Journal of Applied Botany - Angewandte Botanik 75, 144 - 151 (2001), © 2001, Vereinigung für Angewandte Botanik, Göttingen

[1]Institute of Botany, Georgian Academy of Sciences, Tbilisi, Georgia
[2]Department of Geobotany, HHU Düsseldorf, Germany

Changes in Water Relations, Solute Leakage and Growth Characters during Seed Germination and Seedling Development in *Trigonella coerulea* (Fabaceae)

Maia Akhalkatsi[1] and Rainer Lösch[2]

(Received March 28, 2001)

Summary

Changes in water and osmotic potential, water content and dry mass were studied that proceed during seed imbibition, germination, and seedling development under constant environmental conditions in *Trigonella coerulea*. Germination percentage of seeds imbibed in mannitol solutions (0 MPa to -2 MPa) declines with decreasing substrate water potential. Germination is completely suppressed at -1.0 MPa. Germination time becomes prolonged from 48 h at 0 MPa to 96 h at -0.5 MPa. Changes in water relations, water content and dry mass of the seed and seedling tissues, quantified by pressure-volume analysis, can be paralleled with morphogenetic phases and physiological events during germination and seedling growth. The lowest osmotic potential at water saturation, -2.6 ± 0.35 MPa, is reached when the radicle emerges, on the third day after imbibition started. After a transient increase osmotic and water potential decline again when the seedlings have attained the photoautotrophic growth, approx. one week after the imbibition commenced. Only then dry mass of the entire seedling increases whereas during radicle and hypocotyl elongation a decrease of dry weight was obvious. Leakage of phenolic substances and probably also solutes to the surroundings started about six hours after the seeds began to swell, accompanied by a transient decrease in seed water content. Otherwise, seed resp. seedling water contents increased with saturation type kinetics in two steps, the first lasting from seed imbibition until hypocotyl development, the second comprises the time period of the development of cotyledons and primary leaves. Substances released from the imbibing seeds are tannins and various flavonoids. Only seeds releasing these substances germinate successfully, from hard-seeded ones and from imbibed ones without solute exudation never a radicle emerges. Interindividual variation of the water relation parameters is considerable, but general trends of increase and decrease follow always the same pattern. This variation may be an important selection factor under field conditions offering different chances for survival and successful establishment among a population of germinating seeds.

Introduction

Substrate water potential plays a key role in restricting the habitat at the seedling establishment phase of plants and often determines species distribution (STEBBINS, 1974; SWAGEL et al., 1997). The uptake of water by seeds is the initial step to germination and is conditioned by the difference in water potentials between seed and soil. Water potential of dry seeds is very low (ROBERTS and ELLIS, 1989). Different plant species require different threshold values of substrate water potentials for initiation of seed germination (CHOINSKI and TUOHY, 1991). Minimum values of substrate water potentials required for germination are documented, e.g., by FITTER and HAY (1987) and SWAGEL et al. (1997), but for most plants such data are still lacking. During subsequent imbibition, germination and seedling establishment seed tissue water potentials undergo considerable changes (KOLLER and HADAS, 1982; WHALLEY et al., 1998). During germination and seedling growth hydration influences the metabolism of embryo and seedling (LEPRINCE et al., 1992), and plant water relations essentially govern cell and tissue volume extension during these ontogenetic stages (THORNLEY, 1996; LÖSCH, 2001). Various authors (e.g. JOHNSON et al., 1996; LEIVA and FERNANDEZ-ALES, 1998; IKEDA et al., 1999) investigated the influence of environmental stress conditions on species differences in water status during germination and on seedling water relations (EGLI and TEKRONY, 1997). Overviews about the influence of ambient water conditions on imbibing and germinating seeds are given, e.g., by BEWLEY and BLACK (1994) and BRADFORD (1995).

However, not so much attention has been paid to study successive alterations of the parameters of water relations at different developmental stages of seed imbibition, germination and seedling performance. In the present study we document the dynamic changes of water relations that occur in *Trigonella coerulea* seed and seedling tissues in the course of morphogenesis during seed swelling, germination and seedling growth under constant experimental conditions.

Trigonella coerulea is an annual garden spice legume in countries with a (sub)mediterranean-continental climate (LACHASHVILI, 1981). It produces orthodox, endospermous seeds like other species of this genus (BEWLEY and BLACK, 1994). The species shows a moderate hard-seededness. The germination proceeds very quickly (within two days after imbibition) and offers thereby advantages also for laboratory studies of germination processes.

In the present study we subjected under controlled laboratory conditions seeds of *T. coerulea* to mannitol solutions of various water potentials. Seed tissue water relations were determined by pV analysis through all stages from seed coat swelling until the expansion of primary leaves. From these data, time courses of tissue water contents and water potential components were determined and related with the morphogenetic changes during germination and seedling growth. At early stages of imbibition solute leakage can occur from seed tissues into the surrounding medium (BRADFORD, 1995). Different chemical substances are released by imbibing seeds because of membrane damage (LANFERMEIJER et al., 1990; DELL'AQUILA and DITURI, 1995). Phenolic exudations of some legume seeds, which are actively released predominantly from the embryo cells (BEKKARA et al., 1998) can act as chemoattractants and induce transcription of the nodulation genes promoting formation of the *Rhizobium*-legume symbiosis (PHILLIPS et al., 1995; ZUANAZZI et al., 1998). It is not known, whether such a solute leakage is related to the seed tissue water potential. We analyzed therefore changes of water content and tissue dry mass in germinating *T. coerulea* seeds to determine possible interactions between seed water relation dynamics and solute leakage.

Overall, three questions were addressed in this study: 1. To investigate substrate water potential constraints on seed germination in *T. coerulea*; 2. To determine the role of changes in seed osmotic and water potentials, water content and dry mass for germination success and seedling establishment at different developmental phases of seed imbibition, germination and seedling development. 3. Special attention has been paid to possible relationships between solute leakage and water content of the germinated seed.

Material and Methods

Seeds of *Trigonella coerulea* (L.) Ser. (Fabaceae) were collected from plants cultivated under standard conditions on the experimental research farm of the Institute of Botany, Georgian Academy of Sciences, Georgia, in July 1997 and stored in paper bags at room temperature. The germination experiments were carried out in the laboratory of the Department of Geobotany, H. Heine University Düsseldorf, Germany, during February – May of the following year. Seeds were germinated in temperature-controlled (Peltier-thermostat, Driesen & Kern, Germany) trays either with garden soil or on wet or mannitol-soaked filter paper.

Environmental conditions, under which the experiment was conducted, were measured in one-minute intervals, and averages of ten minutes were recorded on a data logger (Squirrel 1200, Grant Instruments, UK). The photoperiod was 15 h, under artificial lightening with Phillips 160 W bulbs providing 28.3 ± 1.2 µmol photons $m^{-2}s^{-1}$ at plant level. Red and far-red photon flux ratio was 1.5 ± 0.04 (SK 215 and SKR 110 sensors, respectively, Skye Instr., UK). Air and soil temperatures were measured with copper-constantan thermocouples. Mean air temperature was 24 ± 1.5 °C during the light period and 22 ± 1.3 °C during the dark period. The soil temperature, measured at a depth of 2 cm below the surface, was 23 ± 0.6 °C. Relative humidity of the air averaged 70% (Vaisala HMP 35, Finnland).

For germination tests, only selected seeds with a black coloured pigmentation in the seed coat and a length between two and 2.5 mm were used. These gave the best germination results as it was proven in preliminary experiments.

To test substrate water potential constraints on germination, seeds were placed in 15 cm diameter Petri dishes on filter paper moistened with 5 ml deionized water, as control, or aqueous solutions of D-mannitol (Merck, Germany). Aqueous solutions of D-Mannitol were used as an osmotic agent to establish definite water potential gradients between the seeds and the surrounding liquid medium. Mannitol concentrations of 0.04, 0.1, 0.2, 0.4, 0.6, and 0.8 mol/l were prepared according to SWAGEL et al. (1997) to generate the correspondent substrate water potentials -0.1, -0.25, -0.50, -1.0, -1.5, and -2.0 MPa. Constant substrate water potential was maintained during the experiment by addition resp. exchange of water and mannitol solutions in two days intervals. One hundred seeds were placed in each Petri dish, and each germination condition was studied with four replicates (one water control and six experimental set-ups at different water potentials). Germinated seeds, with the radicle emerging, were counted daily. Final germination percentage was determined after 7 days from the imbibition by which time no further germination had occurred.

Viability of those imbibed seeds, where radicle emergence failed to occur, was determined by a tetrazolium test (MOORE, 1973). Half sectioned imbibed seeds were placed in an 0.1% aqueous solution of 2,3,5-triphenyltetrazolium chloride (Merck, Germany) in the dark for 24 h at room temperature (22 °C) and then observed under a dissecting microscope for deep pink colour as a positive stain for metabolic activity in the embryo.

To measure parameters of water relations in germinated seeds and seedling tissues at different developmental stages, the seeds were germinated on a humus rich soil (Floraton 1, Germany) in open trays (50 x 30 x 7 cm, depth of the soil 4 cm) that were kept under the constant laboratory conditions, described above. Plants were watered daily to maintain the soil moisture content around 60%. The seedlings were kept 21 days in the trays, and the experiment was repeated three times.

Tissue water potentials were determined by the dew point equilibration method using an HR-33T dew point microvoltmeter (Wescor Inc, U.S.A), calibrated with a range of NaCl solutions of known concentrations. At each measurement time, six individuals were taken from the soil and placed in deionized water for one hour to achieve water saturation. Externally adhering water was removed by blotting the plant material on a filter paper. Then the germinated seeds or little seedlings were placed into sample chambers (Wescor C-52) and enclosed in the sample holder with the thermocouple psychrometer. Vapour equilibration for a sample chamber took 45 min. After water potential determination in the dew point operation mode of the instrument the sample was weighed, allowed for a small water loss and again equilibrated for the next water potential measurement. As a rule, the stepwise desiccation was run until 10 – 12 paired values of decreasing sample water contents and water potentials were reached. Six C-52 sample chambers with *Trigonella* seeds resp. tissues were used as parallels.

Graphical analysis of each of the resulting pressure-volume curves and determination of relative water content, osmotic water potential at water saturation and water potential at zero turgor pressure was performed using a special statistical program based on the non-linear regression procedure of SAS program (ANDERSEN et al., 1991).

Daily observations of germination phenology were conducted with the seedlings growing on soil under laboratory conditions. At each developmental stage, the total length of the germinated seeds or seedlings was measured (n = 20). Measurements of water content and dry mass were carried out with data of the actual fresh weight and the dry weight after drying the samples (n = 50) for 48 h at 105 °C. Water content (in gram water by gram dry mass) was calculated based on: water content = (fresh mass – dry mass)/dry mass. At early stages, as long as the embryo was covered by seed coat in germinated seeds, dry mass was calculated both for whole germinated seeds and for isolated embryos after dissection from the seeds.

For determination of seed exudate components, seeds were imbibed for 24 h on deionized water in Petri dishes. On the next day, the extraction of seed exudates proceeded from four differently prepared specimens: whole seeds, desiccated seed coats, desiccated embryos and pieces of the filter paper, saturated with seed exudate. The material was extracted with methanol for 48 h at 22 °C in the dark. The extracts were filtered, evaporated *in vacuo* (Rotavapor RE 111, Buechi, Switzerland) and dissolved in 5 ml methanol. The extracts together with known standards were separated by thin layer chromatography on silica gel (Merk, TLK, silica gel 60, F_{254}) using the developing system n-butanol–acetic acid–distilled water (4:1:5 by volume). The spots on the plate were monitored under ultraviolet light before and after spraying with the reagents for the detection of phenolic compounds: 5% $AlCl_3$ solution in methanol, 1% solution of diphenylboric acid-ethanolamine complex in methanol and diazotized sulphanilic acid (MARKHAM, 1982). The identity of compounds was confirmed by comparing their R_f values and colour development with that of standard samples. The presence of condensed tannins in the seed coat, embryo and seed exudate was estimated by staining half sectioned imbibed seeds hydrating for 24 h and pieces of the filter paper saturated with the seed exudate with a nitroso reaction (JENSEN, 1962).

Mean values and standard deviations were calculated for every data set. Statistical differences among true mean values were determined using one-way analysis of variance ($P \leq 0.05$) of SAS program (SAS Institute Inc. 1991).

Results

Six developmental stages of seed germination and seedling growth in *T. coerulea* were defined as follows: imbibition, germination, growth, seedling, first leaf and second leaf (Fig. 1). The duration of each phenological stage was determined as a mean duration observed for the individual seeds or seedlings growing on soil (Fig. 1). Seeds began to take up water immediately after soaking. Seed coat rupture and radicle emergence occurred after only 48 h. The seedling elongated rapidly during the first week (Fig. 1). Yellowish cotyledons and the hypocotyl underwent photomorphogenesis and became green and

photosynthetically active at the end of the stage of volume expansion. Cotyledons unfolded and began to enlarge at day 6 after onset of imbibition. They became fully developed at the eighth day.

The germination tests at different substrate water potentials showed the highest mean germination percentage (16 ± 2%) in the control treatment when seeds were soaked on filter paper moistened with deionized water (Fig. 2). Germination percentages declined with decreasing ambient substrate water potential generated by mannitol solutions of different concentrations. Seed germination was almost completely suppressed at -1.0 MPa in the external medium (Fig. 2). The mean percentages of imbibed seeds were not significantly different ($P > 0.05$) from the control seeds, and showed higher values than germination percentages at the same treatment (Fig. 2). Time between soaking and germination increased from 48 h in control seeds to 96 h for seeds germinated at -0.5 MPa of the liquid medium. The comparatively low overall germination rate in this plant species results from a high proportion of hard seeds which do not undergo hydration. The mean percentage of hard seeds in all conducted germination treatments was 58 ± 12.4%.

Dry mass of whole germinated seeds showed no significant changes during imbibition and radicle emergence (Fig. 3). It decreased considerably during the first stages of hypocotyl growth and development of cotyledons. The decrease of the seedling dry mass became reversed only some time after transition to the photoautotrophic growth (Fig. 4). A successive increment in dry mass of entire seedlings occurred after eight days from soaking (Fig. 4). A comparison of dry masses of the isolated embryo and the whole germinated seed showed that dry mass of the embryo was at the beginning much smaller than that of the seed (Fig. 4). It increased during the first five days, until the seed coat became depleted and dropped off. After the seed coat was lost, dry mass of the embryo began to decrease until the eighth day from soaking (Fig 4) by which time the cotyledons had completely expanded.

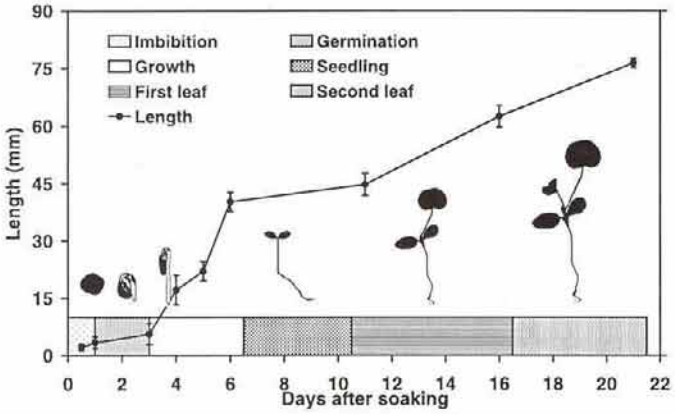

Fig. 1: Mean duration of developmental stages and mean length (incl. standard deviation) at imbibition, germination and seedling growth in *T. coerulea* growing on soil. n = 20. The digital views of the plant material visualise the general morphology of the germinated seed resp. seedling at the corresponding developmental stage.

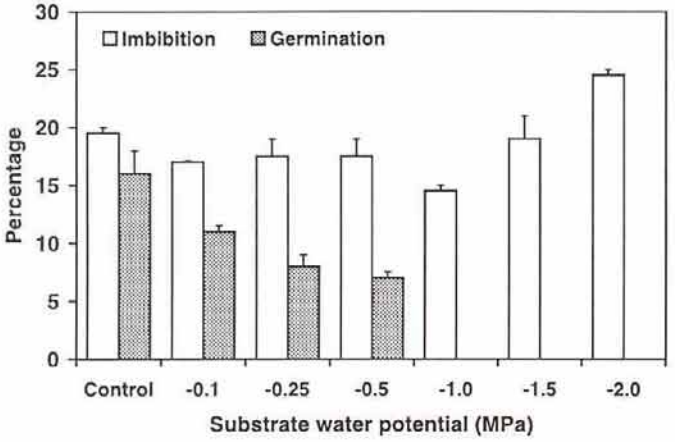

Fig. 2: Mean percentage and standard deviation of imbibed and germinated seeds of *T. coerulea* (out of a total of 100 seeds in each experiment) at different substrate water potentials in the germination medium. D-mannitol was used as osmotic agent to generate different water potentials. The percentages of imbibed and germinated seeds were calculated at the seventh day after soaking by which time no new germination had occurred. n = 4.

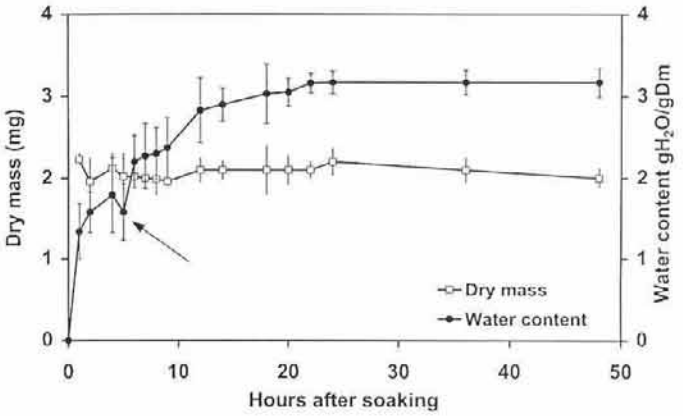

Fig. 3: Mean and standard deviation of dry mass and water content of *T. coerulea* seeds at imbibition and until radicle emergence. An arrow shows the data when solute leakage occurs for the first time. The time of the solute leakage was determined visually when yellowish spots on the filter paper around the germinating seeds were formed. n = 50.

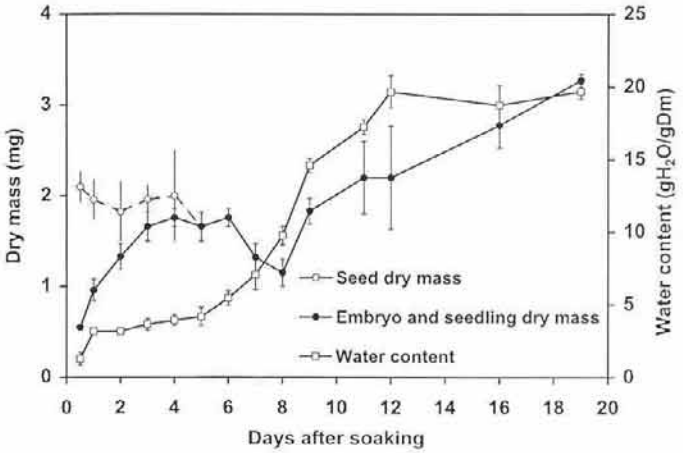

Fig. 4: Mean and standard deviation of dry mass and water content of the germinated seeds, isolated embryos and growing seedlings in *T. coerulea*. For the first five days, as long as the seed coat is available, dry mass was measured separately for the whole seed, containing embryo, seed coat and endosperm, and only for the embryo after its dissecting from a seed. n = 50.

Water content of the germinating seeds increased rapidly during the first day and reached a plateau level 24 h after soaking (Fig. 3). During this period of time, water content was increasing successively. However, five hours after soaking a transient decline of the water content was obvious from the mean values (Fig. 3). Standard deviation of the data is too large to demonstrate this significantly, and the short period of this transient event limited the number of determinations. However, just at this time yellowish coloured spots appeared around the seeds germinating on wet filter paper indicating leakage of seed exudates. Only the seeds where the solute leakage occurred continued the germination further. No radicle emergence was observed in the seeds which had imbibed but did not undergo solute leakage. The tetrazolium test showed that 85 ± 4.3 % of these seeds were also viable. After radicle emergence water content increased continuously (Fig. 4).

Osmotic and water potentials of the seeds germinated on soil decreased rapidly during the early period of imbibition (Fig. 5). Osmotic potential at water saturation was -1.6 ± 0.2 MPa at the plateau phase of water content of imbibed seeds prior to the breaking of the seed coat. At the time of radicle emergence most negative values, -2.6 ± 0.35 MPa, were reached. Similarly, water potential at the zero turgor point showed a strong decline at radicle emergence and reached the minimal value, -3.3 ± 0.15 MPa, at the time when seed coat rupture was significant. Then the naked embryo was partly exposed to a stronger drying gradient to the surroundings. At the same time also the relative water content at turgor loss point showed the minimum value, 76 ± 1,6 %.

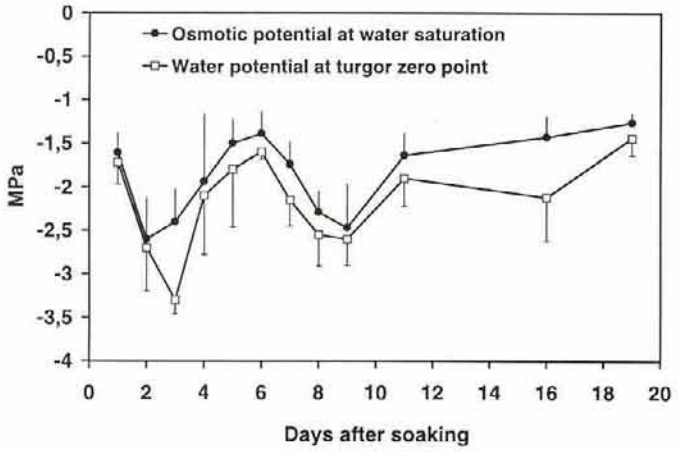

Fig. 5: Mean and standard deviation of osmotic potential at water saturation and water potential at zero turgor point in the course of imbibition, germination and seedling development in *T. coerulea*. n = 6, 12.

During radicle elongation osmotic potential at full saturation and at the turgor loss point increased to significantly less negative values. Thereafter a second decrease of these values followed. This second period of decrease in osmotic and water potentials occured when the cotyledons began enlarging and greening (Fig. 5). From the analysis of individual pV curves some scatter resulted among values of the tissue osmotic potential at water saturation (Fig. 6), even if similarity of the samples in both, time from the soaking and developmental stage, was considered scrupulously. Nevertheless, the mean values of osmotic potential at imbibition and germination are significantly different ($P < 0.001$) from the mean values of osmotic potential at the later stages of development. Mean values of the osmotic potential at the stages of hypocotyl growth, seedling phase, first leaf and second leaf development differ insignificantly from each other ($P > 0.05$).

To test the presence of phenolic compounds within the seed and the seed exudate, alcohol extracts of whole seeds, dissected seed coats, separated embryos, and pieces of the filter paper saturated with the seed exudate were analyzed by thin layer chromatography. Different numbers and locations on the plate of flavonoid spots were revealed with extracts of the seed coat and the embryo, while extracts of the whole seed and the seed exudate contained almost all of these spots. The spots were not much separated from each other and were even overlapping. However, two spots in extracts of the seed coat and the seed exudate could be clearly paralleled with co-chromatographed standards and were identified as vitexin and orientin. Spraying of the plates with these extracts by $AlCl_3$ revealed one fluorescent yellow spot identical to 5-hydroxy-flavonoids. The development of red spots, characteristic for compounds with free phenolic hydroxyl groups, appeared in the embryo extract after spraying the plate with diazotized sulphanic acid. The presence of condensed tannins, estimated with a nitroso reaction, was revealed in the seed coat and the seed exudate when stained directly on the filter paper. In this test the development of a blue colour was considered as indication of the presence of tannins.

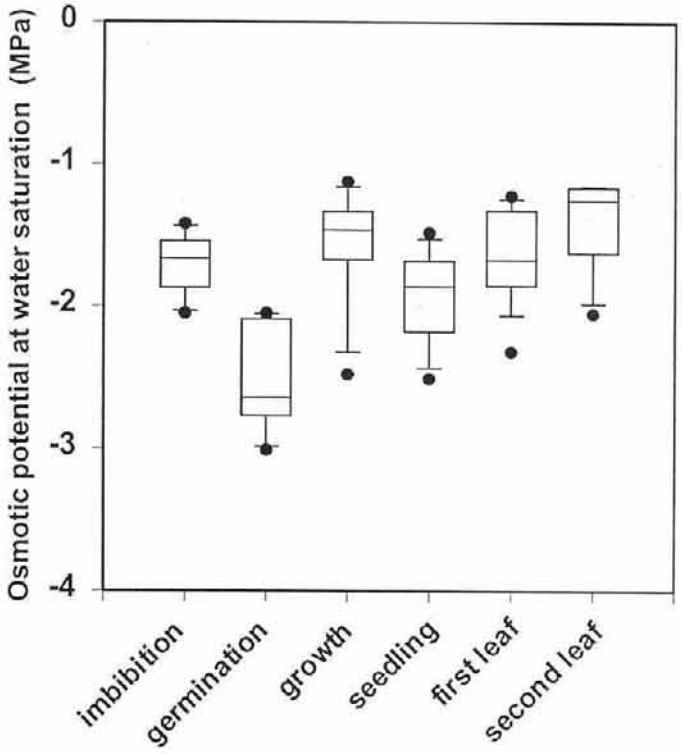

Fig. 6: Osmotic potential at water saturation at different developmental stages of germination and seedling performance in *T. coerulea*. Box plots represent the distribution of data within a treatment. The middle line inside the boxes is median, the 25th and 75th percentiles represent extent of boxes, maximum and minimum values are shown as capped bars, the solid circles represent extreme values exceeding the 1.5-fold box length of the data. n = 12, 15.

Discussion

The investigation of substrate water potential constraints on seed germination of *T. coerulea* has shown that germination rate declined with decreasing substrate water potential. At -1.0 MPa in the external liquid medium the germination was completely inhibited. This threshold value of substrate water potential that inhibits germination, determined here for *T. coerulea* under constant laboratory conditions, is

very near to minimum values of substrate water potential required for germination of other mesophytic crops growing in the field (FITTER and HAY, 1987; CHOINSKI and TUOHY, 1991).

The time for germination of *T. coerulea* seeds is considerably prolonged at substrate water potentials around -0.5 MPa, as compared to control seeds germinated in water. The slowing down does not only affect radicle emergence, but as well seedling growth. The decline in seedling elongation at lower substrate water potentials is accounted for by inhibition of cell expansion, or, at severe water stress, by a declined cell proliferation (TEULAT et al., 1997; NONAMI, 1998). For seedling establishment in the field these changes might be dramatic enough to decide about the individual survival, as a short radicle will be affected heavier by water stress when the soil surface is drying (MEYER and BOYER, 1981; WHALLEY et al., 1998). Hence, during the seedling phase the plant must escape the desiccation, while during the germination phase it is rather drought tolerant (SWAGEL et al., 1997). In consequence, rapid germination and short time for seedling development both will favour plant establishment. This is especially important for plant species distributed in arid and semi-arid habitats, as it is typical for many *Trigonella* species (LACHASHVILI, 1981). Rapid seed germination in *T. coerulea* that requires not more than two days both in experimental conditions and in the field (unpubl. observations) might be an effective adaptation evolved already by the indigenous ancestor of the crop as an adaptation to irregular rainfall events in dry areas of its distribution.

The parameters of water relations in germinated seeds and seedlings of *T. coerulea* showed definite differences both at the various developmental stages and among individuals measured at the same developmental stage. Both, osmotic potential at water saturation and water potential at turgor loss reached the most negative mean values when the radicle emerged. The mean value of osmotic potential at this developmental stage, -2.6 ± 0.35 MPa, was considerably lower than the threshold value of substrate water potential, -1.0 MPa, that inhibited germination. By contrast, substrate water potentials between -1.0 and -2.0 MPa did not prevent imbibition of seeds. Uptake of water by seeds depends on the difference in water potentials between seed and soil, and imbibition largely results from such gradients (ROBERTS and ELLIS, 1989). In consequence, water uptake occurs regardless of whether the seed is dormant or nondormant, viable or nonviable (BEWLEY and BLACK, 1994). In our experiments, most imbibed seeds were viable even where germination failed. In the last named situation the hydration did not lead to radicle emergence indicating that, in all probability, these seeds were dormant. Physiological backgrounds of the developmental arrest and the transition of a seed from the dormant to nondormant state can be manifold and may affect quite heterogeneously the percentages of dormant and nondormant ones within a population of seeds (COHN, 1996).

It is known (FINCH-SAVAGE et al., 1998) that the start of radicle growth functions as a moisture sensitive threshold situation for the whole germination process. During the imbibition period seed water potential must have more negative values than the immediate surroundings. Only after radicle emergence an exploitation for water supply of more distant soil regions becomes possible (LÖSCH, 2001). Even during radicle protrusion out of the seed coat the germinating seed must rely on itself in order to keep the appropriate water potential gradient. This can be acchieved by osmoregulatory solute accumulation that is sufficient to decrease the osmotic potential when the supply of water is becoming limited around the developing roots (MEYER and BOYER, 1981). The strong decrease of osmotic potential at water saturation observed up to the third day of *Trigonella* seed germination may exactly result from this mechanism. However, the extent of osmoregulation is limited by the amount of storage substances that can be mobilized. The osmoregulatory shove that establishes a suitable water potential gradient from the surroundings to the elongating radicle soon must be replaced by foraging for water supply by continued root growth. Individual differences between seeds in the amount of solute mobilization and concentration could be important for a successful further establishment: Such variations in the water status parameters among individual *T. coerulea* seeds were obvious. Osmotic potential at the time of radicle appearance varied between minimal -3 MPa and maximal -2 MPa values. Due to this variability, the difference between seed and actual substrate water potentials will come to different values for the individual plants. This may play a dramatic role for the success of the seed germination and seedling establishment under heterogenous field conditions.

Dry mass of the germinated embryo was reasonably smaller than that of the seed and increased during the first five days after soaking. Besides within the cotyledons the seed of *T. coerulea* contains storage substances in the seed coat and in the endosperm layer surrounding the embryo. Endosperm is absent in most leguminous plants, but it persists in some species, including some of the genus *Trigonella* (BEWLEY and BLACK, 1994). The major source of stored carbohydrate reserves in the dry seed of *T. foenum-graecum* is galactomannan, contained within the endosperm cell walls (REID and BEWLEY, 1979). It was shown in this plant species that the breakdown and mobilisation of the endospermous galactomannan by hypocotyl and cotyledons begins 24 h after soaking and continues up to 120 h, reflected by a concomittant decrease of fresh weight in this tissue (DIRK et al., 1999). In *T. coerulea*, the mobilization of storage substances from the seed coat and the endosperm, indicated by a dry mass accumulation in the embryo, shows a similar time course. After the fifth day, when the seed coat is depleted and becomes removed, dry mass of the growing embryo begins to decrease, probably by catabolic consumption of organic substances.

A net gain of dry mass of the entire seedling is initiated only at the eighth day after seed soaking. The cotyledons unfold and become green two days earlier. It has been suggested (CHORY, 1997) that photomorphogenesis of the seedling represents a developmental pattern that rapidly converts the juvenile plant from the storage material based seed into a photoautotrophic organism. However, it was shown for sunflower seedlings that they lose dry mass, in spite of the fact that cotyledons are photosynthetically active (HEUPEL and KUTSCHERA, 1996). Obviously, the transition from heterotrophic to photoautotrophic growth sustaining the gain of biomass occurs only at a stage of plant development when the cotyledons completed their growth (JUCKNISCHKE and KUTSCHERA, 1998). Moreover, experiments with soybean seedlings have shown (MAREK and STEWART, 1992) that in this species cotyledon photosynthesis never exceeds dark respiration, and photoautotrophic growth occurs only after primary leaf photosynthesis begins. In *T. coerulea*, the seedling dry mass accumulation is initiated after the cotyledons are completely formed. The second osmotic adjustment, expressed by the decrease in osmotic potential at day 6 after soaking, should result in the accumulation of solutes of a cotyledonary origin. In all probability these solutes do not come from storage material, rather they will result from the photosynthesis of the green cotyledons. The photosynthetic capacity of the *Trigonella* leaves has not been determined in this study.

The analysis of the imbibed *T. coerulea* seed extracts confirmed the presence of flavonoids and condensed tannins as they were found also in seed exudates of other leguminous plants (BEKKARA et al., 1998). Different flavonoids are released from the seeds of different plant species playing a role, in a species-specific manner, in interactions between plant hosts, on the one hand, and nitrogen-fixing symbionts, mycorrhizal fungi, parasitic plant roots and microbial pathogens, on the other hand (COOPER-DRIVER and BHATTACHARYA, 1998). We tentatively identified vitexin and orientin in *T. coerulea* seed exudate. Our observations gave no evidence for the presence of quercetin and kaempferol in the seeds. They were found in *T. coerulea* flower extracts (JURZYSTA et al., 1988). But not all spots on the chromatograms could be identified unambiguously, and more detailed studies are

needed to identify completely the phenolic compounds released from *T. coerulea* seeds during the germination. The condensed tannins detected in the seed coats and the seed exudate of *T. coerulea* probably function as protectants of the germinated seeds against fungal and microbial infections, as it is shown for other plant species (KANTAR et al., 1996).

In our observations, solute leakage could be determined visually due to formation of yellowish spots around the seeds germinated on filter paper. It has to be mentioned that only the seeds that have exuded in this way continue germination further. The exudation does not occur in non-germinating imbibed seeds that proved to be viable as determined by the tetrazolium test. These seeds, in all probability, were dormant. However, the mechanism of this dormancy should be different from the hard seed coat dormancy when seeds do not undergo hydration (BASKIN et al., 1998). This type of dormancy was observed for about half the amount of seeds used in germination treatments in the present study. Imbibed seeds remained dormant if no exudation of phenolic substances occured. Only approx. 15 % of all seeds in the best case started germination and released also yellowish substances to the surroundings. We conclude that in imbibed seeds without exudation the developmental program leading to germination is not activated. The exudation of phenolic substances might be an essential part of the gene expression program associated with the transition from the dormant to the germination state (MCCARTY, 1995). The start of leakage of substances out of the seed coats may be accompanied by some water release as it is indicated by a transient decrease in seed water contents during the fifth hour of soaking. We assume that the decline in water content at the time of the first exudation, at least in some measured individuals, might be connected with membrane damages of the imbibed seed cells. Probably they become transiently leaky during the transition from the gel to the liquid-crystalline phase (BEWLEY and BLACK, 1994). Solute leakage might proceed also after membrane stabilization (KEISER et al., 1995; MANSOUR et al., 1998). Seed water content increased during the further course of imbibition until radicle emergence and at later stages of the seedling development. Solute exudation can continue also at later germination stages. In *Sinapis alba* it occurs during the whole period of seedling establishment (COLLINS and ABBAS, 1985), and leakage of phenolic substances is highest during the first two days of *Vicia faba* root growth (BEKKARA et al., 1998).

Plant germination occurs as a sequence of biophysical and biochemical processes that are activated and controlled by plant external conditions and by the plant internal genetic program. Seed and seedling tissue water status is important mediating the environmental situation to the metabolic processes and is influenced, at the same time, by the course of germination events. Water uptake enables the volume enlargement of the plant, release of substances from storage sites to the differentiating tissues or to the surroundings occurs at the same time. The latter phenomenon may be both, an inevitable side-effect of seed tissue reorganization or an essential step in the peculiar germination program. It can be involved in a dormancy-breaking mechanism, it can modify the environment of the developing seedling, or it may function as a messenger for interactions with other organisms. Mobilization of osmotically active solutes inside the seed stabilizes or intensifies the water potential gradient from the surroundings until root water uptake can replace this osmoregulation-based intake of water. Amount and time course of this osmoregulation depend upon the available reserves and their activation. By this way the dynamic changes of the water status are linked to the metabolic processes that proceed under consumption of chemically bound energy and under genetic control. Here, a reasonable species-specifity prevails, but also individuals of one species may show quantitative variations of a qualitatively always uniform pattern of events. Selection by the habitat situation of those plants that develop successfully finds here a prominent point of attachement. A higher or lower provision of reserves by the maternal plants – that in turn may depend on growth conditions and resource distribution to the seeds of a plant (*Trigonella coerulea*: ACHALKAZI and LÖSCH, under submission) – can strongly influence the fate of the offsprings.

Zusammenfassung

Samenkeimung und Keimlingsentwicklung von *Trigonella coerulea* (Fabaceae): Veränderung der Wasserzustandsparameter und Sekundärstoffausscheidung während der ontogenetischen Differenzierung

Während der Samenquellung und Keimung von *Trigonella coerulea* unter kontrollierten Bedingungen wurden die dynamischen Veränderungen von Wasser- und osmotischem Potential, Gewebewassergehalt und -Trockenmasse gemessen und in Beziehung gesetzt zum ontogenetischen Zustand der Pflanzen. Wegen der prinzipiell gegebenen Hartschaligkeit der Samen der Art quillt nur ein knappes Viertel der Samen ein, und selbst bei Auslegen auf mit destilliertem Wasser getränktem Filterpapier keimen nur rund 15 % der Samen. Auf Substrat mit niedrigerem Wasserpotential (verschieden konzentrierte Mannitollösungen) ist der Keimerfolg noch geringer, benötigt längere Zeiten der Einquellung und bleibt bei Substratwasserpotentialen von -1 MPa und niedriger völlig aus. Wenn die Radicula aus der Samenschale hervortritt, ist, nach einem kontinuierlichen Abfall seit Beginn der Einquellung, mit -2,6 ± 0,35 MPa das niedrigste osmotische Potential bei Wassersättigung erreicht (pV-Analyse). Danach steigen die Potentiale wieder an, um nach Ergrünen von Hypokotyl und Keimblättern, rund eine Woche seit Keimungsbeginn erneut abzusinken. Beide Abnahmephasen können als Zeiten der aktiven Osmoregulation gedeutet werden. Bis kurz vor Abschluß der Hypokotylstreckung erhöht der Embryo seine Trockenmasse zu Lasten von Samenschale und Endosperm; während der Kotyledonenentfaltung ist eine Nettoabnahme des Trockengewichtes zu verzeichnen. Der Anstieg des Samenwassergehaltes nach Einquellungsbeginn folgt einer in zwei Stufen verlaufenden Sättigungskinetik. Der erste Plateauwert ist mit rund 3 g H_2O g^{-1} d.m. nach reichlich einem Tag erreicht (Ende der Sameneinquellung) und wird bis zum 6. Tag (Ende der Radiculastreckung) beibehalten. Danach erfolgt ein weiterer, etwas flacherer Anstieg während der Hypokotyl- und Keimblätter-Entwicklung. Er endet knapp zwei Wochen nach Einquellungsbeginn bei einem Wassergehalt von 20 g H_2O g^{-1} d.m., der während der folgenden Primärblattentwicklung konstant bleibt. Rund fünf Stunden nach Einquellungsbeginn kommt es zur Ausscheidung von Tanninen und Flavonoiden, welche dünnschichtchromatographisch näher analysiert wurden. Eine erfolgreiche Fortsetzung des Keimungsablaufes ist notwendigerweise mit dieser Stoffausscheidung verbunden – gequollene Samen ohne derartige Exudation entwickeln sich nicht weiter. Selbst bei der Keimung von äußerlich vergleichbaren Samen herrscht eine erhebliche interindividuelle Variationsbreite der osmotischen Potentiale, Wassergehalte, Trockengewichte usw. vor, bei allerdings stets gleichem Muster in der Veränderung dieser Parameter. In dieser Variationsbreite wird ein bedeutsamer Angriffspunkt für die Selektion unter natürlichen Bedingungen gesehen, welcher den Ausschlag geben kann bei der erfolgreichen standörtlichen Etablierung einzelner Individuen aus der Gesamtheit einer Samenpopulation.

Acknowledgement

The authors thank Prof. A.W. Alfermann for his help with the chromatographic analysis. The study was conducted while the first author was an Alexander von Humboldt Foundation Fellow in Düsseldorf. Support and furtherance from this granting institution is very gratefully acknowledged.

References

AKHALKATSI, M. and LÖSCH, R., 2001: Resource limitation effect on flowering, fruiting, and germination in *Trigonella coerulea* (Fabaceae). (Under submission).

ANDERSEN, M.N., JENSEN, C.R., and LÖSCH, R., 1991: Derivation of pressure-volume curves by a non-linear regression procedure and determination of apoplastic water. J.Exp. Bot. 42, 159-165.

BASKIN, J.M., NAN, X.Y., and BASKIN, C.C., 1998: A comparative study of seed dormancy and germination in an annual and a perennial species of Senna (*Fabaceae*). Seed Scien. Res. 8, 501-512.

BEKKARA, F., JAY, M., VIRICEL, M.R., and ROME, S., 1998: Distribution of phenolic compounds within seed and seedling of two *Vicia faba* cvs differing in their seed tannin content, and study of their seed and root phenolic exudations. Plant Soil 203, 27-36.

BEWLEY, J.D. and BLACK, M., 1994: Seeds. Physiology of Development and germination. Plenum Press, New York, London.

BRADFORD, K.J., 1995: Water Relations in seed germination. In: Kigel, J. and Galili, G. (eds.), Seed development and germination, 351-396. Marcel Dekker, New York.

CHOINSKI, J.S. and TUOHY, J.M., 1991: Effect of water potential and temperature on the germination of four species of African savanna trees. Ann. Bot. 68, 227-233.

CHORY, J., 1997: Light modulation of vegetative development. Plant Cell 9, 1225-1234.

COHN, M.A., 1996: Operational and philosophical decisions in seed dormancy research. Seed Scien. Res. 6, 147-153.

COLLINS, J.C. and ABBAS, M.A., 1985: Ion and water transport in seedlings of mustard (*Sinapis alba* L.). New Phytol. 99, 195-202.

COOPER-DRIVER, G.A. and BHATTACHARYA, M., 1998: Role of phenolics in plant evolution. Phytochemistry 49, 1165-1174.

DELL'AQUILA, A. and DITURI, M., 1995: Deterioration of wheat seeds under subfreezing temperatures and related protein-synthesis in germinating embryos. Seed Scien. Techn. 23, 551-561.

DIRK, L.M.A., VAN DER KROL, R., VREUGDENHIL, D., HILHORST, W.M., and BEWLEY, J.D., 1999: Galactomannan, soluble sugar and starch mobilization following germination of *Trigonella foenum-graecum* seeds. Plant Physiol. Biochem. 37, 41-50.

EGLI, D.B. and TEKRONY, D.M., 1997: Species differences in seed water status during seed maturation and germination. Seed Scien. Res. 7, 3-11.

FINCH-SAVAGE, W.E., STECKEL, J.R.A., and PHELPS, K., 1998: Germination and post-germination growth to carrot seedling emergence: predictive threshold models and sources of variation between sowing occasions. New Phytol. 139, 505-516.

FITTER, A.H. and HAY, R.K.M., 1987: Environmental physiology of plants. Academic Press, San Diego.

HEUPEL, T. and KUTSCHERA, U., 1996: Pigment accumulation, dark respiration and photosynthesis during the greening of sunflower cotyledons. J. Plant Phys. 147, 567-572.

IKEDA, T., OKADA, K., YAMAZAKI, H., OI, R., and IMADA, S., 1999: Growth and water status of cucumber seedlings under altered red/far-red photon flux ratio. J. Jap. Soc. Horticult. Sci. 68, 189-191.

JENSEN, W.A., 1962: Botanical Histochemistry. WH Freeman, San Francisco, London.

JOHNSON, J.M., PRITCHARD, J., GORHAM, J., and TOMOS, A.D., 1996: Growth, water relations and solute accumulation in osmotically stressed seedlings of the tropical tree *Colophospermum mopane*. Tree Physiol. 16, 713-718.

JUCKNISCHKE, A. and KUTSCHERA, U., 1998: The role of the cotyledons and primary leaves during seedling establishment in sunflower. J. Plant Phys. 153, 700-705.

JURZYSTA, M., BURDA, S., OLESZEK, W., and PLOSZYNSKI, M., 1988: The chemotaxonomic significance of laricytrin and medicagenic acid in the tribe *Trigonelleae*. Can. J. Bot. 66, 363-367.

KEISER, J.R., MULLEN, R.E., and HINZ, P.N., 1995: Effects of Ca^{2+}-enriched and Mg^{2+}-enriched germination media on germination and sugar leakage of Ca-deficient soybean seeds. Can. J. Plant Sci. 75, 343-346.

KANTAR, F., HEBBLETHWAITE, P.D., and PILBEAM, C.J., 1996: Factors influencing disease resistance in high and low tannin *Vicia faba*. J. Agricult. Sci. 127, 83-88.

KOLLER, D. and HADAS, A., 1982: Water relations in the germination of seeds. In: Lange, O.L., Nobel, P.S., Osmond, C.B., and Ziegler, H. (eds.), Physiological plant ecology II. Encyclopedia of plant physiology NS 12 B, 401-431. Springer, Berlin.

LACHASHVILI, I., 1981: *Trigonella*. In: Kemularia-Natadze, L. (ed.), Flora of Georgia 7, 51-75. Metsniereba, Tbilisi.

LANFERMEIJER, F.C., KOERSELMAN-KOOIJ, J.W., and BORSTLAP, A.C., 1990: Effect of medium osmolarity on the release of amino acids from isolated cotyledons of developing pea seeds. Planta 181, 568-575.

LEIVA, M.J. and FERNANDEZ-ALES, R., 1998: Variability in seedling water status during drought within a *Quercus ilex* subsp. *ballota* population, and relation to seedling morphology. Forest Ecol. Manage. 111, 147-156.

LEPRINCE, O., VAN DER WERF, A., DELTOUR, R., and LAMBERS, H., 1992: Respiratory pathways in germinating maize radicles correlated with desiccation tolerance and soluble sugars. Physiol. Plant. 85, 581-588.

LÖSCH, R., 2001: Wasserhaushalt der Pflanzen. Quelle & Meyer, Wiebelsheim.

MANSOUR, M.M.F., VAN HASSELT, P.R., and KUIPER, P.J.C., 1998: Ca^{2+} and Mg^{2+}- ATP-ase activities in winter wheat root plasma membranes as affected by NaCl stress during growth. J. Plant Phys. 153, 181-187.

MAREK, L.F. and STEWART, C.R., 1992: Photosynthesis and photorespiration in presenescent, senescent, and rejuvenated soybean cotyledons. Plant Physiol. 98, 694-699.

MARKHAM, K.R., 1982: Techniques of flavonoid identification. Academic Press, London.

MCCARTY, D.R., 1995: Genetic control and integration of maturation and germination pathways in seed development. Annu. Rev. Plant Physiol. Plant Mol. Biol. 46, 71-93.

MEYER, R.F. and BOYER, J.S., 1981: Osmoregulation, solute distribution, and growth in soybean seedlings having low water potentials. Planta 151, 482-489.

MOORE, R.P., 1973: Tetrazolium staining for assessing seed quality. In: Heydeker, W. (ed.), Seed Ecology. Pennsylvania State University Press, University Park.

NONAMI, H., 1998: Plant water relations and control of cell elongation at low water potentials. J. Plant Res. 111, 373-382.

PHILLIPS, D.A., WERY, J., JOSEPH, C.M., JONES, A.D., and TEUBER, L.R., 1995: Release of flavonoids and betaines from seeds of 7 *Medicago* species. Crop Sci. 35, 805-808.

REID, J.S.G. and BEWLEY, J.D., 1979. A dual role for the endosperm and its galactomannan reserves in the germinative physiology of fenugreek (*Trigonella foenum-graecum* L.), an endospermic leguminous seed. Planta 147, 145-150.

ROBERTS, E.H. and ELLIS, R.H., 1989: Water and seed survival. Ann. Bot. 63, 39-52.

SAS Institute Inc., 1991: SAS/STAT User's Guide, Version 6, Fourth Edition, Volume 1, Cary, NC: SAS Institute Inc.

STEBBINS, G.L., 1974: Flowering plants: evolution above the species level. Belknap Press, Cambridge.

SWAGEL, E.N., VAN BERNHARD, A.H., and ELLMORE, S., 1997: Substrate water potential constraints on germination of the strangler fig *Ficus aurea* (*Moraceae*). Am. J. Bot. 84, 716-722.

TEULAT, B., MONNEVEUX, P., WERY, J., BORRIES, C., SOUYRIS, I., CHARRIER, A., and THIS, D., 1997: Relationships between relative water content and growth parameters under water stress in barley: a QTL study. New Phytol. 137, 99-107.

THORNLEY, J.H.M., 1996: Modeling water in crops and plant ecosystems. Ann. Bot. 77, 261-275.

WHALLEY, W.R., BENGOUGH, A.G., and DEXTER, A.R., 1998: Water stress induced by PEG decreases the maximum growth pressure of the roots of pea seedlings. J. Exp. Bot. 49, 1689-1694.

ZUANAZZI, J.A.S., CLERGEOT, P.H., QUIRION, J.C., HUSSON, H.P., KONDOROSI,

A., and RATET, P., 1998: Production of *Sinorhizobium meliloti* nod gene activator and repressor flavonoids from *Medicago sativa* roots. Mol. Plant-Microbe Interact. 11, 784-794.

Address of the authors:
Dr. Maia Akhalkatsi, Institute of Botany, Georgian Academy of Sciences, Kojori road 1, 380007 Tbilisi, Georgia. E-mail: maia.akhalkatsi@usa.net.
Prof. Dr. Rainer Lösch, Department of Geobotany, H.Heine-University Düsseldorf, Universitätsstraße 1, D-40225 Düsseldorf, Germany. E-mail: loesch@uni-duesseldorf.de. (Author for correspondence).