CO_2 ASSIMILATION AND TRANSPIRATION BALANCE IN SPECIES OF GENUS *VITIS* CULTIVATED *IN VIVO* AND *IN VITRO*. ESTIMATION OF STOMATAL AND CUTICULAR TRANSPIRATION *IN VITRO*.

QUOTIENT D'ASSIMILATION DU CO_2 ET DE LA TRANSPIRATION DES ESPÈCES DU GENRE *VITIS* CULTIVÉES *IN VIVO* ET *IN VITRO*. ESTIMATION DE LA TRANSPIRATION STOMATALE ET CUTICULAIRE *IN VITRO*.

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Abstract : The influence of genotype on leaf gas exchange was investigated for *Vitis riparia* and *Vitis rupestris* plants cultured *in vivo* and *in vitro*. Gas exchange was measured at constant temperature and photosynthetic photon flux density and at varying relative humidity from 75 p. cent to 65 p. cent.
Differences of transpiration and CO_2 assimilation rates between genotypes were observed that did not depend upon conditions. Water use efficiency was strongly controlled by the genotype.
Linear correlations between assimilation, transpiration and relative humidity were used to estimate extra stomatal (cuticular) and stomatal transpiration of *in vitro* plants. Cuticular transpiration of *in vitro* plants was also measured directly during the darkness. Our results confirmed the indirect estimates of cuticular transpiration elaborated with a linear regression model. Results also show that cuticular transpiration of *in vitro* grown plants may be relevant and is strongly influenced by the genotype. Similarly, the sensitivity of plantlets to transplantation from *in vitro* to *in vivo* conditions may be strongly related by the genotype.

Résumé : Nous avons étudié l'influence du génotype sur les échanges de gaz au niveau de la feuille de *Vitis riparia* et *Vitis rupestris* cultivées *in vitro*. Les échanges de gaz ont été mesurés à une température et à une densité photosynthétique de flux photonique constantes et à une humidité relative comprise entre 75 et 65 p. cent. Parmi les génotypes, on a observé des différences de transpiration et de quotient d'assimilation de CO_2 dépendant guère des conditions. La capacité d'utilisation de l'eau a été fortement contrôlée par le génotype.
On a utilisé des corrélations linéaires entre l'assimilation, la transpiration et l'humidité relative afin de mesurer la transpiration extra-stomatale (cuticular) et stomatale des plantes *in vitro*. On a aussi mesuré la transpiration cuticulaire des plantes *in vitro* directement dans le noir. Nos résultats ont confirmé les estimations indirectes de la transpiration cuticulaire qu'on a élaboré avec un modèle de régression linéaire. Les résultats ont montré également que la transpiration cuticulaire des plantes élevées *in vitro* peut être importante et qu'elle est très influencée par le génotype. De même, la sensibilité des plantules à la transplantation de la condition *in vitro* à celle *in vivo* peut être fortement influencée par le génotype.

Key words : *in vitro* and *in vivo* culture, stomatal and cuticular transpiration, *Vitis riparia*, *Vitis rupestris*

Mots-clés : *in vitro* et *in vivo* culture, transpiration stomatale et cuticulaire , *Vitis riparia*, *Vitis rupestris*

INTRODUCTION

The genetic control of physiological variables that influence photosynthesis is a crucial aspect in the plant's adaptability to a changing environment. The experimental study of such genotypic effects requires a large sample number and strictly controlled environmental conditions such as soil medium, relative humidity and light intensity. Generally, this aim is best achieved by using *in vitro* cultures. On the other hand, *in vitro* grown plants may respond quite differently to changes in their environment than *in vivo* grown plants. For example, *in vitro* grown plants of a number of species have been found to have less deposition of epicuticular wax

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(GROUT and ASTON, 1977), a lower photosynthetic efficiency (GROUT and ASTON, 1978 ; GROUT and MILLAM, 1985) and their stomata are more likely to malfunction than those of their *in vivo* counterparts (BRAINERD and FUCHIGAMI, 1982). Therefore, results from studies of physiological traits such as drought resistance have to be carefully interpreted depending on the plants' environmental background (FALQUE *et al.*, 1991 ; YUE *et al.*, 1992). Besides, the methods for studying such traits have to be carefully assessed as well. An useful technique for studying plant responses to a changing environment is an open gas exchange system (LAFORGE *et al.*, 1990, DÜRING and HARST, 1996). It allows the control and maintenance of steady state conditions such as CO_2, temperature, irradiance and relative humidity.

In recent years, IACONO *et al.* (1992) and LIMA DA SILVA *et al.* (1996) showed that the photosynthetic activity of *in vitro* plants of genus *Vitis* accounts for 20 p. cent only of the level of field and greenhouse grown plants. DÜRING and HARST (1996) demonstrated that rates of photosynthesis of *in vitro* plantlets are limited by low CO_2 concentration in the headspace of the vessels and low light intensity ; besides, they proved that stomata of *in vitro* plantlets do respond to light intensity changes but, unlike photosynthesis, their reactions are delayed and stomatal closure is incomplete in the dark. These observations do not take into account that leaf transpiration must be considered as the addition of stomatal and cuticular transpiration. Cuticular transpiration, in fact, seems a very important factor. SANTRUCEK J. and SLAVIK B. (1990a, 1990b) proposed already a method for estimating the apparent cuticular transpiration by restricting stomatal diffusion by a mass flow of air in the opposite direction. They used a modified leaf chamber of an open gas system where relative humidity was maintained constant. More recently, HOAD *et al.* (1996) proposed a leaf disc method that enables measurements with intact cuticles and a high relative humidity content for periods of up to 12 days.

In the present study we aimed to investigate the physiological responses to changes in relative humidity of *in vitro* and *in vivo* grown plants. The genetic control of physiological parameters associated to water loss was also studied, in order to better understand the process leading to *in vivo* acclimation of *in vitro* grown plants. Our investigation has been conducted on *Vitis rupestris* and *Vitis* riparia, two genotypes which already proved to be adapted for genetic and physiological studies in our previous experience. Here we estimated the cuticular transpiration indirectly in relation to relative humidity changes : thus determine its significance for the behavior of *in vitro* plantlets to *in vivo* plants. At our knowledge this are the first data about cuticular contribution on leaf transpiration in *in vitro* plantlets in the *Vitis* genus.

MATERIALS AND METHODS

Plant material

The tests were conducted on free virus *Vitis riparia* cv. Michaux and *Vitis rupestris* cv. Scheele genotypes. *In vivo* plants were selected from our ampelographic collection. Five year old adult plants of uniform size were chosen.

As for *in vitro* plants, 6 well-developed micropropagated plantlets of both genotypes were employed. Plantlets were propagated by dissecting the apices of *in vivo* grown plants. After cutting, the apices were sterilized for 15 min in a 0.8 p. cent v/v sodium hypochlorite solution. Then, they were cultured in Magenta boxes containing NN medium (NITSCH and NITSCH, 1969) supplemented with 15 p. cent sucrose and 0.8 p. cent agarose. Plantlets were incubated at 25°C and a 16-h photoperiod, under 70 µmol Q m^{-2} s^{-1} cool white light. A 2-month old culture produced well developed plantlets.

CO_2 assimilation (P_n) and transpiration (E) were measured in an open gas exchange minicuvette system (H. Walz, D-8521 Effeltrich, Germany). CO_2 (350 ppm), temperature (25°C) and radiation (PPFD 700 µmol Q m^{-2} s^{-1}) were kept constant in the cuvette, whilst relative humidity was varied from 75 p. cent to 65 p. cent, recording gas exchange values at each 1 p. cent R.H.

Gas exchange of *in vivo* grown plants was assessed on 6 adult leaves from shoots, chosen in the middle cordon part, on the 8^{th} and 11^{th} node respectively. Measurements were conducted during the first week of July (3 leaves) and during the second week of August (3 leaves) from 10.00 a.m. to 12.00 a.m. The levels of leaf water potential (Φ) and relative water content (RWC) for both genotypes were also recorded. The average of Φ and RWC was -1.58 MPa and 84 p. cent respectively for *Vitis riparia* and -1.64 MPa and 85.5 p. cent for *Vitis rupestris* - in the region where the experience has been conducted these values are usually recorded by irrigated *in vivo* vines.

Whole shoots of *in vitro* plants were inserted into the cuvette to measure gas exchange, while the root systems were maintained in the synthetic medium. In order to compare *in vitro* and *in vivo* measurements,

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gas exchange data are expressed on a Dry Matter basis (g DW), instead of Leaf Area.

Water use efficiency was calculated as the ratio between assimilation (P_n) and transpiration (E) (WUE = P_n/E= µmol CO_2 mmol H_2O^{-1}).

Cuticular transpiration was estimated from gas exchange values of both *in vivo* and *in vitro* cultures using a model derived from the data. In order to validate the model, transpiration of six *in vitro* plantlets of each genotype was measured in the dark (stomata may be considered closed) at the same levels of temperature and relative humidity.

RESULTS AND DISCUSSION

I - COMPARISON BETWEEN GENOTYPES AND CULTURE CONDITIONS

Vitis riparia always had a higher transpiration rate (E) than *Vitis rupestris* but their assimilation rate (P_n) was similar regardless of the culture conditions. This in turn led to a higher water use efficiency (WUE) in *Vitis rupestris* as compared with *Vitis riparia* (table I).

Culture condition noticeably affected gas exchange (table II). *In vitro* cultured plants always had a much higher transpiration rate than *in vivo* cultured plants but the reverse was true when their P_n were compared. Consequently, WUE was reduced from 8.7 *in vivo* to 1.4 µmol CO_2 mmol H_2O^{-1} *in vitro*.

TABLE I
Influence of genotypes on gas exchange parameters.
(average of both culture conditions at 65 and 75 p. cent relative humidity, n = 60)

Tableau I - Influence des génotypes sur les paramètres d'échanges de gaz
(moyenne des deux conditions de culture à une humidité relative entre 65 et 75 p. cent, n = 60).

Genotype	P_n (µmol CO_2 g $DW^{-1}s^{-1}$)	E (mmol H_2O g $DW^{-1}s^{-1}$)	WUE (µmol CO_2 mmol H_2O^{-1})
V. riparia	0.210 a	0.200 a	1.05 b
V. rupestris	0.220 a	0.100 b	2.22 a

Numbers in columns within data followed by the same letter are not significantly different at p=0.05 according Scheffe test.

TABLE II
Influence of culture conditions on gaz exchange parameters.
(average of both genotypes at 65 and 75 p. cent relative humidity, n = 60)

Tableau II - Influence des conditions de culture sur les paramètres d'échange de gaz
(moyenne des deux génotypes à une humidité relative entre 65 et 75 p. cent, n = 60)

Culture Conditions	P_n (µmol CO_2 g $DW^{-1}s^{-1}$)	E (mmol H_2O g $DW^{-1}s^{-1}$)	WUE (µmol CO_2 mmol H_2O^{-1})
In vitro	0.212 b	0.150 a	1.40 b
In vivo	0.309 a	0,035 b	8,70 a

Numbers in columns within data followed by the same letter are not significantly different at p=0.05 according Scheffe test.

TABLE III
Influence of genotype and relative humidity on gas exchange parameters
(average of both culture conditions, n = 30).

Tableau III - Influence du génotype et de l'humidité relative sur les paramètres d'échange de gaz
(moyenne des deux conditions de culture, n = 30).

Genotype	Relative Humidity (%)	P_n (µmol CO_2 g $DW^{-1}s^{-1}$)	E (mmol H_2O g $DW^{-1}s^{-1}$)	WUE (µmol CO_2 mmol H_2O^{-1})
V. riparia	65	0.258 a	0.287 a	0.90 c
	75	0.178 b	0.093 b	1.91 b
V. rupestris	65	0.218 a	0.125 b	1.74 b
	75	0.204 a	0.074 c	2.73 a

Numbers in columns within data followed by the same letter are not significantly different at p = 0.05 according Scheffe test.

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There was no significant interaction between culture conditions and the level of relative humidity. However, there was a significant interaction between genotype and relative humidity for both assimilation and transpiration rates. A decrease in relative humidity was associated with increased P_n and E in both genotypes (table III). While a change in relative humidity did not significantly increase P_n in *Vitis* rupestris, it did it, at the opposite, in *Vitis riparia* from 0.178 to 0.258 µmol g $DW^{-1}s^{-1}$. The transpiration rate responded to a change in relative humidity with a more than 3-fold increase in *Vitis riparia* while the increase in *Vitis rupestris* was less than 2-fold.

A significant interaction was also observed between genotype and culture conditions. *Vitis riparia* showed a relatively larger increase in CO_2 assimilation rate between *in vitro* and *in vivo* cultured plants than *Vitis rupestris* did. Similarly a decline in the transpiration rate between *in vitro* and *in vivo* grown plants was smaller in *Vitis rupestris* than in *Vitis riparia* (table IV).

There was no interaction among genotype, relative humidity and culture conditions. WUE was always higher in *V. rupestris* than *V. riparia* (figure 1) in both culture and relative humidity conditions because E was lower (figure 2).

II - RELATIONSHIPS AMONG CO_2 ASSIMILATION RATE, TRANSPIRATION AND RELATIVE HUMIDITY

A decrease in relative humidity resulted in an increase of P_n and E in both species. E responded more strongly in *Vitis riparia* than in *Vitis rupestris* as is indicated by the slope of the regression line (table V). Furthermore, E of *in vitro* grown plants was always higher than E of *in vivo* grown plants regardless of

TABLE IV
Influence of genotype and culture conditions on gas exchange parameters
(average of 65 and 75 p. cent relative humidity, n = 30)

Tableau IV - Influence du génotype et des conditions de culture sur les paramètres d'échange de gaz
(moyenne de l'humidité rélative entre 65 et 75 p. cent, n = 30)

Genotype	Culture conditions	P_n (µmol CO_2 g $DW^{-1}s^{-1}$)	E (mmol H_2O g $DW^{-1}s^{-1}$)	WUE (µmol CO_2 mmol H_2O^{-1})
V. riparia	*in vitro*	0.215 c	0.208 a	1.04 c
	in vivo	0.349 a	0.042 c	8.25 a
V. rupestris	*in vitro*	0.209 c	0.103 b	2.23 b
	in vivo	0.269 b	0.029 c	9.35 a

Numbers in columns within data followed by the same letter are not significantly different at p = 0.05 according Scheffe test.

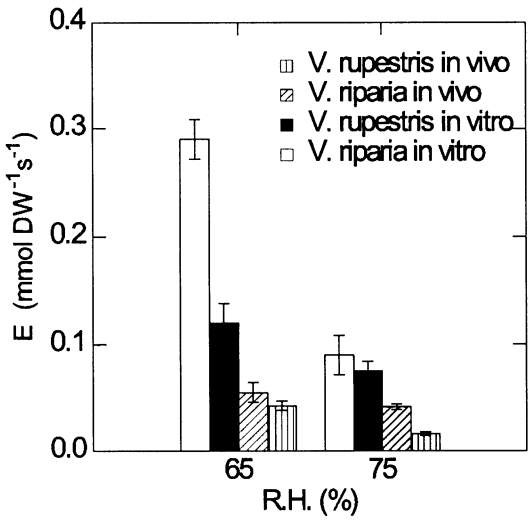


Fig. 1 - Influence of genotype, relative humidity (R.H.) and culture conditions on water use efficiency (WUE).

Fig. 1 - Influence du génotype, de l'humidité relative (R.H.) et des conditions de culture sur l'efficacité de l'utilisation de l'eau (WUE).

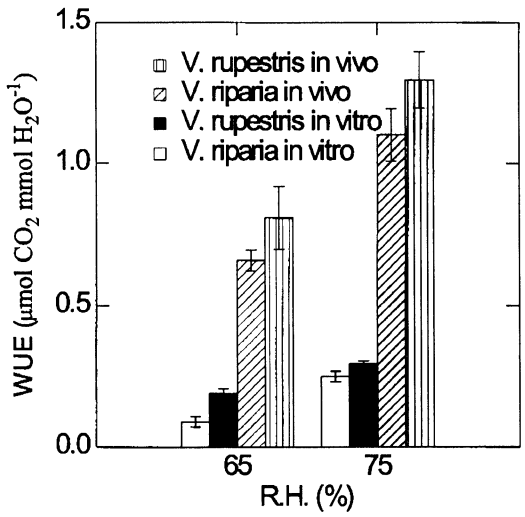


Fig. 2 - Influence of genotype, relative humidity (R.H.) and culture conditions on transpiration (E).

Fig. 2 - Influence du génotype, de l'humidité relative (R.H.) et des conditions de culture sur la transpiration (E).

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TABLE V
Linear regression between transpiration, CO_2 assimilation rate and relative humidity in *in vitro* and *in vivo* cultures

Tableau V - Régression linéaire entre la transpiration CO_2, le taux d'assimilation et l'humidité relative dans les cultures *in vitro* et *in vivo*

Genotype	Culture conditions	DF	Dependent variable	$y = a \pm bx$	R_2
V. riparia	*in vitro*	65	E	1.610- 0.020 R.H.	0.89
	in vivo	65		0.215- 0.0025 R.H.	0.85
	in vitro	65	P_n	0.511- 0.002 R.H.	0.88
	in vivo	65		0.801- 0.001 R.H.	0.86
V. rupestris	*in vitro*	65	E	0.477- 0.005 R.H.	0.90
	in vivo	65		0.147- 0.001 R.H.	0.86
	in vitro	65	P_n	0.309- 0.001 R.H.	0.88
	in vivo	65		0.309- 0.0006 R.H.	0.87

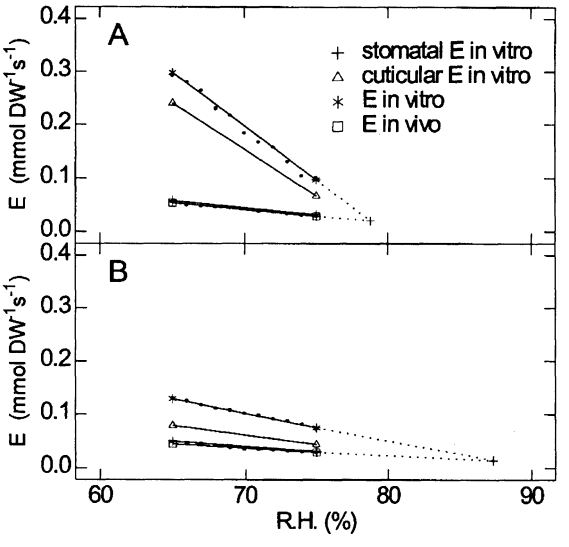


Fig. 3 - Stomatal and cuticular transpiration (E) estimated *in vitro* for *Vitis riparia* (A) and *Vitis rupestris* (B).
Dot lines represent the extrapolations of the regression lines. Each point is the average of 6 data.

Fig. 3 - Transpiration stomatale et cuticulaire (E) *in vitro* de *Vitis riparia* (A) et de *Vitis rupestris* (B).
Les lignes à point représentent les extrapolations des lignes de regression. Chaque point représente une moyenne de six données.

changes in relative humidity. The rate of P_n increase, in response to a lower relative humidity *in vitro*, was relatively lower than that of rate of E which resulted in decreased WUE of *in vitro* plants. This behavior suggests that plants grown *in vitro* lost a larger proportion of E directly through the cuticle rather than the stomata. In fact, data collected in the darkness on different *in vivo* grown plants showed that night transpiration (mainly cuticular transpiration) represents 3-4 p. cent of the day transpiration (stomatal + cuticular transpiration) at relative humidity 50-60 p. cent (data not shown).

III - ESTIMATION OF CUTICULAR TRANSPIRATION *IN VITRO*

In order to study cuticular transpiration, we used information obtained from *in vivo* and *in vitro* gas exchange measurements. Results show that the slope of the linear regression line describing the response of E and P_n to a change in relative humidity is steeper for *in vitro* as compared to *in vivo* plants of both genotypes (table V).

We assumed that the cuticular E rate measured *in vivo* at relative humidity higher than 65 p. cent, was negligible. As a consequence, at the interception point of the extended regression lines of *in vitro* and *in vivo* plants, their E is equal. For *Vitis riparia* the point of interception between the linear regressions of E *in vivo* and *in vitro* is at relative humidity 78.78 p. cent and E is 0.020 mmol g $DW^{-1}s^{-1}$ (figure 3a) while for *Vitis rupestris* this results at relative humidity 87.35 p. cent and E is 0.014 mmol g $DW^{-1}s^{-1}$ (figure 3b).

If E *in vivo* is mainly controlled by stomata, the same control can be hypothesized for *in vitro* plantlets at high relative humidity (> 75 p. cent). Therefore, the WUE of *in vitro* and *in vivo* plants at this point should be modified only by P_n. Furthermore, since no water stress effect was shown by *in vitro* plantlets during gas exchange measurements from 75 to 65 p. cent of relative humidity, if E was controlled by stomata alone, the ratio between WUE of *in vitro* and *in vivo* plants should remain the same at comparatively low relative humidity (65 p. cent) and for any given genotype. However, we observed a lower WUE *in vitro* as compared to *in vivo* plants. This suggests that the ratio is changed because the rate of E through the cuticle, as a proportion of total E, is relatively much higher *in vitro* than *in vivo* plants and WUE decreased accordingly. Then :

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$$1)\ \frac{WUE_{in\ vitro\ R.H.}}{WUE_{in\ vivo\ R.H.}} = \frac{WUE_{in\ vitro\ i}}{WUE_{in\ vivo\ i}}$$

where :

$WUE_{in\ vitro\ R.H.}$ = WUE *in vitro* at specific R.H. value ;
$WUE_{in\ vivo\ R.H.}$ = WUE *in vivo* at specific R.H. value ;
$WUE_{in\ vivo\ i}$ = WUE *in vitro* in the interception point ;
$WUE_{in\ vivo\ i}$ = WUE *in vivo* in the interception point ;

Based on the previous assumptions, we can estimate the relative contribution of cuticular versus stomatal transpiration according to the following model. Considering P_n *in vitro* and knowing WUE *in vivo* (linked to stomata control), it is possible to estimate stomatal E *in vitro* and, subtracting it from total E *in vitro*, obtain cuticular E *in vitro*. In fact, from formula 1) we obtain :

2) $WUE_{st.\ in\ vitro\ R.H.} = (WUE_{in\ vitro\ i}/WUE_{in\ vivo\ i}) * WUE_{in\ vivo\ R.H.}$;
3) $E_{st.\ in\ vitro\ R.H.} = P_{n\ in\ vitro\ R.H.}/WUE_{st.\ in\ vitro\ R.H.}$;
4) $E_{cut.\ in\ vitro\ R.H.} = E_{tot.\ in\ vitro\ R.H.} - E_{st.\ in\ vitro\ R.H.}$;

where :

$WUE_{st.\ in\ vitro\ R.H.}$ = WUE estimated *in vitro* without cuticular loss at specific R.H. value
$P_{n\ in\ vitro\ R.H.}$ = Assimilation *in vitro* at specific R.H.value
$E_{st\ in\ vitro\ R.H.}$ = Estimated stomatal E *in vitro* at specific R.H. value
$E_{tot\ in\ vitro\ R.H.}$ = Measured total E *in vitro* at specific R.H. value
$E_{cut.\ in\ vitro\ R.H.}$ = Estimated cuticular E *in vitro* at specific R.H. value

In *Vitis riparia* stomatal E *in vitro* is estimated 0.058 mmol g $DW^{-1}s^{-1}$ at relative humidity 65 p. cent and 0.030 mmol g DW^{-1} s^{-1} at relative humidity 75 p. cent (figure 3a) while the measured values of total E are respectively 0.298 and 0.096 mmol g $DW^{-1}s^{-1}$ (table VI).

Therefore, cuticular E is estimated 0.240 mmol g $DW^{-1}s^{-1}$ at relative humidity 65 p. cent and 0.067 mmol g $DW^{-1}s^{-1}$ at relative humidity 75 p. cent. Cuticular E in *Vitis riparia*, when relative humidity decreases from 75 to 65 p. cent, represents from 69.8 to 80.0 p. cent of total E. Stomatal E in *in vitro* culture is higher than E in *in vivo* culture especially at low relative humidity.

Following the same hypothesis, it is also possible to estimate cuticular and stomatal E in *Vitis rupestris* (figure 3b). Stomatal E is 0.050 mmol g DW^{-1} s^{-1} at relative humidity 65 p. cent and 0.031 mmol g $DW^{-1}1s^{-1}$ at relative humidity 75 p. cent. Cuticular E is 0.079 and 0.044 mmol g $DW^{-1}s^{-1}$ at 65 and 75 p. cent of relative humidity respectively. When relative humidity decreases from 75 to 65 p. cent cuticular E represents from 58.7 to 61.2 p. cent of total E (table VI). Cuticular E in *Vitis riparia* at relative humidity 65 p. cent increases 3.58 times in comparison to the values measured at relative humidity 75 p. cent, while in *Vitis rupestris* it increases 1.04 times. Stomatal E in *in vitro* culture is similar to E in *in vivo* culture no matter what relative humidity. In *Vitis rupestris* stomatal E is lower than in *Vitis riparia* : hence, stomata control in this genotype is very strong.

The results of the estimated cuticular E *in vitro* are confirmed by E data measured in both genotypes in the darkness, that is with stomata mainly closed. In fact, E values were only slightly higher than the estimated values (table VI).

CONCLUSIONS

There is strong evidence that photosynthetic response to relative humidity changes of *Vitis riparia* and *Vitis rupestris* grown either *in vivo* or *in vitro* are similar. In fact, transpiration (E) *in vitro* was always higher than E *in vivo* while CO_2 assimilation rate (P_n) *in vitro* was lower. Therefore, water use efficiency (WUE) *in vitro* was always lower than WUE *in vivo*. *Vitis riparia* in both culture conditions had a lower WUE than *Vitis rupestris* especially under decreasing relative humidity. Our data are in agreement with what recently reported by LIMA Da SILVA *et al.* (1996).

TABLE VI
Measured and estimated cuticular and stomatal transpiration in *in vitro* and *in vivo* conditions

Tableau VI - Mesure et estimation de la transpiration cuticulaire et stomatale en conditions *in vitro* et *in vivo*

Genotype	*in vivo* E (mmol H_2O g $DW^{-1}s^{-1}$)		*in vitro* total E (mmol H_2O g $DW^{-1}s^{-1}$)		*in vitro* cutic. E (mmol H_2O g $DW^{-1}s^{-1}$)		*in vitro* stom. E (mmol H_2O g $DW^{-1}s^{-1}$)		cutic. E (dark) (mmol H_2O g $DW^{-1}s^{-1}$)	
R.H. (%)	65	75	65	75	65	75	65	75	65	75
V. riparia	0.054	0.030	0.298	0.096	0.240	0.067	0.058	0.030	0.241	0.070
V. rupestris	0.045	0.013	0.129	0.075	0.079	0.044	0.050	0.031	0.085	0.048

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In our investigation the cuticular and stomatal E values of *in vitro* cultures have been estimated by considering negligible the cuticular E measured *in vivo*. Indeed, data obtained in the darkness for *in vitro* grown plantlets confirms this hypothesis. According to the estimations effected through our model, the different rates of cuticular transpiration measured in the dark may be explained with a relatively slower stomatal response to environmental changes of *in vitro* grown plants (DÜRING and HARST, 1996). In this regard, it has been proved that stomata of *in vitro* grown plantlets have sever morphological and functional malformations (SALLANON *et al.*, 1993) ; however, we believe that cuticular contribution in total leaf transpiration may be relevant, and in some cases higher than what stomata alone allow.

In fact, our estimation method proves that cuticular E is a crucial factor in the control of water loss *in vitro* (from 58 to 80 p. cent of the total E). Besides, in our investigation, genotype proved to be a relevant component. Considering that cuticular E *in vitro* at relative humidity 75 p. cent is similar in both genotypes but is very different at relative humidity 65 p. cent (in *Vitis riparia* 3 times higher than in *Vitis rupestris*), it is possible to conclude that genotypes affect the number and size of stomata but also the morphological leaf tissue constitution as reported by ZAMBONI *et al.* (1985) and these differences are probably maintained *in vitro*.

Our own observations indicate that for both genotypes of the genus *Vitis*, water loss occurring during *in vivo* acclimation of *in vitro* plantlets is strongly associated to cuticular E that limits the plant survival at relative humidity 65-75 p. cent. As a consequence, transplanting procedures have to be carefully assessed for a certain genotype, in order to limit the cuticular transpiration which is one of the most crucial factors during *in vitro* culture.

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