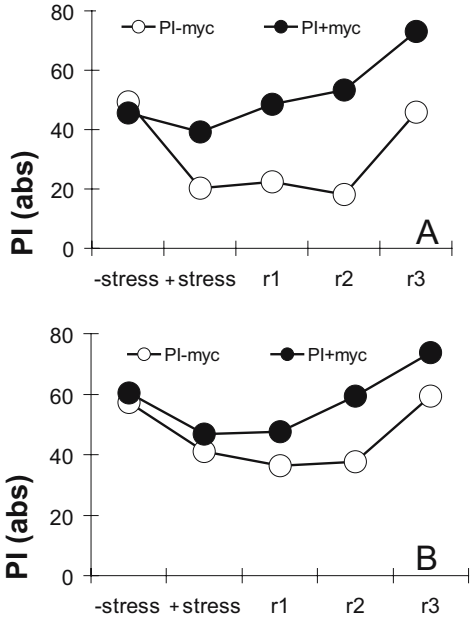


**Fig. 6** Performance index [PI (abs)] of 6-month-old rose plants cv. New Dawn inoculated with *G. intraradices* (*PI +myc*) and without mycorrhiza (*PI −myc*) before (− stress), during severe stress (+ stress), and during recovery (r1, r2, r3). Data are from the short-term drought experiment IIA and B, respectively

survival rate was 100% for all plants. Symptoms of reversible wilting could not be differentiated between +myc and −myc plants until the recovery phase. Growth data on shoot length supported the uniform outer appearance of the plants (Fig. 3), which were free from growth promotion due to AM at that stage.

### *Proline content*

Stressed plants always exhibited a significant higher proline content in the leaves, independently from mycorrhiza formation. This typical stress response in all plants confirmed proline as a general marker for drought stress (Fig. 4). In parallel to non-mycorrhizal plants, the increase in proline content could not specifically indicate AM-induced enhancement of stress tolerance.

### *Vitality probed by chlorophyll a fluorescence*

In experiment IIA, the main effect of the increasing drought stress tolerance of +myc plants was observed as a change in the polyphasic shape of the chlorophyll *a* fluorescence transient O-J-I-P (Fig. 5, experiment IIA). The differences between +myc plants and −myc plants started in the time phase of 1–40 ms (I step) and lasted up to the point of maximum fluorescence values (P step).

The effect of drought clearly led to reduced PI values for −myc plants, whereas +myc plants maintained higher PI levels. After re-watering, PI values of −myc plants continued to decrease, whereas +myc plants started to recover (Fig. 6, experiment IIA). On further recovery stages, PI values of +myc plants and −myc plants levelled off again to a prestress level or even higher. The performance of +myc plants was essentially less impaired over the whole stress period. Data assessed at the beginning under moderate stress (not shown) matched the shape of the respective curves.

In experiment IIB, variations in the shapes of the fluorescence curves were less pronounced (Fig. 5, experiment IIB). However, the small but constant differences between stressed +myc plants and −myc plants resulted in more stable and slightly higher performance indices of mycorrhizal plants, although the mycorrhizal enhancement of stress tolerance was delayed and less intense than in experiment IIA (Fig. 6, experiment IIB).

The values of parameter $F_v/F_m$ (maximum quantum efficiency of primary photochemistry of photosystem II) did not vary considerably from 0.8 throughout the three steps of treatment (prestress, stress, poststress); this parameter did not contribute to the characterization of a mycorrhiza-induced tolerance effect neither in experiment IIA nor B.

During the experimental phases, the DI rates varied depending on mycorrhizal status: In experiment IIA, −myc plants showed a higher rate of DI, whereas +myc plants reaction was more balanced, as is shown by the parameter DI/RC (Fig. 7, experiment IIA). In addition, this parameter reflected that the stress tolerance enhancing effect in +myc

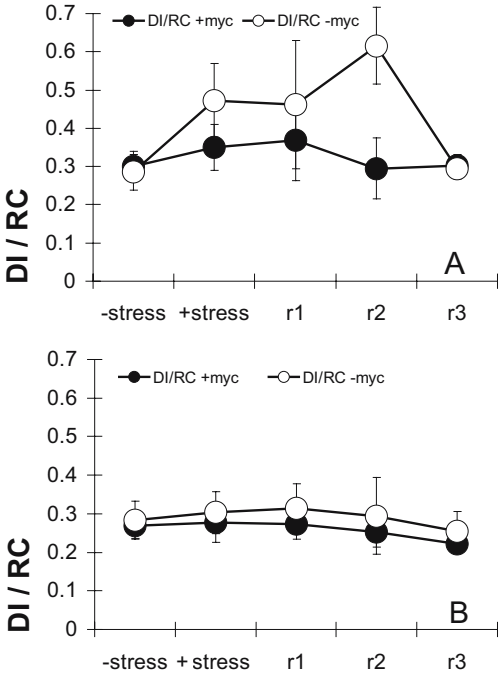


**Fig. 7** Dissipation rates DI/RC (mean±SD) of 6-month-old rose plants cv. New Dawn inoculated with *G. intraradices* (*DI/RC +myc*) and without mycorrhiza (*DI/RC −myc*) before stress (− stress), during severe stress (+ stress), and during recovery (r1, r2, r3). Data are from the short-term drought experiment IIA and B, respectively; *n*=8

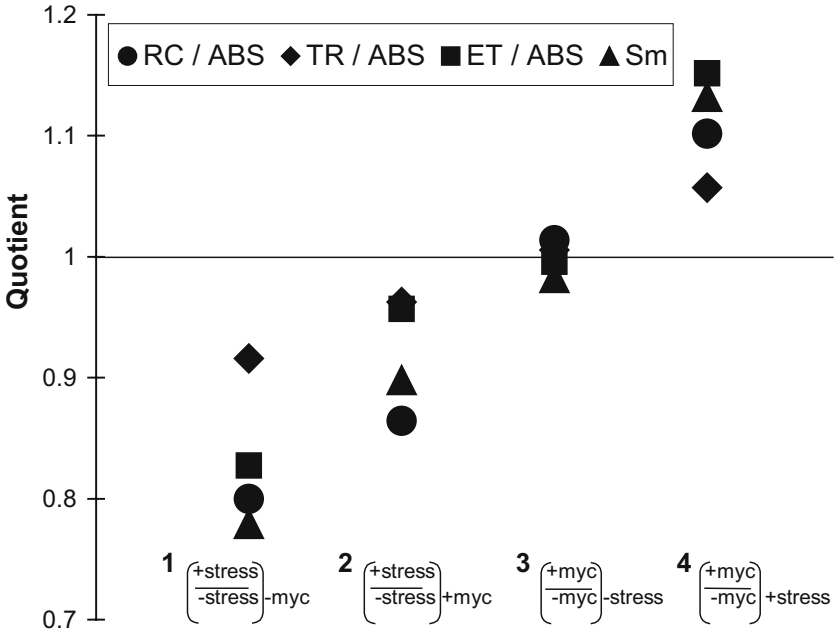


**Fig. 8** Quotients of data from JIP test parameters RC/ABS, ET/ABS, TR/ABS, and $S_m$ in four different situations (*1* to *4*), reflecting a comparative information on reactions of 6-month-old rose plants cv. New Dawn inoculated with *G. intraradices* or without mycorrhiza in the short-term drought experiment IIA. *1* Non-mycorrhizal plants, 'stressed' divided by 'non-stressed'; *2* mycorrhizal plants, 'stressed' divided by 'non-stressed'; *3* unstressed plants, 'mycorrhizal' divided by 'non-mycorrhizal'; *4* stressed plants, 'mycorrhizal' divided by 'non-mycorrhizal'

plants was less expressed in experiment IIB (Fig. 7, experiment IIB).

The sensibility of photosynthetic activity against drought stress in repetition A of experiment II is illustrated in Fig. 8 with results stemming from four parameters: comparative information on the reaction of rose plants in four different situations is reflected by the quotients of the JIP test parameters RC/ABS, ET/ABS, TR/ABS and $S_m$. The experimental conditions +stress, −stress, +myc and −myc were combined as the following situations: 1, $[+\text{stress}/-\text{stress}]_{-\text{myc}}$; 2, $[+\text{stress}/-\text{stress}]_{+\text{myc}}$; 3, $[+\text{myc}/-\text{myc}]_{-\text{stress}}$ and 4, $[+\text{myc}/-\text{myc}]_{+\text{stress}}$. The quotients have the value 1 if the values of −myc plants and +myc plants are identical (demonstrated in situation 3 without stress). In a comparison of non-mycorrhizal (situation 1) and mycorrhizal plants (situation 2) all parameters demonstrate

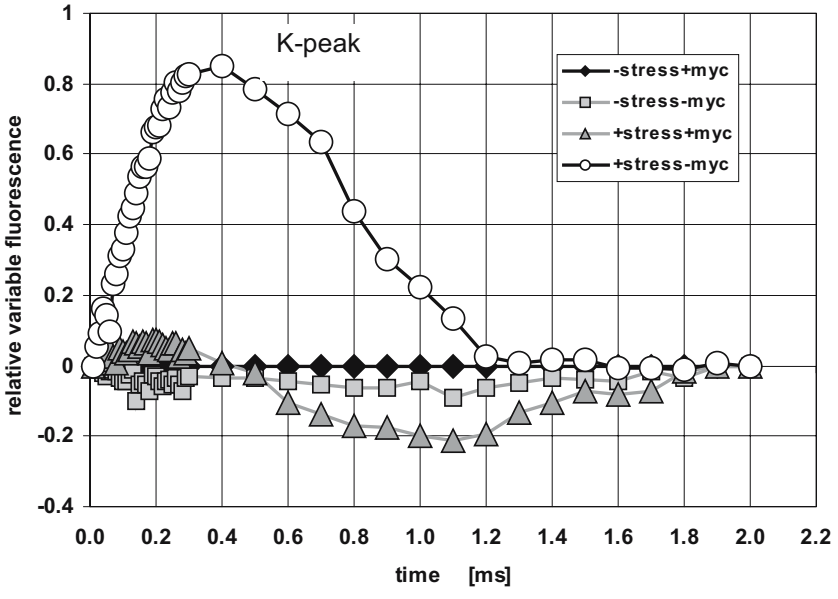


**Fig. 9** Occurrence of a K peak in the chlorophyll *a* fluorescence kinetics of 6-month-old rose plants without mycorrhiza (−myc), compared to plants inoculated with *G. intraradices* (+myc) under short-term drought stress (+stress) or unstressed (−stress) (experiment IIA). Fluorescence intensities are normalized between 0.05 and 2 ms (relative fluorescence intensities between 0 and 1) and plotted on a linear time scale with the program 'Biolyzer'. The differences of the four samples to the reference sample of non-stressed plants without mycorrhiza (−stress −myc) are plotted with an amplification of 16

that the latter reacts less drastically to drought. The comparison of non-stressed (situation 3) and stressed (situation 4) plants underlines that the buffering effect of mycorrhiza develops only under stress. Within the four parameters used, TR/ABS=$F_v/F_m$ was the least reactive. RC/ABS, however, was a more flexible factor with the capacity to indicate mycorrhizal-dependent structural changes in the photosynthetic apparatus.

Finally, an appearance of the K step in the shape of the fluorescence transient curve was detected only for stressed −myc plants (Fig. 9), indicating instability of the oxygen-evolving complex and therefore an instability of the electron transport chain on the donor side of PS II.

## Discussion

The term "plant tolerance" embraces a wide range of adverse factors, including biotic and abiotic stress (D'Arcy et al. 2000). Stress-buffering capacities, either based on genetic background or induced by symbiotic organisms, are of decisive importance for the plant to adapt to environmental challenges. Drought tolerance of mycorrhizal plants can be influenced by altered physical parameters of the soil: an increased root conductance and an altered relation of substrate water capacity to water tension led to an improved plant performance in flax (*Linum usitatissimum* L.) under stress (Reichenbach and Schönbeck 1995). In addition, a desiccating mycorrhizal soil can maintain substantially higher water contents as compared to a non-mycorrhizal soil because of the aggregating outcome of mycorrhizal hyphae on soil structure (Augé et al. 2001). In our experiments, these effects were minimized by simultaneous observation and balancing of soil water content since the main goal of the study was to focus on the fungal effect on plant metabolism and on the assessment of drought tolerance performance. Likewise, the fertilization level for standard rose production was adjusted to avoid an additional outcome of AM-dependent nutrient (P) supply effects on drought tolerance. Consequently, a reliable assessment of drought tolerance effects could be undertaken.

The visual scoring of drought symptoms revealed that mycorrhizal rose plants showed significantly less damage under severe water-deficit conditions. The positive effect of mycorrhiza was most noticeable if plants were subjected to a prolonged drought stress period (experiment I, water regimen D). Although shoot length measurements are only partly representative of shoot growth, shoot fresh weight or dry mass could not be recorded under our experimental conditions, as these plants were further cultivated for transplantation to the open field. However, these shoot length data supported the results of visual drought damage scoring in both experiments: long-term drought treatments (B to D) in experiment I reduced growth more in non-mycorrhizal plants. In experiment II, in which wilt symptoms were reversible, the short-term stress had no influence on growth of any plants.

Proline content was used as an indicator of drought tolerance of drought-stressed plant tissue, as proline accumulation helps to maintain high osmotic levels in plant cells suffering from water deficit (Chaves et al. 2002; Mohamed et al. 2000; Rhodes et al. 1998). In our experiments, the proline content of rose leaf tissues was confirmed as a general drought-stress marker, but it was less adequate to designate AM-induced enhancement of stress tolerance. Porcel and Ruiz-Lozano (2004) demonstrated the accumulation of higher proline levels in soybean mycorrhizal roots and lower contents in mycorrhizal shoots compared to non-mycorrhizal plants under drought conditions. A more detailed analysis of both root and shoot samples during early drought stress phases would be necessary to evaluate whether roses exhibit similar patterns.

Because of the key role of photosynthesis in plant metabolism, related parameters have been investigated in studies of plant stress responses. Davies and Santamaria (2000) proposed stomata functioning and gas exchange as useful techniques for plant quality assessment in young micropropagated plants. Similarly, in an ectomycorrhizal association drought stress tolerance was attributed partly to specific physiological mechanisms based on chlorophyll content and gas exchange (Morte et al. 2000). However, the holistic chlorophyll content does not deliver any information about the functionality of the chlorophyll molecules. Assessments of gas exchange under drought impact also have their drawbacks when the plant manifests partial or complete closing of the stomata (Jones 1998). For example, non-mycorrhizal rose plants differed from mycorrhizal ones in that the former maintained higher stomatal conductance only during the beginning of soil drying (Augé and Duan 1991). If the metabolism of a plant is disturbed due to water deficiency, redundant energy has to be dissipated to avoid lethal damage of plant tissues (Ott et al. 1999). Dissipation results via non-photochemical processes like heat or chlorophyll fluorescence (Govindjee 1995); the corresponding processes modify the polyphasic rise in chl *a* fluorescence (Strasser et al. 1995). Physiological or structural features of stress may therefore be monitored by analysis of the chl *a* fluorescence kinetics (Strasser et al. 2004).

In our study on micropropagated rose plants, the JIP test parameters appeared to be suitable tools for detection of drought tolerance-enhancing effects of mycorrhiza and for characterization of underlying photosynthetic reactions. The intensity of electron trapping revealed differences between fluorescence transients of +myc plants and −myc plants in repetition A of the short-term drought experiment. The derived PI illustrated the enhanced vitality of +myc plants under drought and the reduction of the photosynthetic activity of −myc plants.

To study in more detail mechanisms of drought stress tolerance, selected single parameters of the JIP test were analysed. Comparisons of four basic physiological parameters under different treatments revealed clear AM-dependent modifications in photosynthesis. The stress-buffering effect of mycorrhiza was obvious in stress conditions. In particular, the parameters $S_m$, RC/ABS and ET/ABS increased in mycorrhizal plants. This might be due to less deactivation of RC in the presence of mycorrhiza under

water stress conditions, which cause perturbations in electron transfer, so that the number of active RC controlled the intensity of the photosynthetic reactions. $S_m$, measuring the pool of electron transporters between PS II and PS I, displayed a decreased electron transport between these photosystems in −myc plants.

Under a water stress impact, values of TR/ABS, RC/ABS and ET/ABS were higher for +myc plants ([+myc/−myc]$_{+stress}$): mycorrhiza led to an improved function of more active reaction centres and increased electron efflux. Inactive RC did not contribute to electron transport; in this case RC/ABS somehow conformed with ET/ABS. Consequently, the regulating element for electron flux was the density of active RC: the sensitivity of RC determined how much trapped but redundant energy was emitted during drought stress and how much surplus energy charged the electron transport chain in +myc plants. This may explain the higher photosynthetic activity and performance of the +myc plants under drought.

Another characteristic of photosynthetic changes induced by AM was the disappearance of the stress-induced K step in the O-K-J-I-P kinetics at 300 μs. A reduced electron delivery from the water splitting system could be the reason for this down-regulation of electron transport (Srivastava et al. 1997). Since the water-stressed +myc plants showed no enhanced K peak, they must possess a more stable electron donor site of PS II. The appearance of a K peak, similar to −myc plants, has been described as a stress indicator of general character (Guissé et al. 1995). In addition, it corresponds to a partial uncoupling of the oxygen-evolving complex (OEC) at PS II resulting in a lack of electrons coming from the donor side (Strasser 1997).

The results demonstrate the positive effect of mycorrhiza on photosynthetic yield under drought stress: the drought tolerance effect was detected in PS II in parameters of structure such as yields and ratios of rate constants (e.g. the flux ratio ET/ABS), and in parameters of specific functions (e.g. energy fluxes per reaction centre, RC/ABS). Electron-transport activity of water-stressed +myc plants remained more efficient, and stress-related discharge was lower. Furthermore, the results indicate that drought stress led to a reduction of active ($Q_A$ reducing) RC and an uncoupling of OEC: AM promoted electron flow from PS II to PS I and beyond it to the Calvin cycle. It is still ambiguous whether the two mechanisms are correlated or independent of one another.

Differences between plant reactions consequent to short-term drought impact in experiment II raise speculations about a threshold level of stress intensity that might be necessary for the induction of these tolerance mechanisms. Although the gentler stress in experiment IIB (characterized by longer duration and higher soil moisture content) resulted in similar visible wilting symptoms as in experiment IIA, there seem to be different physiological responses of the +myc plants as the tolerance mechanisms detected in the photosynthetic activity were underexpressed. This encourages further investigations with graduated stress scenarios and sophisticated methods in order to discern signals for and limits of AM as tolerance inducer. Overall, these findings contribute to advances in the knowledge of AM-induced drought stress tolerance. Due to more balanced physiological processes, the mycorrhizal symbiosis can lead to an improved photosynthetic performance and thus to enhanced plant survival under drought. Whether the long-lasting symbiosis can sustainably improve the performance of rose plants longer than one vegetation period is being investigated. Furthermore, it will be necessary to validate drought tolerance mechanisms in nursery and field trials conducted under agricultural conditions.

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