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METHODOLOGICAL ASPECTS AND EXPERIMENTAL CONDITIONS IN THE USE OF FLUORESCENCE AS AN INDEX OF WATER STRESS IN CUT LEAVES FROM *GENUS VITIS*

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Summary : *Fluorescence emission is linked to leaf physiological conditions. In particular water stress modifies emission curves strongly. But fluorescence is also influenced by some factors which usually arise in experimental vine breeding programmes. The present research studies some variables that usually characterize the trials on genus* Vitis*: rootstocks and graftings.*

The time of measurement is very important and the fluorescence variables (absolute value) are not correlated with leaf water content. It needs a standardisation by time. The gross relation between fluorescence (after standardisation) and RWC is significant but it is modified by the rootstock behaviour. The Variable Fluorescence is strongly linked to leaf water content, even though the Maximum rate of Fluorescence Quenching shows a variable correlation with decreasing RWC. The genotype controls that variable better than Variable Fluorescence.

INTRODUCTION

The study of chlorophyll fluorescence can represent a valid method for verifying the occurence of different kinds of abiotic stresses (KRAUSE et WEIS, 1984; HETHERINGTON et SMILLIE, 1984; LICHTENTHALER, 1988) and particularly water stress (HAVAUX et LANNOYE, 1983; HAVAUX et *al.*, 1984a, 1984b; PASTORE et *al.*, 1988b, 1988c).

Being proved the link between fluorescence and carbon assimilation (WALKER, 1988; MARTINIELLO et BLUM, 1989), that would make up a decisive step from plant water status to its actual physiological condition relating to the photosynthetic system. In particular the detailed investigation of fluorescence emission profiles gives very interesting information about some aspects of the photosynthetic system (PAPAGEORGIOU, 1975; GENTY et *al.*, 1989).

Some Authors have already shown that fluorescence studies on grapevine can be very useful (DOWNTON, 1984; DOWNTON et MILLHOUSE, 1985; STEIN et *al.*, 1986). They gave determining roles to both fast and slow phases of the curve. IACONO et *al.* (1990) already proposed to use the slow phase to make early screenings of genotypes which can tolerate drought at different extents.

However these studies abstract from the possible factors that can modify fluorescence emission, some of which, particularly in the agronomic sector, determinine the research results. The aim of this work is to verify the relation between leaf water content and chlorophyll fluorescence emission, with particular consideration of the role of some variability factors that can often occur in the experimental designs on grapevine.

MATERIALS AND METHODS

We compared 8 hybrid rootstocks of genus *Vitis* ungrafted (field A) and cv. *Chardonnay* cl. 130 SMA (*Vitis vinifera*) grafted on the same rootstocks (field B). The rootstocks were chosen after their parents in order to maintain the current variability of viticulture.

3309 C	(*Vitis riparia x Vitis rupestris*)
140 Ru	(*Vitis berlandieri x Vitis rupestris*)
1103 P	(*Vitis berlandieri x Vitis rupestris*)
Kober 5BB	(*Vitis berlandieri x Vitis riparia*)
SO4	(*Vitis berlandieri x Vitis riparia*)
420A	(*Vitis berlandieri x Vitis riparia*)
161-49	(*Vitis berlandieri x Vitis riparia*)
41 B	(*V. vinifera cv. Chasselas x V.berlandieri*)¡

Sampling-homogeneous leaves (5-6th internode) were collected at predawn, were hydrated in cold water until they reached constant weight. At RWC equal 100 p. cent, fluorescence was measured (Fluorometer Model SF 30, Richard Brancher Ltd., Ottawa, Canada) in 670 nm. monochromatic light and 36 $\mu Em^{-2}s^{-1}$ (time 0), after 30 minutes adaptation to darkness. Some leaves were kept at full turgor, while others were tested after 2 hours (time 1) and 4 hours (time 2) dehydration. After, the leaves were rehydrated for 3 hours (time 3). The experiment was carried out in the dark, at 25°C and 60 p. cent RH. Relative Water Content and chlorophyll fluorescence were measured at each step.

The investigated parameters were calculated as follows :

RWC = (FW-DW)/(TW-DW);

where RWC = Relative Water Content; FW = Fresh Weight; DW = Dry Weight; TW = Turgid Weight;

VF = (MF-F0)/F0;

where VF = Variable fluorescence; MF = Maximun Fluorescence; F0 = Fluorescence at time 0 sec;

MFQ = (MF-SF)/(ST-MT); (We used this algorithm because many leaves, as the stress status progresses, do not show the second peack) (figure I)

where MFQ = Maximun rate of Fluorescence Quenching; SF = Second Fluorescence value recorded by the instrument after MF; MT = time at MF; ST = time at SF.

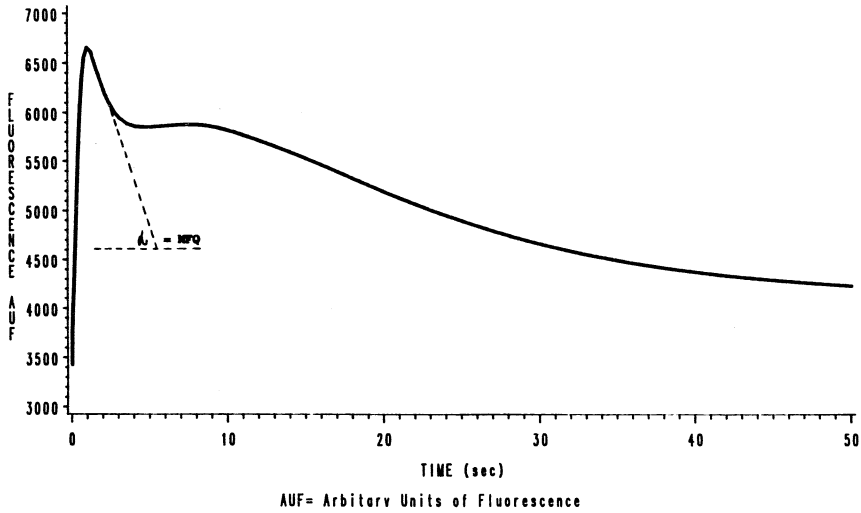


Fig. 1 — Fluorescence emission after dark adaptation. MFQ explanation

RESULTS

I — SELECTION OF PARAMETERS THAT CHARACTERIZE DEHYDRATED AND REHYDRATED STATUS OF LEAVES

The Anova results show a significant influence of the time (0, 1, 2, 3), field (A, B: ungrafted and grafted genotypes) and time*field interaction for RWC and VF. MFQ is not significantly modified by the same variability factors (table I). Therefore VF does not show a clear behaviour in relation to time (table II). Data suggested to use the information from the constantly hydrated leaves to consider the variations occurring during the day (figure II)

We calculated :

$VF\_s = VFi \pm (VF_1 * (VFt_1 - VFt_o) / VFt_o)$;

where VF_s = Variable Fluorescence standardized by the time of measurement; VFi = Variable Fluorescence at time i; VFti = Variable Fluorescence of hydrated leaves at time i; VFt_o = Variable Fluorescence of hydrated leaves at time 0;

$MFQ\_s = MFQ_1 \pm (MFQ_1 * (MFQt_1 - MFQt_o)/MFQt_o)$;

where MFQ_s = Maximum rate of Fluorescence Quenching standardized by the time of measurement; MFQi = Maximum rate of Fluorescence Quenching at time i; MFQti = Maximum rate of Fluorescence Quenching of hydrated leaves at time i; $MFQt_o$ = Maximum rate of Fluorescence Quenching of hydrated leaves at time 0.

In table I, we notice that MFQ_s is significantly influenced by factor time. Table II shows that the VF and MFQ standardisation by time modifies the results in function of the dehydration and rehydration time. Particularly after 2 and 4 hours of dehydration, VF_s and MFQ_s decrease as RWC content decreases. Rehydrated leaves show significantly higher values.

TABLE I

Influence of field (ungrafted and grafted genotypes) and time of dehydration on RWC and fluorescence variables

TABLEAU I — Rôle du champ (génotypes greffés et non-greffés) et du temps de déhydratation sur RWC et les paramètres de la fluorescence

Factors Variability	RWC		VF		MFQ		VF_s		MFQ_s	
	Sum of Squares	Signif. F	Sum of Squares	Signif. F	Sum of Squares	Signif. F	Sum of Squares	Signif. F	Sum of Squares	Signif. F
Field	448.54	0.0001	0.730	0.0001	0.383	0.3901	0.604	0.0001	0.450	0.2369
Time	8904.02	0.0001	0.266	0.0049	1.762	0.3462	1.569	0.0001	13.348	0.0001
Flield*Time	759.58	0.0001	0.747	0.0001	2.415	0.3385	0.624	0.0001	1.967	0.1930
Total	13186.42		5.168		113.76		5.759		81.597	

II — EVALUATION OF THE DIFFERENCES BETWEEN FIELDS AND ROOTSTOCKS

In figure III, the interaction field*time is plotted. During dehydration, field B (grafted genotypes) shows less water loss than field A (ungrafted genotypes), even if RWC values become closer to each other after rehydration. VF_s, at full turgor already, is lower in field A (ungrafted genotypes) and this difference is constant during all the experiment.

Also MFQ_s is lower in field A (ungrafted genotypes) at full turgor, but during dehydration the difference decreases and increases again during rehydration.

In Field A (ungrafted genotypes), VF_s, MFQ_s and RWC discriminate the rootstocks significantly. Only for RWC the time interaction is significant. In field B (grafted genotypes), MFQ_s does not discriminate the rootstocks and there are no significant effects due to time interaction (table III). In the ungrafted genotypes, 41B and 140 Ru and 420A show the

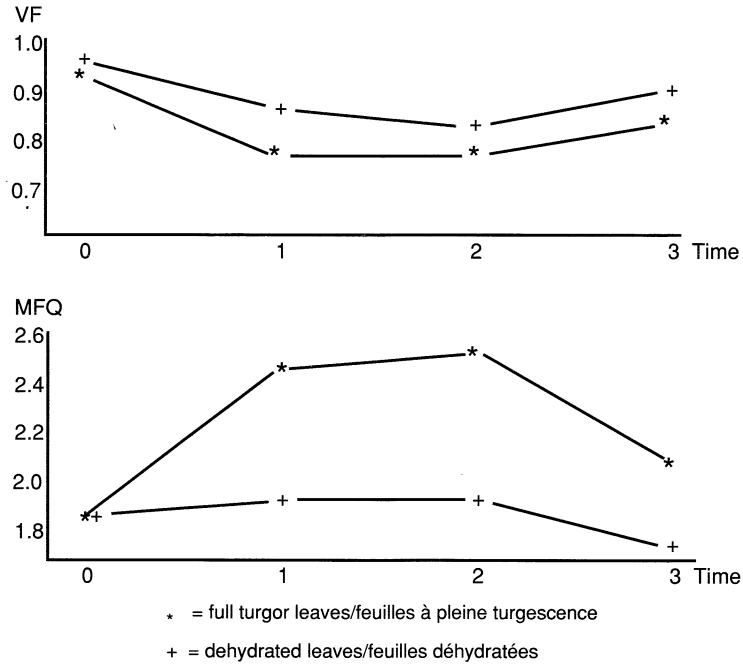


Fig. 2 — Variation of fluorescence variables in full turgor and dehydrated leaves.
Fig. 2 — Changement des paramètres de la fluorescence mesurés sur des feuilles à pleine turgescence et sur des feuilles déshydratées

TABLE II

Variation of RWC and Fluorescence variables during dehydration and rehydration (numbers in columns within dates followed by the same letter are not significantly different at P=0.05 according to Duncan's new multiple range test)/

TABLEAU II — Changement du RWC et des paramètres de fluorescence pendant les phases de déhydratation et de réhydratation (les valeurs ayant la même lettre ne diffèrent pas significativement au test de Duncan , p = 0,05)

		RWC	VF	MFQ	VF_s	MFQ_s
Time	0	100.0 a	0.95 a	1.84 a	0.96 a	1.84 a
	1	90.6 c	0.89 b	1.98 a	0.76 c	1.26 c
	2	83.5 d	0.87 b	2.02 a	0.74 c	1.18 c
	3	96.4 b	0.86 b	1.80 a	0.84 b	1.57 b

highest RWC values, even though 1103 P shows one of the lowest one. The same rootstock has also the lowest VF_s value. 161-49, with the highest VF_s value, shows the lowest MFQ-s value. In the grafted genotypes the differences are less evident: 420A confirms high RWC value. 1103 P shows a completely different behaviour between fields, in fact Chardonnay grafted on this rootstock shows the highest RWC value (table IV). It is possible to notice that there is not relation between water status parameters measured on ungrafted and grafted genotypes because *V. vinifera* leaves control water loss in a very strong way.

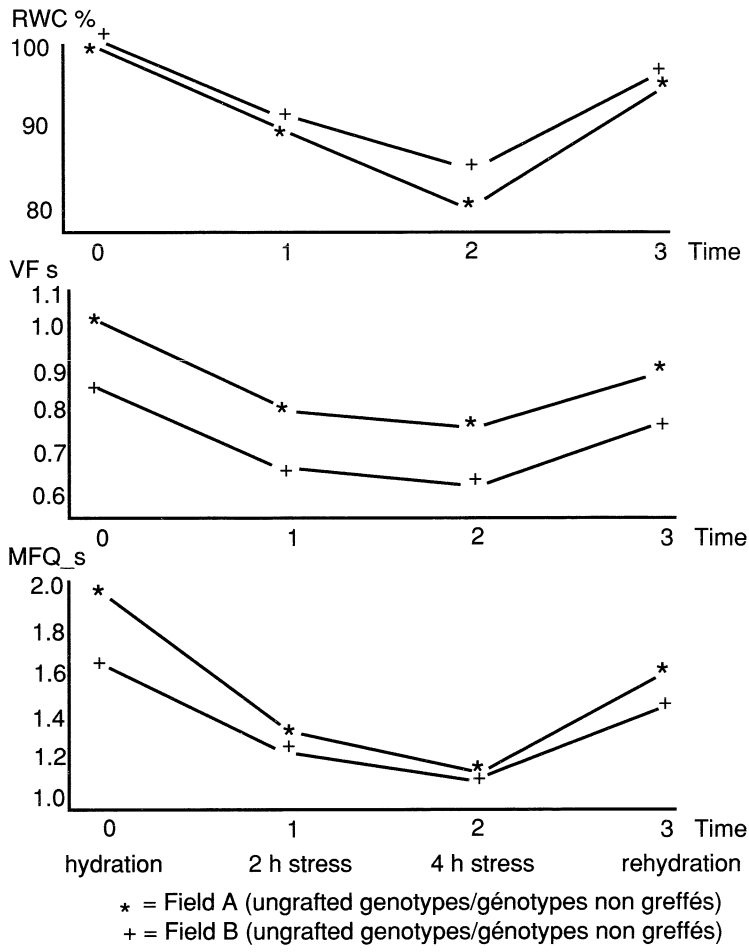


Fig. 3 — Variations of RWC and fluorescence variables in relation to the time and the field.

Fig. 3 — Changement de RWC et des paramètres de la fluorescence en fonction du temps et du champ

TABLE III

Role of the rootstocks in RWC and fluorescence variables control in the two fields. Field A = ungrafted genotypes; Field B = grafted genotypes

TABLEAU III — Rôle du porte-greffe sur le contrôle du RWC et des paramètres de la fluorescence dans les deux champs. Field A = génotypes non greffés; Field B = génotypes greffés

Variability factors			RWC		VF_s		MFQ_s	
			Sum of Squares	Signif. F	Sum of Squares	Signif. F	Sum of Squares	Signif. F
Field	A	Rootstock	886.296	0.0001	1.059	0.0001	4.455	0.0123
		Time	5968.445	0.0001	0.837	0.0001	11.167	0.0001
		Roots.*Time	656.828	0.0249	0.070	0.9995	4.298	0.5902
		Total	8741.660		2.905		36.278	
Field	B	Rootstock	168.248	0.0008	0.257	0.0203	2.307	0.5902
		Time	3184.071	0.0001	0.729	0.0001	3.463	0.0457
		Roots.*Time	112.819	0.6033	0.122	0.9896	6.457	0.7745
		Total	3996.212		2.249		44.868	

TABLE IV

Influence of field on rootstocks behaviours (numbers in columns within dates followed by the same letter are not significantly different at P=0.05 according to Duncan's new multiple range test). (Field A = ungrafted genotypes; Field B = grafted genotypes)

TABLEAU IV — Rôle du champ sur la conduite du porte-greffe (les valeurs ayant la même lettre ne diffèrent pas significativement au test de Duncan , p = 0,05). Field A = génotypes non greffés; Field B = génotypes greffés

Rootstocks	Field A			Field B		
	RWC	VF_s	MFQ_s	RWC	VF_s	MFQ_s
3309 C	85.68 e	0.798 ef	1.398 abc	92.08 c	0.739 b	1.450 a
140 Ru	94.07 a	0.835 de	1.636 ab	93.65 abc	0.856 a	1.422 a
1103 P	88.69 de	0.723 f	1.658 ab	95.70 a	0.777 ab	1.500 a
Kober 5BB	92.82 abc	0.896 bcd	1.493 abc	94.45 ab	0.734 b	1.479 a
SO4	89.64 cd	0.857 cde	1.762 a	92.81 bc	0.706 b	1.519 a
420A	93.48 ab	0.986 b	1.271 bc	95.16 a	0.761 ab	1.599 a
161-49	90.40 bcd	1.077 a	1.091 c	93.84 abc	0.800 ab	1.118 a
41 B	93.98 a	0.951 bc	1.722 a	94.80 ab	0.852 a	1.461 a

III — Relationships between VF_s, MFQ_ and RWC per specific rootstocks

A significant, positive relation between fluorescence parameters and RWC is shown in both fields (table V). The straight line slopes are very similar in both fields, even though data ranges are different; the regressions are significant if the rehydrated leaves values are also considered.

TABLE V

Gross relation between fluorescence variables and RWC in relation to the field (Field A = ungrafted genotypes; Field B = grafted genotypes)

TABLEAU V — Relation générale entre les paramètres de la fluorescence et le RWC en fonction du champ. Field A = génotypes non greffés; Field B = génotypes greffés

Field	Rootstocks	Linear Model y (*) x	Signif. F	Angular Coeff. b
A	all	VF_c = RWC	0.0001	0.0098
	all	MFQ_c= RWC	0.0001	0.026
B	all	VF_c = RWC	0.0001	0.014
	all	MFQ_c= RWC	0.0073	0.027

(*)= Linear model : Y=a+bX where Y = dipendent variable, X = indipendent variable, a = intercept, b = angular coefficient (slope).

By analysing separately the dehydration and rehydration periods, it is possible to deepen the result analysis (table VI). During the first 2 hours of dehydration, linear regressions between VF_s, MFQ_S and RWC are strongly significant and the estimated straight line slopes are similar in both fields. From 2 to 4 hours dehydration, MFQ_s is not linked to RWC, whereas VF_s decreases by decreasing of RWC (figure IV). The links between MFQ_s and RWC are re-established during rehydration even though the rates of VF_s and MFQ_s restoration are lower than those assessed during the first dehydration period (the absolute slope values are at lowest) (figure V).

IV — RELATIONSHIPS BETWEEN VF_S, MFQ_S AND RWC PER SPECIFIC ROOTSTOCKS

It is clear that also the specific rootstocks contribute to modify the relations between VF_s, MFQ_s and RWC. Particularly VF_s is more linked to RWC than MFQ_s in both field. By removing the rehydration period from the statistical analysis, in field A (ungrafted genotypes) the relation between VF_s and RWC is verified for all the rootstocks while the linear model does not improve in field B (grafted genotypes). It is so possible to define VF_s as a good indicator of leaf water status (table VII).

The different significance of the linear model applied, stresses the different genotypic relation between leaf water content and fluorescence emission after adaptation to darkness.

On the other hand, MFQ_s is less linked to RWC so that also in field A (ungrafted genotypes) some rootstocks (140 Ru, 161-49, 41B SO4) do not have a linear behaviour. In field B (grafted genotypes), the linear regression is only verified in *Vitis vinifera* grafted on 420A and this behaviour is confirmed when rehydration data are removed from the statistical analysis. 420A shows the highest straight line slope (data not shown) indicating a strong link between RWC and slow transients of fluorescence emission curve that reflect reactions at the level of enzyme regulation (KRAUSE et WEIS, 1984).

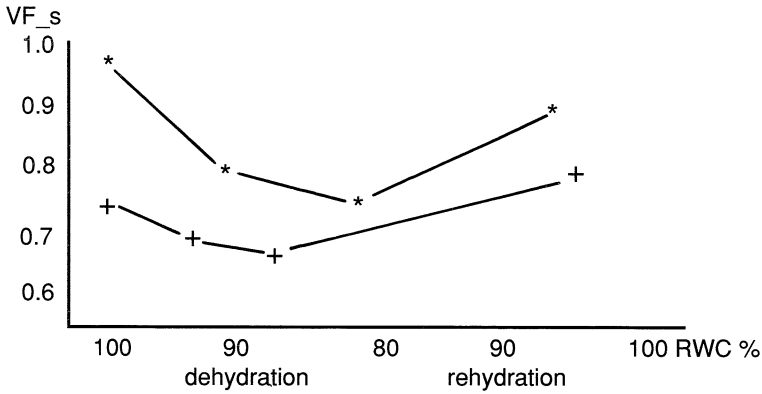
TABLE VI

Relations between fluorescence variables and RWC in relation the time of measurement and the field. (Field A = ungrafted genotypes; Field B = grafted genotypes)

TABLEAU VI — Relation générale entre les paramètres de la fluorescence et le RWC en fonction du temps de mesurage et du champ. Field A = génotypes non greffés; Field B = génotypes greffés

Time	Field	Rootstocks	Linear Model y (*) x	Signif. F	Angular Coeff.
0-1	A	all	VF_s = RWC	0.0001	0.017
		all	MFQ_s= RWC	0.0008	0.044
0-1	B	all	VF_s = RWC	0.0001	0.022
		all	MFQ_s= RWC	0.0447	0.044
1-2	A	all	VF_s = RWC	0.0043	0.006
		all	MFQ_s= RWC	0.9502	0.000
1-2	B	all	VF_s = RWC	0.0111	0.007
		all	MFQ_s= RWC	0.6989	0.007
2-3	A	all	VF_s = RWC	0.0002	0.007
		all	MFQ_s= RWC	0.0198	0.017
2-3	B	all	VF_s = RWC	0.0001	0.011
		all	MFQ_s= RWC	0.0459	0.022

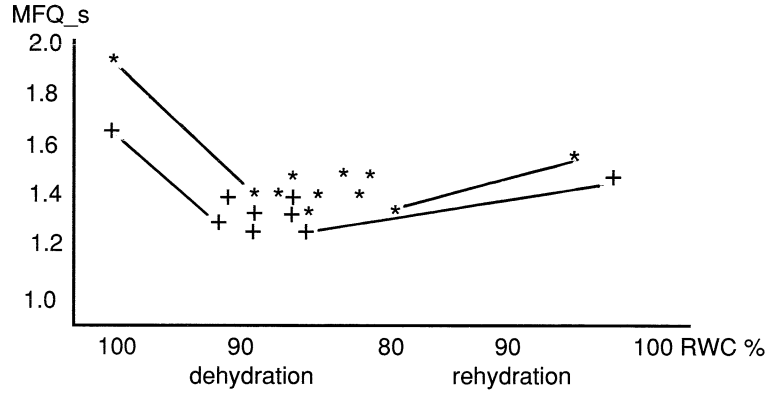
(*)= Linear model : Y=a + bX where Y = dipendent variable, X = indipendent variable, a = intercept, b = angular coefficient (slope)



* = Field A (ungrafted genotypes/génotypes non greffés)
+ = Field B (ungrafted genotypes/génotypes non greffés)

Fig. 4 — Relation between VF_s and RWC
during the different phases of the trial in relation to the field.

Fig. 4 — Relation entre VF_s et RWC
pendant les différentes phases des essais en fonction du champ.



* = Field A (ungrafted genotypes/génotypes non greffés)
+ = Field B (ungrafted genotypes/génotypes non greffés)

Fig. 5 — Relation between VF_s and RWC
during the different phases of the trial in relation to the field.

Fig. 5 — Relation entre VF_s et RWC
pendant les différentes phases des essais en fonction du champ.

TABLE VII

Relations between Fluorescence variables and RWC for the specific rootstocks evaluated in relation with the FIELD with and without the rehydration period. (Field A = ungrafted genotypes; Field B = grafted genotypes)

TABLEAU VII — Relation générale entre les paramètres de la fluorescence et le RWC pour les différents porte-greffe évalués en fonction du champ et avec et sans période de réhydratation. Field A = génotypes non greffés; Field B = génotypes greffés

		With rehydration period				Without rehydration period			
Roots.	Linear Model (*) Y X	Field	Signif. F	Field	Signif. F	Field	Signif. F	Field	Signif. F
1103 P	VF_s = RWC	A	0.0001	B	0.0029	A	0.0479	B	0.0375
	MFQ_s= RWC		0.0331		0.6157		0.0176		0.5118
140 Ru	VF_s = RWC	A	0.0808	B	0.0010	A	0.0329	B	0.0027
	MFQ_s= RWC		0.2622		0.8579		0.1919		0.5555
161-49	VF_s = RWC	A	0.0535	B	0.1135	A	0.0131	B	0.0691
	MFQ_s= RWC		0.7521		0.2794		0.1724		0.3980
3309 C	VF_s = RWC	A	0.0113	B	0.0460	A	0.0276	B	0.0141
	MFQ_s= RWC		0.0011		0.0677		0.0032		0.1249
41 B	VF_s = RWC	A	0.0251	B	0.093	A	0.0210	B	0.0218
	MFQ_s= RWC		0.4206		0.2599		0.5618		0.2114
420 A	VF_s = RWC	A	0.0872	B	0.0539	A	0.0205	B	0.0835
	MFQ_s= RWC		0.0484		0.0461		0.0305		0.0381
K 5bb	VF_s = RWC	A	0.0042	B	0.0006	A	0.0138	B	0.0024
	MFQ_s= RWC		0.0437		0.1032		0.1195		0.1204
SO4	VF_s = RWC	A	0.0006	B	0.0025	A	0.0001	B	0.0046
	MFQ_s= RWC		0.1261		0.0952		0.2016		0.3651

(*)= Linear model : Y=a + bX where Y = dipendent variable, X = indipendent variable, a = intercept, b = angular coefficient (slope)

DISCUSSION

The evaluation of water availability in leaves cut from genus *Vitis* carried out by means of fluorescence analysis must necessarily be combined with a parallel study on leaves kept at constant full turgor. Considered as test, these have been used to standardise the dehydrated leaves data by time. Other Authors (PASTORE et *al.*, 1988a) already showed a diffe rent fluorescence emission between night and day, but they deduced that the differences are negligible during the day. In genus *Vitis*, these variations are instead able to modify the dehydration and rehydration results. After the standardisation of data by time, both VF_s and MFQ_s decrease when RWC decreases. This gross relation is significant for both ungrafted rootstocks and *V. vinifera* cv. Chardonnay, proving that this is a good model.

Notwithstanding this, some factors are very important to modify this relation. *V. vinifera* cv. Chardonnay controls the leaf water content better than the sampled rootstocks. In the same way, the european genotype shows lower VF_s and MFQ_s values.

The relationships between VF_s, MFQ_s and RWC are also influenced by the physiological leaf conditions: VF_s always decreases when RWC decreases, whereas MFQ_s shows this behaviour only during the first dehydration period.

Therefore VF_s characterizes better than MFQ_s the leaf water content, while MFQ_s, after the first decrease of RWC to 80 p. cent, is strongly controlled by the genotype, grafted or ungrafted. During rehydration, the relations are restored but MFQ_s does not reach the same values shown during dehydration. These deductions are supported by the evaluation of the specific genotype role. In fact only ungrafted 140 Ru and 161-49 and 420A are genotypes that during rehydration do not regain the values recorded during hydration: the linear regression model applied with rehydration data is not significant. Because VF_s represents the fast transients of the fluorescence emission curve that contain information on primary electron transport reactions (KRAUSE et WEIS, 1984), this result is very important in the evaluation of the recovery capacity of the specific genotypes.

Only *V. vinifera cv Chardonnay* grafted on 420A shows a significant linear regression between MFQ_s and RWC, while this relation is not verified in the other graftings. Enzyme regulation reactions can justify this result. From a general point of view the genotype can control this parameter no matter what the rootstocks it is grafted on.

CONCLUSIONS

In *V. vinifera cv. Chardonnay*, the relations between fluorescence and RWC are only verified when the data are standardised by time of measurement. By considering that during the day, the fluorescence variations are trifling; this means that during dehydration the genus *Vitis* leaves do not modify substantially their photosynthetic system characteristics. Therefore the factors that can modify the relations between VF_s, MFQ_s and RWC must be considered carefully in order not to fall into evaluation mistakes that are more important for MFQ_s less linked to RWC than VF_s.

VF_s and MFQ_s values are strongly linked to the genotype and *V. vinifera*, even if it shows a higher water content control, presents low fluorescence values.

The specific rootstocks prove to be different because they do not always show similar links between VF_s, MFQ_s and RWC: above all during rehydration some of them show difficulties to restore the values recorded during dehydration. These differences are not noticed in *V. vinifera cv. Chardonnay*, which shows MFQ_s almost constant when dehydration status increases (RWC 90 to 80 p. cent), so resulting a drought tolerant genotype and not too much influenced by rootstock. These results do not include the functions of control that the rootstocks can exert through the root system and that regulate the stomata closure.

Therefore, water status must not be considered in his absolute value but also considering the leaf physiological condition. VF_s containing information on primary electron transport reactions is always linked to RWC a part leaf water status. On the other hand, MFQ_s, displaying enzyme regulation reactions, is strongly linked to the genotype when water deficit increases.

Fluorescence, is a valid method in the genetic characterization studies and in the drought tolerance screenings because it is linked to photosynthetic efficency. Considering the genetic control it also needs a dynamic evaluation by TIME of measurement and by the tested genotype to control the numerous factors that modify VF_s and MFQ_s, and change their behaviour.

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