BIOLOGIA PLANTARUM (PRAHA)

30 (5) : 393—396, 1988

BRIEF COMMUNICATION

Osmotic Adjustment in Tobacco Plantlets

JANA POSPÍŠILOVÁ

Institute of Experimental Botany, Czechoslovak Academy of Sciences,
Flemingovo nám. 2, 160 00 Praha 6, Czechoslovakia

Abstrac t. Using *Nicotiana tabacum* L. plantlets cultivated *in vitro* as a model system it was proved tha t osmotic adjustment may be caused by a decrease in water potential of substrate as well as by a decrease in air humidity.

Osmotic adjustment in plants as a mechanism of maintaining a certain level of growth and development under water stress or salinity has been observed and studied for many years. Nevertheless, the physiological and biochemical events leading to osmotic adjustment as well as the factors which trigger it are still not quite clear. The decrease in osmotic potential of plants to the level lower than would correspond to a decrease in their water content is usually caused by a large number of simultaneously acting environmental factors. The aim of this contribution was to differentiate between the effect of substrate water potential and the effect of air humidity.

Seedlings of tobacco (*Nicotiana tabacum* L. cv. White Burley) were cultivated in a growth cabinet [irradiance (400—700 nm) at leaf level 40 μmol m^{-2} s^{-1}, day/night temperature 31/26 °C, air humidity 92—97 %], in glass vessels on solid agar Murashige and Skoog medium of half concentration without saccharose or with 2 % of saccharose (besides having a purely nutritive effect the cultivation media influenced plantlet growth through their osmotic properties, and the concentration of saccharose contributed greatly to final media water potentials). Plantlets were transferred to corresponding media every fortnight, as hydrolysis into monosaccharides or consumption by plantlets could affect the concentration of saccharose. After six weeks when plantlets had five leaves half of the vessels (30) was opened for two days and thus air humidity around leaves (measured by a Jumo Humitherm sensor) decreased to 67—73 %.

Received April 11, 1988; accepted April 25, 1988

TABLE 1

Average values of characteristics of water relations in tobacco plantlets cultivated on media without saccharose or with 2 % of saccharose and under air humidity of 95 % or for the last two days exposed to air humidity of 70 %

Air humidity [%]	Concentration of saccharose [%]	Water potential of medium [MPa]	Leaf water potential [MPa]	Leaf osmotic potential [MPa]	Leaf pressure potential [MPa]
95	2	−0.55 ± 0.01	−0.70 ± 0.02	−0.88 ± 0.03	0.18 ± 0.02
70	2	−0.63 ± 0.05	−1.00 ± 0.06	−1.13 ± 0.04	0.13 ± 0.04
95	0	−0.37 ± 0.05	−0.55 ± 0.04	−0.74 ± 0.03	0.19 ± 0.02
70	0	−0.44 ± 0.03	−0.65 ± 0.03	−0.84 ± 0.04	0.19 ± 0.04

The differences in leaf water and osmotic potentials between plantlets grown in the medium with and without saccharose were statistically significant under both air humidities. Similarly, the differences in leaf water potentials and leaf water and osmotic potentials between plantlets grown continuously under air humidity 95 % or exposed to lower air humidity were statistically significant when cultivated on the medium without saccharose or with 2 % of saccharose, respectively. On the other hand, the differences in leaf pressure potentials were not statistically significant.

Leaf water and osmotic potentials as well as water potential of the media were measured with a droplet thermocouple psychrometer arranged with a Keithley Microvolt Ammeter 150 B at a temperature of 25 ± 0.002 °C. The water potential was determined on living leaves and the osmotic potential was determined after the same leaves had been frozen (−18 °C, 16 h) and thawed. The pressure potential was calculated as the difference between the water and osmotic potentials (see POSPÍŠILOVÁ 1975, for detail). Values of water potential were checked by measuring the leaves *in situ* with a Wescor Dew Point Hygrometer HR-33T.

Plantlets cultivated *in vitro* are a good model system for studying the osmotic adjustment caused only by changes in water potential of substrate as their transpiration rate is extremely low due to the high air humidity and low irradiance. In closed vessels the water potential of the medium without saccharose was −0.37 MPa and that of the medium with 2 % of saccharose was −0.55 MPa. The water potential of plantlet leaves corresponded to that of the medium (Table 1). The difference between water potentials of leaves and of the medium was 0.15 and 0.18 MPa in plantlets grown on medium with and without saccharose, respectively. The lower water potential of leaves grown on a medium with 2 % of saccharose compared with that in leaves of plantlets grown on a medium without saccharose was mostly due to a decrease in their osmotic potential and not in their pressure potential. Thus osmotic adjustment occurred in tobacco plantlets (Fig. 1A) and under this extremely low transpiration rate it was caused only by the decrease in water potential of the substrate.

Similarly, the decrease in water potential of the medium by addition of 3 % of saccharose caused a decrease in water and osmotic potentials of potato shoots to −0.95 and −1.15 MPa, respectively, and this osmotic adjustment quickly disappeared after transplanting the plantlets into pots with sand and

nutrient solution (water potential of the substrate was −0.35 MPa) (Pospíšilová *et al.* 1988). Increased concentration of saccharose to 2, 5, 10 and 15 % caused the osmotic adjustment in pea seed embryos (Wang *et al.* 1987) but the osmotic adjustment in plant regenerants cultivated *in vitro* has still not been observed.

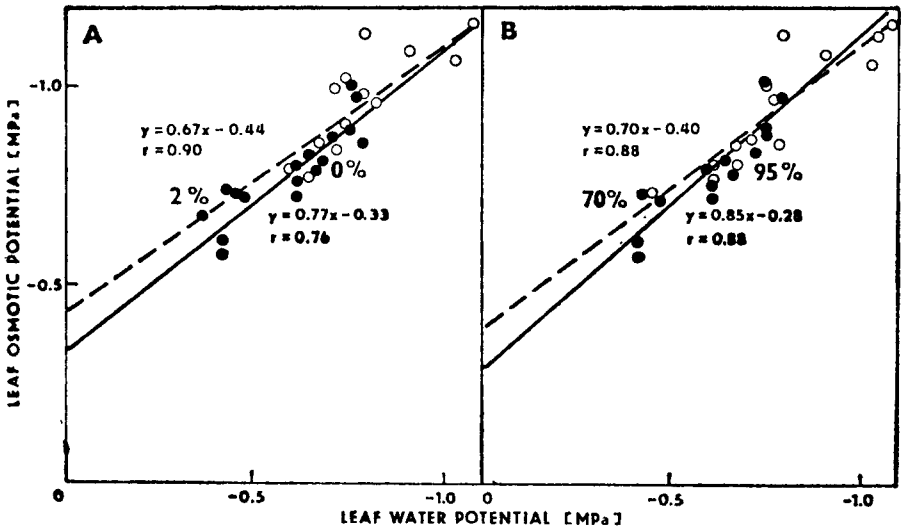

Fig. 1. Regression lines of the relationship between leaf osmotic potential and leaf water potential of tobacco plantlets cultivated on medium without saccharose or with 2 % of saccharose (A) and under air humidity 96 or 70 % (B).

The osmotic adjustment was not a specific effect of saccharose as a similar decrease in water and osmotic potentials of tobacco plantlet leaves (to −0.64 and −0.83 MPa, respectively) was caused by a decrease in osmotic potential of the medium to −0.50 MPa due to an increase in the concentration of mineral elements by using the full Murashige and Skoog medium (but without saccharose). Osmotic adjustment was measured only in cell cultures and it was also induced by different substances: NaCl (Heyser and Nabors 1981, Binzel *et al.* 1985, Daines and Gould 1985, Hasegawa *et al.* 1986), polyethylene glycol (Handa *et al.* 1983, 1986), mannitol and sorbitol (Pritchard *et al.* 1986).

Decrease in air humidity after opening the vessels for two days caused an increase in transpiration rate and thus further decrease in leaf water potential (Table 1). Due to a higher transpiration rate also the difference between water potentials of leaves and the medium increased to 0.21 and 0.37 MPa in plantlets grown on a medium without saccharose and with 2 % of saccharose, respectively. The decrease in water potential was also mostly caused by decrease in osmotic potential and the difference between leaf osmotic potentials of plantlets grown on a medium with 2 % of saccharose and without saccharose was below 70 % air humidity, twice (0.29 MPa) that under air humidity of 95 % (0.14 MPa) even if the differences between water potentials of media increased only from 0.18 to 0.19 MPa. Thus the osmotic

adjustment in tobacco plantlets (Fig. 1B) can be caused not only by a decrease in water potential of substrate but also by increased transpiration rate due to a decrease in air humidity.

Therefore when studying osmotic adjustment in the field it is important to pay attention not only to the substrate moisture and salinity level but also to the environmental conditions affecting the transpiration rate.

Acknowledgement

The author wishes to express sincere thanks to RNDr. M. Ondřej, CSc. for the provision of plant material.

REFERENCES

BINZEL, M. L., HASEGAWA, P. M., HANDA, A. K., BRESSAN, R. A.: Adaptation of tobacco cells to NaCl. — Plant Physiol. **79** : 118—125, 1985.

DAINES, R. J., GOULD, A. R.: The cellular basis of salt tolerance studied with tissue cultures of the halophytic grass *Distichlis spicata*. — J. Plant Physiol. **119** : 269—280, 1985.

HANDA, S., BRESSAN, R. A., HANDA, A. K., CARPITA, N. C., HASEGAWA, P. M.: Solutes contributing to osmotic adjustment in cultured plant cells adapted to water stress. — Plant Physiol. **73** : 834—843, 1983.

HANDA, S., HANDA, A. K., HASEGAWA, P. M., BRESSAN, R. A.: Proline accumulation and the adaptation of cultured plant cells to water stress. — Plant Physiol. **80** : 938—945, 1986.

HASEGAWA, P. M., BRESSAN, R. A., HANDA, A. K.: Cellular mechanisms of salinity tolerance. — HortScience **21** : 1317—1324, 1986.

HEYSER, J. W., NABORS, M. W.: Osmotic adjustment of cultured tobacco cells (*Nicotiana tabacum* var. Samsun) grown on sodium chloride. — Plant Physiol. **67** : 720—721, 1981.

POSPÍŠILOVÁ, J.: Development of water stress in kale leaves of different insertion levels. — Biol. Plant. **17** : 392—399, 1975.

POSPÍŠILOVÁ, J., SOLÁROVÁ, J., ČATSKÝ, J., ONDŘEJ, M., OPATRNÝ, Z.: The photosynthetic characteristics during the micropropagation of tobacco and potato plants. — Photosynthetica **22** : 35—39, 1988.

PRITCHARD, H. W., GROUT, B. W. W., SHORT, K. C.: Osmotic stress as a pregrowth procedure for cryopreservation. 2. Water relations and metabolic state of sycamore and soybean cell suspensions. — Ann. Bot. **57** : 371—379, 1986.

WANG, T. L., SMITH, C. M., COOK, S. K., AMBROSE, M. J., HEDLEY, C. L.: An analysis of seed development in *Pisum sativum*. III. The relationship between the r *locus*, the water content and the osmotic potential of seed tissues *in vivo* and *in vitro*. — Ann. Bot. **59** : 73—80, 1987.