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**NIGHTTIME TRANSPIRATION IN GRAPEVINE: GENETIC DETERMINISM AND
PHYSIOLOGICAL BASES**
**TRANSPIRATION NOCTURNE CHEZ LA VIGNE : DETERMINISME
GENETIQUE ET BASES PHYSIOLOGIQUES**

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Abstract

In the face of increased water scarcity which accompanies climate change, improving water use efficiency (WUE) becomes crucial for sustaining viticulture in the Mediterranean area. The leeway to improve WUE depends on how water losses are coupled with biomass gain. As water loss at night is not associated with any carbon gain, its reduction could be a good strategy to limit waste of water without altering photosynthesis rate in the daytime. However, genetic and physiological bases of the variations in night transpiration are still unclear. The present study aimed at elucidating whether and how variations in night transpiration are genetically controlled in grapevine. A 2-year experiment was conducted on the F1 progeny from a cross between Syrah and Grenache using a phenotyping platform coupled to a controlled-environment chamber. Potted plants of the whole progeny were analysed for night transpiration in well-watered conditions. A high genetic variability was found enlightening for the first time the genetic control of water losses at night. We further explored the physiological bases of this genetic variability. 15 genotypes were chosen within the progeny for their contrasting transpiration at night. By feeding detached leaves with abscisic acid, we showed that stomata remain partially open at night, and that this residual opening differs among genotypes. We took advantage of the hypostomatic feature in grapevine leaves to study the cuticular conductance on the adaxial face. We found a significant genetic control of cuticular conductance, which accounted for up to 30% of the total leaf transpiration at night. These results open new avenues to breed grapevine for lower water losses at night, whose genetic variability lies both in stomata and cuticle.

Keywords: *Vitis vinifera*, night transpiration, water use, stomata, cuticle.

Résumé

Face à la raréfaction des ressources hydriques qui accompagne les changements climatiques, il devient crucial d'augmenter l'efficacité d'utilisation de l'eau, notamment dans les systèmes viticoles en zone méditerranéenne. Cependant, le couplage entre gain de biomasse et consommation d'eau par la plante limite les marges d'amélioration. C'est pourquoi la réduction de l'utilisation de l'eau la nuit, en l'absence de photosynthèse, est une cible intéressante. Cette étude visait l'analyse de la variabilité génétique de la transpiration nocturne chez la vigne et l'identification des processus physiologiques sous-jacents. Une expérimentation a été répétée 2 années sur une descendance F1 issue du croisement des variétés Syrah et Grenache. La descendance cultivée en pots a été analysée en conditions de bonne irrigation à l'aide d'une plate-forme de phénotypage couplée à une chambre climatique. Une forte variabilité génétique de la transpiration nocturne (rapportée à la surface foliaire) a été observée dans la descendance et, pour la première fois, des déterminants génétiques de ce caractère ont été localisés sur le génome. Les bases physiologiques de cette variabilité ont été recherchées chez 15 génotypes de la descendance. En alimentant des feuilles détachées avec une solution très concentrée d'acide abscissique pour induire la fermeture stomatique, nous avons montré qu'il existait une ouverture résiduelle des stomates la nuit avec des différences significatives entre génotypes. De plus, les pertes d'eau à travers la cuticule de la face adaxiale, dépourvue de stomates, représentaient une part non négligeable des pertes totales avec des différences entre génotypes. Il apparaît donc possible de sélectionner des variétés avec de faibles pertes d'eau la nuit en jouant à la fois sur les contributions stomatiques et cuticulaires.

Mots-clés: *Vitis vinifera*, transpiration nocturne, utilisation de l'eau, stomate, cuticule

1. Introduction

Water stress is thought to be the main environmental constraint for vine growth and grape production under Mediterranean conditions (Araus, 2004). In the face of future drought scenario, reducing water use and increasing water use efficiency (WUE, the balance between biomass production and water costs) have become research priorities for viticulture sustainability (Morison et al., 2008). In plants, photosynthesis and growth are tightly coupled to water use because carbon acquisition and energy capture require high leaf area and open stomata which imply transpirational water losses. This makes not trivial any gain in WUE. However, transpiration at night (E_n) can be substantial and could be reduced without negative impact on photosynthesis, offering one way to modulate WUE. E_n represents 5-30% of daytime transpiration depending on species and ambient conditions (Caird et al., 2007; Dawson et al., 2007). Recent investigations in natural accessions of *Arabidopsis thaliana* revealed genetic variation for E_n (Christmann et al., 2008), opening promising avenues for selection. In grapevine, water losses at night have been scarcely studied. Contrary to the simplistic view that stomata close at night, several studies have demonstrated that stomata can remain partially opened during the night allowing for significant water losses (Rogiers et

al., 2009). Compared to stomata, the cuticle surrounding them at the leaf surface has very low water permeability in *Vitis* species (Flexas et al., 2009). However, when considering low water losses at night, the cuticle may represent a substantial part of the total water loss. The present study aimed at elucidating whether and how variations in night transpiration are genetically controlled in grapevine, and exploring the respective roles of stomata and cuticle in E_n variability.

2. Material and Methods

Plant material

A pseudo-F1 progeny of 186 two year-old genotypes was obtained as the first generation from a reciprocal cross between the grapevine cultivars Syrah and Grenache (Adam-Blondon et al., 2004). In February 2010, 20 clones of each offspring and the parents were grafted on 110Richter rootstock (*V. berlandieri* × *V. rupestris*) and then cultivated outside with ferti-irrigation in individual pots containing a 30:70 (v/v) mixture of a loamy soil and organic compost.

Measurement of transpiration rates at night on whole plants

Three (in 2012) and two (in 2013) replicates (clones) of each offspring and the parents were studied as previously described (Coupel-Ledru et al., 2014) into the PhenoArch phenotyping platform in a greenhouse at Montpellier, France. For each genotype, the replicates were maintained under well-watered conditions by automatized, daily watering in the platform. Individual, whole-plant leaf area was estimated from daily images taken in the platform imaging cabin.

Transpiration rate at night was measured on the whole progeny while plants were taken off the platform and placed in a controlled-environment chamber. Pots were bagged to prevent evaporation from the soil. Plants were submitted to a dark period with a similar timing to that prevailing in the greenhouse. Air temperature and relative humidity (RH) were measured every 30 s (HMP35A probe, Oy, Helsinki, Finland). Temperature was set to an average of 20 °C and Vapour Pressure Deficit (VPD) was maintained by manipulating RH to reach 1.5 ± 0.2 kPa during the first 7 hours and 0.8 ± 0.2 kPa during the next 5 hours. Each pot was weighed with 0.1 g accuracy (Sartorius balance, IB 34 EDEP, Goettingen, Germany) at the beginning and end of the night period. Weight losses were used to calculate average night transpiration rates on a leaf area basis (E_n).

Single effect of genotype was tested by ANOVA. Broad sense heritability (H^2) was calculated and QTL detection was performed on BLUPs with MapQTL 4.0 software as previously described (Coupel-Ledru et al., 2014).

Experiments on detached leaves

A panel of 15 genotypes (5 potted plants per genotype) was selected within the progeny based on their contrasted behaviours for night transpiration. They were grown outside for one additional year and pruned to produce one, unbranched leafy axis with their inflorescences removed. In July 2014, plants were transferred to a controlled environment chamber one night prior to measurements in order to ensure low-transpiring conditions.

The day of measurements, leaves were excised from plants in dark conditions and their petioles were immediately immersed in individual 5 mL containers filled with a filtered (0.2 μm), degassed control solution [$2 \text{ mol m}^{-3} \text{ KH}_2\text{PO}_4$, $1 \text{ mol m}^{-3} \text{ MES}$, $0.4 \text{ mol m}^{-3} \text{ Ca}(\text{NO}_3)_2$] adjusted to pH 6.5. Petioles were tightly sealed to the containers caps. Each leaf in its container was placed in the chamber in dark conditions, with VPD maintained at 1.5 ± 0.2 kPa and temperature at 22°C. Transpiration rate in the dark was determined by weighting leaves fed with solution in their container every 20 min over a 1h30 time period.

Abscisic acid (synthetic (\pm)-ABA) was then added to the solutions to reach varying concentrations of (+)-ABA (16, 32, 64, 128 and 256 $\mu\text{mol m}^{-3}$). Average transpiration in ABA, still in the dark, was determined as previously described once the weight declined at a stabilized rate (which occurred about 45 minutes after adding ABA).

For another set of leaves, after 1h30 in control solution, the abaxial face was completely coated with Vaseline which stopped water loss on this face (preliminary results not shown). Transpiration rate was quantified during a 2h30 time period as described above.

Single genotype and interaction genotype X treatment effects were tested by using analysis of variance models (ANOVA). All statistical analyses were performed with R packages (R Development Core Team, 2012).

3. Results and discussion

Night transpiration is under genetic control

Mean genotypic values of night transpiration rate ranged from 0.04 to 0.16 $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ (Fig.1), consistent with a previous report (Rogiers et al., 2009) where E_n measured for Grenache averaged 0.08 $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$. Cumulated E_n values over the course of the whole night could thus reach 180 g m^{-2} leaf area, similar to the substantial water losses (200 g m^{-2}) found by Escalona et al. (2013). ANOVA revealed a highly significant effect of the genotype ($p < 0.001$) on E_n . Genotypic means measured for E_n were well correlated between years (not shown), consistent with the high values found for broad sense heritability in 2012 (0.7) and in 2013 (0.52). This indicated a strong control of the genotype on night transpiration in well-watered conditions in grapevine. More than 7 significant QTLs were detected for E_n on the consensus map, each one accounting for 9 to 12% of the total variance. Two genomic regions of particular interest on linkage groups 4 and 13 contained most of these QTLs with stable localizations between 2012 and 2013. A genetic origin for E_n variation had previously been reported in *Arabidopsis thaliana* (Christmann et al., 2008), encouraging further characterization within and among populations and species. Our results evidenced for the first time a genetic control of water losses at night in grapevine by identifying the underlying locus.

Stomata remain partially open at night

Transpiration rate measured on detached leaves in control solution averaged $0.077 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ for the 15 genotypes (Table 1) which was similar to mean E_n determined for the whole progeny on entire plants ($0.08 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$, Fig.1). Thus, E_n recorded on detached leaves was a relevant proxy for plant night transpiration. A high genetic variability was found for E_n on detached leaves in control solution (Table 1) consistent with the strong genetic control of whole plant E_n underlined above. Increasing [ABA] in the feeding solution resulted in decreased E_n and minimal E_n was observed for [ABA] higher than $128 \mu\text{mol m}^{-3}$ (Fig.2). This concentration was retained to trigger stomatal closure on detached leaves of the 15 genotypes. ANOVA revealed a highly significant effect of ABA treatment on E_n ($p < 0.001$, Table 1) indicating that stomata were partially opened before ABA treatment including in these dark conditions. Previous reports also showed significant stomatal opening during the night in irrigated grapevines (Caird et al., 2007; Rogiers et al., 2009). With the ABA concentration used in the present study, residual E_n was on average $0.025 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$, e.g. 32% of maximal E_n observed in control solution. The still significant rate of water loss in ABA-treated leaves exhibited a high genetic control (Table 1). Moreover, the significant genotype X treatment interaction (Table 1) suggests that residual opening of stomata at night was genetically variable and independent on residual transpiration through the cuticle. However, it remains unclear whether residual E_n of ABA-treated leaves was still due to marginal opening of stomata or exclusively due to water losses through the cuticle.

Water loss through the cuticle is under genetic control and accounts for a significant part of night transpiration

Given that grapevine is hypostomatic (e.g. stomata are present only on the abaxial face), water losses measured through the adaxial face while the abaxial face was waterproofed necessarily occurred through the cuticle. ANOVA evidenced a highly significant and substantial effect of the waterproofing treatment on E_n (Table 1). Most importantly, we found a highly significant effect of the genotype on the rate of water loss through the adaxial cuticle thereby evidencing for the first time a genetic control on cuticle permeability to water. Residual E_n with the waterproofing treatment was on average $0.011 \text{ mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$ (Table 1), representing about 50% of E_n measured in ABA-treated leaves. Assuming that cuticular transpiration is of the same magnitude on both faces of the leaf (which cannot be experimentally verified), we could then consider that total cuticular transpiration (on both faces) is of the same magnitude as that measured on ABA-treated leaves. This suggests that the ABA concentration used ($128 \mu\text{mol m}^{-3}$) induced nearly full stomatal closure and that water losses through the cuticle of both faces could account for up to 32% of total night water losses.

4. Conclusions

We showed that night transpiration in grapevine is under a high genetic control and identified for the first time stable QTLs involved in this variability. We examined the respective roles of stomata and cuticle in water losses during the night. We found that stomata remain partially opened at night and that this residual opening is under genetic control. We also showed a significant role of cuticle in water losses at night together with a genetic control on this component. Both stomata and cuticle could therefore be considered as targets to breed grapevine for lower water losses at night.

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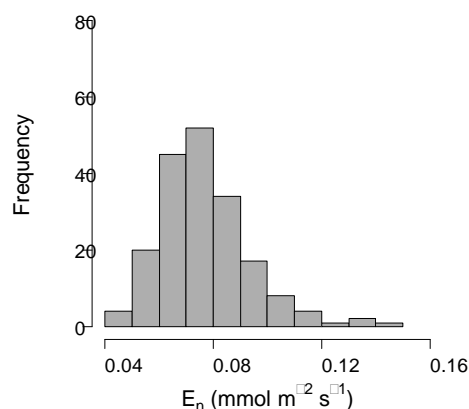


Figure 1: Distribution of mean genotypic values of night transpiration (E_n) measured on the Syrah X Grenache progeny in 2012 and 2013 (mean of 5 plants per genotype in total) under the well-watered treatment. Frequency stands for the number of genotypes (188 in total) for each E_n interval.

Figure 1: Distribution des moyennes génotypiques de la transpiration nocturne (E_n) mesurée sur plantes entières de la population Syrah X Grenache, en 2012 et 2013 (moyenne calculée pour 5 plantes par génotype au total), en conditions bien irriguées. Fréquence en nombre de génotypes (188 au total) pour chaque intervalle de E_n .

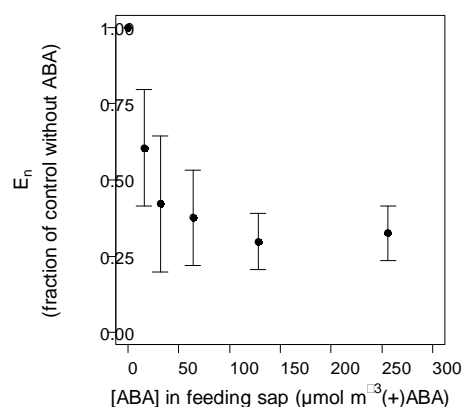


Figure 2: Night transpiration (E_n) measured on detached leaves of Grenache as a function of ABA concentration in the feeding solution. Values are normalized by E_n measured in the control solution (without ABA) for each leaf. Mean \pm standard error for 5 leaves measured at each concentration.

Figure 2: Transpiration nocturne (E_n) mesurée sur des feuilles détachées de Grenache alimentées par des solutions avec différentes concentrations d'ABA. Valeurs de E_n rapportées à la transpiration mesurée pour chaque feuille dans la solution témoin (sans ABA). Moyennes \pm écart-type de 5 feuilles mesurées à chaque concentration.

Table 1. Night transpiration measured on detached leaves either fed with a control solution, or fed with solutions of highly concentrated abscissic acid ((+)-ABA 128 $\mu\text{mol m}^{-3}$) or else with the abaxial face waterproofed by vaseline application and fed with control solution. For each treatment, effect of treatment, genotype (15 genotypes analyzed) and genotype by treatment interaction have been tested by ANOVA. $N > 5$ leaves for each combination genotype X treatment. *** indicates highly significant effect with $P \leq 0.0001$ (NA when not applicable).

Table 1. Transpiration nocturne mesurée sur feuilles détachées alimentées par une solution témoin, ou par une solution concentrée en acide abscissique ((+)-ABA 128 $\mu\text{mol m}^{-3}$), ou encore en solution témoin avec l'imperméabilisation complète de la face abaxiale par application de vaseline. Pour chaque traitement, effet du traitement, du génotype (15 génotypes analysés) et de l'interaction, avec $N > 5$ feuilles pour chaque combinaison. *** indique un effet hautement significatif à $P \leq 0.0001$ détecté par ANOVA (NA quand non applicable).

	E_n (mean \pm s.e.) ($\text{mmol m}^{-2} \text{s}^{-1}$)	Effect of treatment	Effect of genotype	Effect of genotype X treatment interaction
Control	0.077 ± 0.35	NA	***	NA
ABA	0.025 ± 0.01	***	***	***
Waterproofed abaxial face	0.011 ± 0.005	***	***	***