Journal of Plant Physiology 185 (2015) 84–92

Contents lists available at ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.com/locate/jplph

Physiology

Physiological parameters and protective energy dissipation mechanisms expressed in the leaves of two *Vitis vinifera* L. genotypes under multiple summer stresses

Alberto Palliotti[a,*], Sergio Tombesi[a], Tommaso Frioni[a], Oriana Silvestroni[b], Vania Lanari[b], Claudio D'Onofrio[c], Fabiola Matarese[c], Andrea Bellincontro[d], Stefano Poni[e]

[a] *Dipartimento di Scienze Agrarie, Alimentari e Ambientali, Università di Perugia, Borgo XX Giugno 74, 06128 Perugia, Italy*
[b] *Dipartimento di Scienze Agrarie, Alimentari ed Ambientali, Università Politecnica delle Marche, Via delle Brecce Bianche, 60131 Ancona, Italy*
[c] *Dipartimento di Scienze Agrarie, Alimentari ed Agro-Ambientali, Università di Pisa, Via del Borghetto 80, 56124 Pisa, Italy*
[d] *Dipartimento per l'Innovazione dei Sistemi Biologici, Agroalimentari e Forestali, Università della Tuscia, Via S. Camillo de Lellis, Viterbo, Italy*
[e] *Dipartimento di Scienze delle Produzioni Vegetali Sostenibili, Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, 29100 Piacenza, Italy*

ARTICLE INFO

Article history:
Received 13 April 2015
Received in revised form 6 July 2015
Accepted 7 July 2015
Available online 31 July 2015

Keywords:
Photosynthesis
Xanthophylls
Chlorophyll fluorescence
Grapevine
Multiple summer stresses
Catalase activity

ABSTRACT

Photosynthetic performances and energy dissipation mechanisms were evaluated on the anisohydric cv. Sangiovese and on the isohydric cv. Montepulciano (*Vitis vinifera* L.) under conditions of multiple summer stresses. Potted vines of both cultivars were maintained at 90% and 40% of maximum water availability from fruit-set to veraison. One week before veraison, at predawn and midday, main gas-exchange and chlorophyll fluorescence parameters, chlorophyll content, xanthophyll pool and cycle and catalase activity were evaluated. Under water deficit and elevated irradiance and temperature, contrary to cv. Montepulciano and despite a significant leaf water potential decrease, Sangiovese's leaves kept their stomata more open and continued to assimilate CO_2 while also showing higher water use efficiency. Under these environmental conditions, in comparison with the isohydric cv. Montepulciano, the protective mechanisms of energy dissipation exerted by the anisohydric cv. Sangiovese were: (i) higher stomatal conductance and thermoregulation linked to higher transpiration rate; (ii) greater ability at dissipating more efficiently the excess energy via the xanthophylls cycle activity (thermal dissipation) due to higher VAZ pool and greater increase of de-epoxidation activity.

1. Introduction

Under field conditions, plants are rarely affected by a single abiotic stress but rather from multiple environmental limiting factors which might include excessive light and temperature, too low relative humidity, water shortage, etc. (Mittler, 2006). Usually, when combined, these abiotic stresses have synergistic effects, causing a significant decrease in CO_2 fixation capability due to stomata closing and/or to impairment of photochemical reactions that limit growth, yield and fruit composition up to threatening plant's survival. This situation is very frequent in semi-arid environments, typical of countries of the Mediterranean basin where grapes are grown since long time, or in other overseas countries where grape growing is more recent (i.e., Australia, Chili and some areas of Argentina).

It is known that environmental stresses lead to a chain of morpho-structural, physiological, biochemical and molecular changes which influence plant growth and yield (Chaves, 2002; Wang et al., 2003). According to the species and to the timing and intensity of abiotic stresses, different mechanisms have been evolved, at both molecular and cellular level, to increase tolerance just after the onset of stress conditions. Under these conditions, the gene expression profiles result significantly altered, especially in specific functional categories such as “antioxidative response”, “signaling” and “protein metabolism” and it improves thereby the

---

Abbreviations: P_n, net photosynthesis; g_s, stomatal conductance; WUE_i, intrinsic water use efficiency; Chl, chlorophyll; ETR, electron transport rate; NPQ, non photochemical quenching; P_r, photorespiration; F_v/F_m, maximum photochemical efficiency; J_c, electron flow to carboxylation; Φ_{PSII}, actual maximum quantum yield of PSII; CAT, catalase; H_2O_2, hydrogen peroxide; Ψ_l, leaf water potential; V, violaxanthin; A, antheraxanthin; Z, zeaxanthin; WW, well-watered; WS, water-stressed.

[*] Corresponding author. Fax: +39 75 5856255.
E-mail address: alberto.palliotti@unipg.it (A. Palliotti).

http://dx.doi.org/10.1016/j.jplph.2015.07.007
0176-1617/

abiotic stress defense pathways (Carvalho et al., 2011). Drought and high temperatures cause oxidative stress and consequently may determine the denaturation of structural and functional proteins. However, usually these abiotic stresses activate a different categories of genes coding for heat-shock proteins, chaperones, osmoprotectants, free radical scavengers, acquaporins and ion transporters as well as proteins which protect the membranes (Wang et al., 2003). Recently, Rocheta et al. (2013) has reported that under acute heat stress the cv. Aragonez expresses a huge number of genes with high energetic costs, whereas under water deficit it reduces the gene expression to a minimum and increases only those genes necessary to survive.

There is a fairly shared consensus that the *Vitis vinifera* L., due to the more than 10,000 varieties cultivated worldwide (Alleweldt and Dettweiler, 1992), displays large variation in terms of tolerance to drought which is primarily related to stomatal behavior (Escalona et al.,1999; Chaves and Oliveira, 2004) as between-genotype variation in photosynthetic rate seems to be minor under both well watered and water deficit conditions (Chaves et al., 1987; Schultz, 1996; Bota et al., 2001).

As it has been previously reported, the two red grape cultivars most commonly cultivated in Italy, i.e., Sangiovese and Montepulciano currently grown on more than 100,000 ha acreage, show intraspecific differences in morpho-physiological characteristics and productive performances under non limiting water conditions (Palliotti et al., 2014). Unlike Montepulciano, which under drought is classified as a isohydric genotype, the near-anisohydric Sangiovese retains higher whole vine carbon fixation, thereby being considered a genotype more adapted to drought (Palliotti et al., 2008, 2009, 2014; Tombesi et al., 2014).

Under drought conditions, stomatal regulation versus water loss determines the level of carbon gain and therefore plant growth, productivity and survival. Recently, it has emerged that the mechanisms that allow the various varieties to adapt to summer stresses are mediated by stomatal sensitivity and are genetically controlled (Medrano et al., 2003; Beis and Patakas, 2012; Hochberg et al., 2013; Palliotti et al., 2014). According to stomata kinetics under drought, *V. vinifera* embraces varieties classified as isohydric, which are capable of maintaining a fairly constant midday leaf water potential regardless of soil water availability by means of early stomatal closure, and anisohydric, which maximize photosynthetic gain by maintaining stomata open despite a large leaf water potential drop (Tardieu and Simmoneau, 1998). In truth, a whole range of responses between completely isohydric and anisohydric behaviors can be detected in different plant species, and the two strategies can even occur within different cultivars of the same species or as a function of different water stress intensity, duration and time of application as well as interactions with excessive irradiance and temperature (Poni et al., 2007; Chaves et al., 2010). This is likely to depend on the fact that some responses were defined using one- or two-year old vines, often without clusters, and/or with water stress artificially applied after the end of growth. After the end of growth, responses to drought usually are limited to only short-term adjustment responses mainly at leaf level. Indeed, adjustment processes at plant level, such as plant architecture and hydraulic, occurs only when the stresses are experienced during the shoot and root growth (Chaves et al., 2002; Schultz, 2003; Choat et al., 2010; Palliotti et al., 2014).

This study investigates physiological changes and some energy dissipation mechanisms showed by primary leaves of outdoor grown vines of cvs. Sangiovese and Montepulciano during the hottest hours of summer days under concurrent light, heat and water stresses. Catalase activity and one of the major marker of oxidative stress, i.e. the leaf bulk of H_2O_2 concentration, as well as total chlorophyll content and xanthophyll-cycle pigments were analyzed at midday and compared with values measured at predawn.

2. Materials and methods

2.1. Experimental conditions and trial layout

This study was conducted in 2011 on eight-year-old potted (60 L) vines of *V. vinifera* L. cv. Sangiovese (clone VCR30) and cv. Montepulciano (clone R7) grafted onto 1103 Paulsen rootstock and grown in an outdoor area close to the Faculty of Agriculture of the University of Perugia (Region of Umbria, central Italy, 42°58′N, 12°24′E, elevation 405 m a.s.l.). All the pots were filled with loam soil with a field capacity of 30.2% [(vol water/vol soil) × 100] and a wilting point of 16.7%. At the end of February, each vine was pruned to retain 4 spurs with 2 buds each. All shoots were oriented up-right using suitable stakes. Ten vines per cultivar were used and maintained at about 90% of maximum water availability (WW, well-watered vines) and 10 vines received, from fruit-set to veraison, 40% of maximum water availability (WS, water-stressed vines). During water limitation, all stressed pots were covered with a plastic film to avoid interference due to rainfall and soil water evaporation. The water supply per pot was determined by monitoring the soil water content with a Diviner 2000® capacitance probe (Sentek Environ. Tech., Australia) using access tubes.. In each pot, in June, July and August the water was supplied every day at 20:00 h.

2.2. Leaf water potential, gas-exchange and chlorophyll fluorescence measurements

In 2011, one week before veraison, gas exchange readings of single leaves from both Sangiovese and Montepulciano vines were taken at midday (between 13:00 and 14:00 h) using a portable, open system, LCA-3 infrared gas analyzer (ADC Bio Scientific Ltd., Herts, UK). The system was equipped with a broad leaf chamber with a 6.25 cm^2 window and all readings were taken at ambient relative humidity with an air flow adjusted to 350 $mL\,min^{-1}$. For each cultivar, twenty average-size leaves, chosen between nodes 14 and 16 of primary shoots, were sampled under saturating light (PAR > 1400 μmolphotons $m^{-2}\,s^{-1}$). Net photosynthetic rate (P_n) and stomatal conductance (g_s) were calculated from inlet and outlet CO_2 and H_2O concentrations. Intrinsic water use efficiency (WUE_i) was then calculated as Pn/g_s ratio. Just after the gas-exchange measurements, the leaf temperature was evaluated on the same leaves (20 per each treatment and variety) with an infrared thermometer (Mod. TM909L9, Assi-control, Italy) and the leaf dark respiration (R_d) was measured in the dark (PAR level of 0 μmolphotons $m^{-2}\,s^{-1}$) obtained by covering the broad leaf chamber with a black sheet. The time needed to reach the steady state was between 2 and 6 min according to leaf temperatures, in accordance with Zufferey et al. (2000) during this time the leaf temperatures equilibrated with ambient temperature. Moreover, between 13:00 and 14:00 h, leaf water potential (Ψ_l) was measured on ten leaves for each cultivar and treatment using a portable pressure chamber (model 1000, PMS Instruments Co., USA). In order to highlight possible instability of the photochemical apparatus, on the same leaves sampled for gas exchange, modulated chlorophyll fluorescence measurements were carried out around midday (between 13:00 and 14:00 h) using a field-portable pulse modulated fluorometer (FMS-2, Hansatech Instruments, Norfolk, UK). Leaves were dark adapted for 30 min to obtain open PSII centers using the instrument leaf-clips to ensure maximal photochemical efficiency. The fiber optic and its adaptor were fixed to a ring located over the leaf-clip at approximately 1 cm from the sample