BIOLOGIA PLANTARUM (PRAHA)

23 (3) : 209—213, 1981

Desiccation Tolerance Changes in Winter Rape Leaves Grown under Different Environmental Conditions

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Abstract. The results of studies performed on winter rape leaves (*Brassica napus* L. var. *oleifera* L., cv. Górczański) indicated that independently of the plant cultivation conditions and of the leaf growth rate, all leaves showed significant increase in desiccation tolerance at the beginning of leaf expansion. The increase in tolerance was correlated with the formation of a central vacuole in the mesophyll cells. Therefore, the high desiccation tolerance of vacuolised cells in comparison to the non-vacuolised ones is supposed to be due to the higher ability of the former to avoid cytoplasm dehydration. Development of frost tolerance in the leaves during autumn does not seem to be causally related to desiccation tolerance changes.

According to many investigators (see LEVITT 1972), development of drought resistance in plants often correlates with plant frost tolerance. A common basis for both phenomena is generally accepted: a high stability of cell components under dehydration conditions. Frost tolerance of winter rape leaves was previously found (SIKORSKA and KACPERSKA-PALACZ 1979) to be a three-step process; one of these steps was due to frost-induced tissue dehydration. Therefore, the present work was undertaken to examine how the development of tissue dehydration resistance corresponds to frost hardiness changes. Since dehydration resistance may be due either to higher dehydration avoidance or/and to higher dehydration tolerance of the tissue (LEVITT 1972), we used in the studies the previously described method (DŁUGOKĘCKA and KACPERSKA-PALACZ 1978) which allows distinction between these two features of the tissue.

MATERIAL AND METHODS

The experiments were performed on winter rape plants (*Brassica napus* L., var. *oleifera* L. cv. Górczański) grown either under out-door conditions or in light and temperature controlled rooms (the phytotron). Plants grown out of doors were sown in the University experimental field either in autumn

Received June 6, 1980; accepted October 8, 1980

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(1976 and 1977) or in spring (1978). Phytotron plants were grown in boxes filled up with garden soil, under 12 h day ($\sim$ 45 000 erg cm^{-2} s^{-1}, fluorescent tubes "white" 40 W Polam, Poland), at 20 °C (day) and 15 °C (night) and relative humidity of about 60%.

Analyses were performed on the leaf blades collected consecutively from the 1st to the 6th leaf. Sampling of the respective leaf blades started when the blade reached a surface area of about 2 cm^2.

Fresh weight and surface area of one leaf blade were determined (in 10 replicates) during the leaf growth. Surface area was estimated with photocopy technique.

Resistance to desiccation was determined in leaf discs (14 mm in diameter), cut off from the young leaf blades or from the apical part of the older ones. They were floated on distilled water for 2—3 h to regain the full turgidity before the desiccation test. Desiccation of the discs was performed over $CaCl_2$ under vacuum for 10, 15, 20, 40 or 60 min.

Tissue ability to avoid dehydration was estimated according to DŁUGOKĘCKA and KACPERSKA-PALACZ (1978) from changes in the leaf fresh weight during desiccation: the amount of water lost from 1 leaf disc per 1 min during a desiccation period which caused 50% tissue injury was taken as a criterion of the tissue ability to avoid dehydration.

Desiccation tolerance of the leaf discs was estimated according to DŁUGOKĘCKA and KACPERSKA-PALACZ (1978) by determination of water deficit causing 50% tissue injuries. Injuries caused by desiccation were determined with the electric conductivity method (DEXTER *el al.* 1932) and index (I_D) was calculated according to formula:

$$L_D = \frac{L_D - L_0}{100 - L_0} [\%] .$$

Where:

I_D — injury index; L_D — electrolytic leakage from desiccated tissue expressed as percentage of the total electrolyte content; L_0 — electrolytic leakage from the control tissue expressed in the same way.

Water saturation deficits (WSD) were estimated in leaf discs (14 mm in diameter, 10 discs in each replicate of three), according to Stocker's formula:

$$WSD = \frac{W_s - W_a}{W_s - D_w} \times 100 ,$$

where: W_s — water content in the tissue fully saturated with water; W_a — actual water content (in the desiccated tissue); D_w — tissue dry weight.

Standard deviation was calculated when necessary, to evaluate the statistical significance of differences.

RESULTS

Fig. 1 shows that desiccation tolerance of the young leaf was very low at the beginning of October: critical WSD was about 30%. The tolerance markedly increased during October, both in 1976 and in 1977 seasons. Dur-

Abbreviations used: I_D = injury index; WSD = water saturation deficit.

ing winter months the tolerance level was practically constant and fell in the critical WSD range of 70% to 80%. By the end of February a tolerance decrease was observed.

In 1977 the level of desiccation tolerance was found to be less stable and somewhat lower than that noted in 1976: the mean values of critical WSD were about 75% and 85%, respectively.

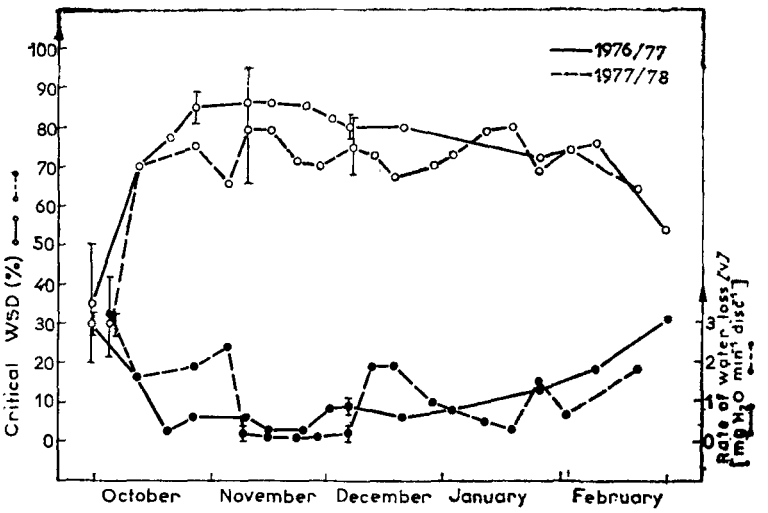

Fig. 1. Changes in dehydration tolerance (critical WSD) and dehydration avoidance (the rate of water loss, v) in winter rape leaves (leaf no. 3 and no. 4) grown during winter under out-door conditions. The leaf expansion was accomplished by the end of October.

Tissue ability to avoid dehydration was also found to increase during October, particularly in 1976: the rate of water loss under desiccation conditions decreased from 3 mg of water min^{-1} $disc^{-1}$ in October to about 0.5—1.0 mg in November. In 1977 marked fluctuations of this parameter were noted throughout the winter season.

A rapid increase in the desiccation tolerance observed at the beginning of two winter seasons might be due either to the plant acclimation to cold or to the regular pattern of leaf development. In order to distinguish between these two possibilities, the leaves of plants not exposed to the cold hardening conditions were analysed for growth and drought resistance changes.

As can be seen in Fig. 2, the growth intensity of the studied leaves was different. It depended on the leaf position and on the cultivation conditions. The expansion of the first true leaves (nos. 1 and 2, Fig. 2 A and B, respectively) was not as intensive as that of the leaves nos. 5 and 6 (Fig. 2 C, D, respectively) and it was accomplished by the twelfth day of the experiment. At that time the leaf surface area attained 10 and 20 cm^2 in leaves grown in the phytotron and in the field, respectively. After two weeks of growth, the surface area of leaves nos. 5 and 6 and also that of nos. 3 and 4 grown in the phytotron (Fig. 2 D) reached 40 cm^2 while of those grown under out-door conditions acquired about 130 cm^2 (Fig. 2 C).

Independently of the cultivation conditions and of the leaf position on the plant, all leaves under examination showed a more or less pronounced

increase in desiccation tolerance (critical WSD increased from 50—70% to 75—85%) during the first few (4 to 9) days of their expansion (Fig. 2). That level of desiccation tolerance was previously observed in the autumn leaves (see Fig. 1). During further leaf growth the desiccation tolerance begun to decrease, mainly in leaves nos. 1 and 2 (Fig. 2 A and B). The decrease was probably due to the early senescence of these leaves.

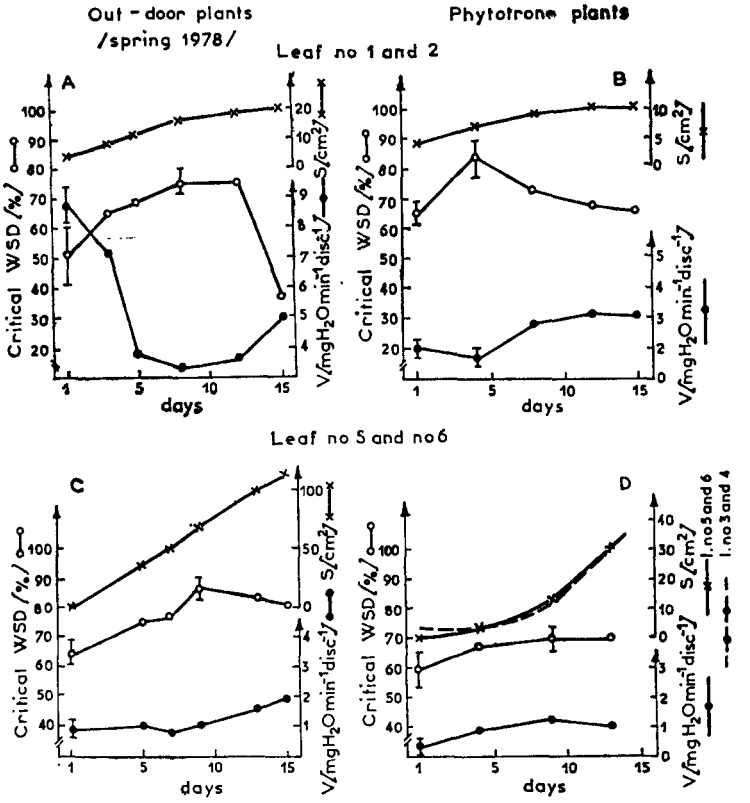

Fig. 2. Comparison of dehydration resistance (critical WSD corresponds to dehydration tolerance and the rate of water loss to dehydration avoidance) and growth intensity changes in the winter rape leaves grown under out-door or phytotron conditions.

During the leaf growth, changes observed in the tissue ability to avoid dehydration were rather small (Fig. 2). In most of the studied leaves, the intensity of water loss under desiccation conditions was rather low at the beginning of the experiment and slowly increased during further leaf growth. This is thought to be a result of the increasing leaf transpiration area. Only the first true leaves derived from plants grown outdoors showed a very high ability to loose water during early stages of their development (about 9 mg min^{-1} $disc^{-1}$), but this ability decreased to 3 mg min^{-1} $disc^{-1}$ after 5 days of growth. It is possible that determinations of dehydration avoidance in the other leaves were performed too late to observe this phenomenon.

Since the increase in desiccation tolerance took place during the first few days of leaf growth and was accomplished before the end of leaf expansion,

studies on changes in the anatomical-cytological leaf structure were undertaken using leaves nos. 3 and 4. The pattern of growth of these leaves closely resembles the growth pattern of the leaf no. 5 and no. 6 (see dotted line in Fig. 2 D).

The results of the anatomical studies indicate that during 9 days of growth a bilayer palisade parenchyma was formed and intercellular spaces developed mainly in the spongy parenchyma (compare phot. A and B in Fig. 3). Large vacuoles were formed in the central part of many cells and the cytoplasm which initially filled up the cell was subsequently pushed to the cell walls.

DISCUSSION

Independently of the cultivation conditions and of the leaf growth pattern, all leaves under examination showed significant increase in desiccation tolerance at the beginning of leaf expansion. The increase in tolerance seems to be correlated with the formation of a central vacuole in the cells. This may indicate that this vacuole may serve as a water reservoir protecting the cytoplasm against dehydration under conditions of a severe and rapid dehydration when an equilibrium is not yet established. Thus, the high desiccation tolerance of the vacuolized cell in comparison to the non-vacuolized one may be of the avoidance type, according to LEVITT's (1972) terminology.

Comparison of the data presented here on leaf desiccation tolerance changes during the winter season with the results of the previous studies on leaf frost tolerance changes (SIKORSKA and KACPERSKA-PALACZ 1979) indicates that desiccation tolerance develops much earlier than frost resistance. Since desiccation tolerance is found to be an attribute of a specific stage of leaf development and frost tolerance was shown to be the result of leaf growth cessation and of specific metabolic changes occurring at the low temperature (SIKORSKA and KACPERSKA-PALACZ 1979), the two phenomena do not seem to be causally related. However, a higher desiccation tolerance at the leaf might be of importance for its freezing tolerance: higher resistance of protoplasts to frost-induced dehydration is one of the aspects of plant frost tolerance (LEVITT 1978).

Acknowledgement

The skilful assistance of mgr Elżbieta Wawrzyniak in microscopic studies is gratefully acknowledged.

This work was supported by Grant No. FG-Po-310 from the US Department of Agriculture, Agricultural Research Program, Public Law 480 and by the Project No. II/7, 4.1. financed by The Polish Academy of Science and Ministry of Science, Higher Education and Technology.

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Fig. 3 at the end of the issue.

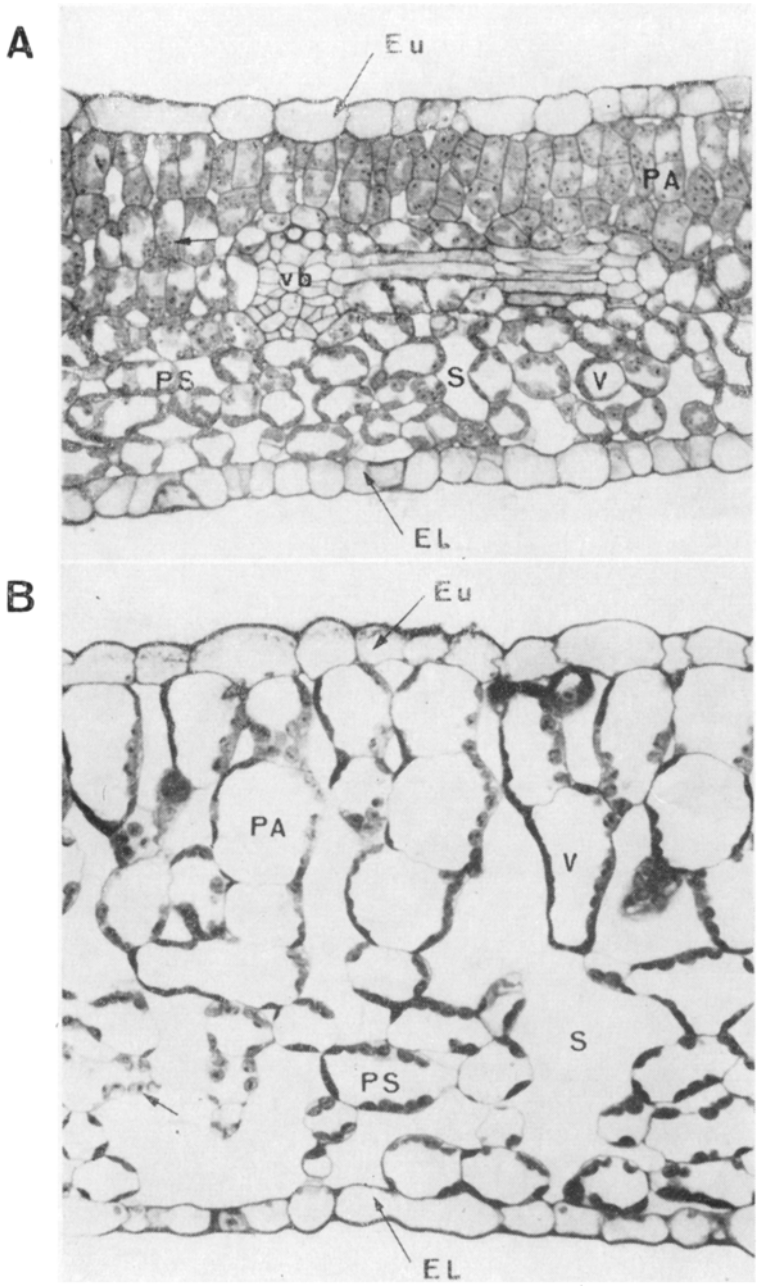

Fig. 3. Transverse sections of rape leaf showing differences in anatomical and cytological structure of mesophyll; leaves were harvested at the time when leaf blade surface area reached 3 cm^2 (A) or 15 cm^2 (B). (× 1250, prepared by E. Wawrzyniak). — Specimens fixed in 2% aqueous $KMNO_4$ solution for 1 h at room temperature, dehydrated in ethanol and embedded in Epon (after Luft). Sections stained in Toluidine Blue (in borax, 1%) for 2 min. — PA — palisade parenchyma, PS — spongy parenchyma, Eu — upper epidermis, EL — lower epidermis, S — intercellular space, V — vacuole, vb — vascular bundle, arrow — starch grains.