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WATER DEFICIT RESPONSE OF NEW DISEASE RESISTANT GRAPEVINE VARIETIES: EFFECT ON PLANT PHYSIOLOGY

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Abstract

Climate change represents a challenge that we are already facing today, in which water stress plays a very important role.

In fact, the growing periods of drought are increasingly worrying wine producers, who find the harvest with ever lower yields. The quality of the grapes is also affected, in fact the increases in temperature and the scarcity of water in some areas have increased the average values of sugar concentration within the bunches of grapes, which translate into increases in the final alcohol content of the wine .

From the results of these problems, the aim of our study arises, using resistant varieties in which 2 of the 4 genotypes belong to the VDQA variety category, for the production of wines with low alcohol content.

The aim of the project was to see how hybrid varieties, all belonging to the same breeding program, can respond to different imposed water stresses, in terms of gas exchange, final yield and dry matter content, trying to highlight the differences between VDQA and non-VDQA varieties.

Riassunto

Il cambiamento climatico rappresenta una sfida che già oggi stiamo affrontando, in cui lo stress idrico gioca un ruolo molto importante.

Di fatti i crescenti periodi di siccità stanno sempre di più preoccupando i produttori di vino, che si ritrovano alla vendemmia con rese sempre più basse. Anche la qualità dell'uva ne risente, infatti gli aumenti di temperatura e la scarsità di acqua in alcune zone, hanno incrementato in valori medi di concnetrazione zuccherina all'interno dei grappoli di uva, che si traducono con aumenti della gradazione alcolica finale del vino.

Dai risultati di questi problemi, nasce lo scopo del nostro studio, utilizzando varietà resistenti in cui 2 dei 4 genotipi appartengono alla categoria delle varietà VDQA, per la produzione di vini a basso contenuto alcolico.

L'obbiettivo del progetto è stato quello di vedere come delle varietà ibride, tutte appartenenti allo stesso programma di breeding, possano rispondere a diversi stress idrici imposti, in termini di scambi gassosi, resa finale e contenuto in sostanza secca, cercando di evidenziare delle differenze tra le varietà VDQA e non VDQA.

1. Introduction

1.1. Water scarcity issues

The scientific evidence is clear: the climate is changing, affecting societies and the environment, and one of the consequences of climate change concerns the availability of water (IPCC, 2018).

Water is a renewable but finite natural resource and anthropogenic climate change has contributed to the increased likelihood and severity of the impact of droughts in many regions, significantly altering the processes affecting water quality and quantity. Furthermore, the growth of the population and the expansion of industrial activities have strongly contributed to the massive use of water resources. In fact, according to research data from the World Bank of Water Resources Group, by 2030, the global demand for water will exceed the available reserves by the 40%, and it remains to be understood, as reported by the World Resources Institute, where all that water will be drawn from (IPCC, 2022; Hassanjabbar et al. 2022).

Climate change can affect water resources through changes in the amount and patterns of precipitation, impact on water quality through changes in runoff, river flows, retention and thus loading of nutrients, and through extreme events like floods and droughts (OECD, 2014).

The agricultural sector consumes the largest quantities of water globally (UNESCO, UN-Water, 2021). Currently it uses the 11% of the world's land surface for crop production, and accounts for the 70% of all water withdrawn from aquifers, streams and lakes (FAO, 2011; AQUASTAT, 2014). This is reason why agriculture is significantly affected by climate change, mostly in terms of water availability (N. Mbava et al., 2020).

Focusing on drought and the scarcity of water, we know that this phenomenon happens in many parts of the world every year and is considered a key threat for the 21st century (Liu, 2022; UNESCO, 2012), often with devastating effects on crop production. In fact, drought is considered as one of the main limiting factors, as it strongly influences most of the plant's functions (Moussa, 2008).

For these reasons, prudent management of water resources is of paramount importance due to growing human water demands (such as agricultural use, domestic consumption, and hydropower generation) and environmental/ecological requirements (Hassanjabbar et al. 2018).

1.1Water deficit in plants: focus on perennial fruits

Water deficit (WD, commonly known as drought) can be defined as the absence of adequate moisture necessary for normal plant functioning and to complete the life cycle. It influences plant physiological processes and functions and affects shoot growth, as well as fruit development (Moussa 2008; Rahmati M. et al., 2015).

However, deficit irrigation is also frequently adopted to improve water-use efficiency (WUE) and is considered an alternative to traditional irrigation scheduling approaches that fully meet plant water requirements (Ruiz-Sánchez, M.C., 2010; Howell 2001; Fereres and Soriano 2007). In this sense, the sensitivity of fruit growth to water deficit is variable from plant to plant, as well as it depends on the state of growth of the fruit, but also duration and intensity of water stress (H.-B. Shao et al., 2008; M.E. Berman, T.M. Dejong, 1996). That is why the relationship between water application and biomass or yield production, which is usually linear for herbaceous crops (Hanks 1983), can behave differently in tree crops and vines depending on the moment of the stress (Fereres et al. 2012).

The water content of the plant is extremely important for sugar accumulation in fruit, and many studies have found that reduced irrigation and water stress change the sugar content in fruits (Wei-Feng Ma et al., 2022). In fact, photosynthesis is negatively impacted by water deficit (from a specific WD2 threshold depending on the species). The inhibition of photosynthesis by water deficit depletes the daily production of photosynthetic products (sucrose) (Ruan, Jin, Yang, Li, & Boyer, 2010), while increasing the presence of toxic metabolites, and generation of reactive oxygen species (ROS), which eventually results in cell and whole plant death (K. Pandey et al., 2021).

WD2 also induces osmotic stress, which further worsens to turgor loss leading to increase in solute and sugar concentration (Francaviglia et al., 2013; Arji et al., 2016).

According to Wang et al. (2019), medium water stress (MS, 50%–60% θf, where θf denotes soil moisture) and light water stress (LS, 60–70% θf) treatments improve fruit quality by improving the contents of total soluble solid (TSS) and soluble sugar (fructose, glucose and sorbitol) (Fig. 1). But MS and LS treatments applied in stage I (from stage of fruit set to mature) reduced fruit weight, while LS treatment applied in stage II (from stage of fruit enlargement to mature) did not affect fruit weight (Fig.2).

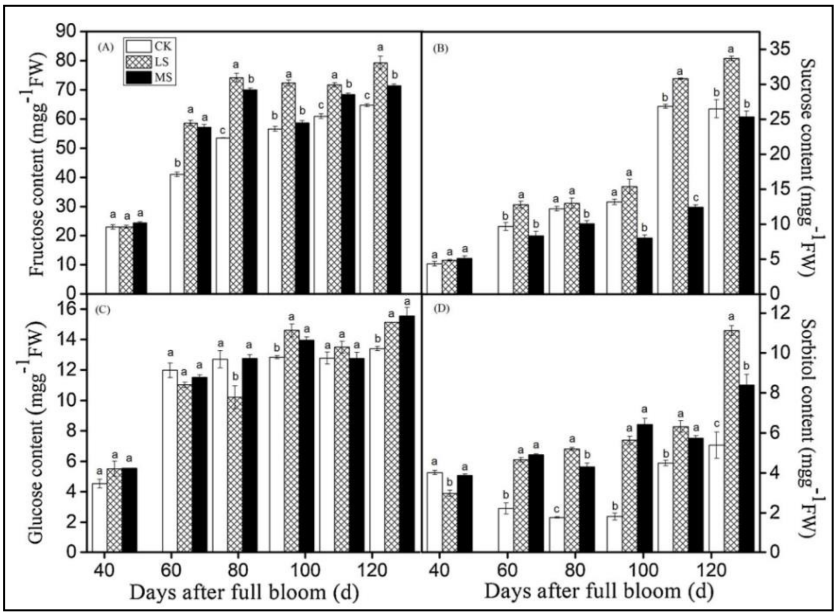


Fig. 1*: Soluble sugar content of 'Gala' apple fruit under conventional irrigation (CK), light water stress (LS), and medium water stress (MS). Water managements were applied from stage of fruit set to mature (stage I). (A): fructose content, (B): sucrose content, (C): glucose content, (D): sorbitol content. Bar graphs (within each date) that do not share a letter (a, b, c) are significantly different (Tukey, $P < 0.05$)* (Yuanji Wang et al., 2019).

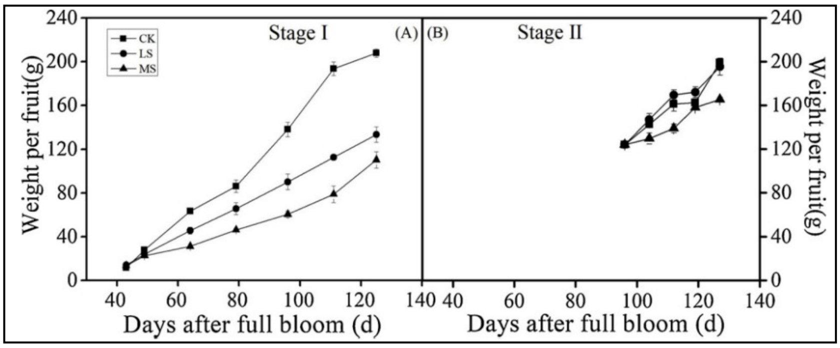


Fig. 2*: The weight per fruit of 'Gala' apple in stage I (A) and stage II (B) and the fruit diameter in stage I (C) and stage II (D) under conventional irrigation (CK), light water stress (LS), and medium water stress (MS). Stage I: water managements were applied from stage of young fruit to mature. Stage II: water managements were applied from stage of fruit expanding to mature.* (Yuanji Wang et al., 2019).

Although abiotic stresses at severe levels affect the growth and development of plants, it has been suggested that mild levels of stresses can be applied to induce the secondary metabolism, stimulating the adaptation of plants to such conditions (Cogo et al., 2011, Ellen Cristina Perin, 2019 Krol et al., 2014). In fact, it should be emphasized that water stress positively and negatively influences the quality of the fruit, such as soluble solid contents (Lopez et al., 2011), depending on the species and the phenological phase of fruit development.

In conclusion, DI 75 did not affect fruit mass, sugars and organic acids in both cultivars. DI 33 and DI 58 increased accumulation of total sugars, sucrose and sorbitol in both cultivars with acceptable fruit mass (>85 g) while severe DI treatment (DI 33) decreased glucose and fructose at harvest in 'Spring Bright' but, increased glucose in 'Summer Bright'.

There are several hypotheses that can explain the different accumulation of sugars under WD2, the first one taken into consideration by Bordonaba & Terry (2010), says that the increase in sugar concentration could be associated with limited vegetative growth and photosynthesis shift - towards the fruit, due to an attempt by the plant to reduce the osmotic potential through the accumulation of solutes. In case of severe drought stress, dehydration may function as an additional cause of solute accumulation in fruit (Yakushiji et al., 1998; Bordonaba & Terry, 2010). Although in most cases, the increases in soluble solids can be attributed to the decrease in water content of the fruits (solute concentration), some studies have reported increased active synthesis or a lower demand from the vegetative part due to their sensibility to WD, that is higher than the photosynthetic activity, in fact under water deficit, relationships between C availability and sink organ growth are weaken or are modified, suggesting release of the influence of C availability on sink organ growth (Muller et al., 2011). Therefore, the increase in soluble solids may not be explained solely by a concentration effect (Stefanelli, Goodwin, & Jones, 2010).

Mills, Behboudian, and Clothier (1996, 1997) reported a different response in apple sugar accumulation to deficient irrigation applied at different periods of fruit growth. In fact, the total sugar content in ripe, sparsely irrigated apples during the entire season from 55 days after full bloom (DAFB) to final harvest was higher than in control (fully irrigated) apples. In contrast, the sugar content in apples when lightly irrigated at 55 DAFB until re-irrigation at 100 DAFB was lower than in the control, although a large increase in sugar content was found at the end of the stress period (100 DAFB). The authors suggest that this could be due to dilution of the sugar concentration by the irrigation process applied to the previously stressed plant.

In addition, they also reported that under-watered apples at the end of the season from 105 DAFB through harvest showed no changes in sugar concentration compared to control samples. This indicates that fruit sugar concentration is modified by a water deficit imposed early in the season, but less affected by one imposed later in the season. DI applied near fruit ripening stage has the greatest positive influence on glucose and fructose accumulation in tomato fruits (Ripoll et al., 2014).

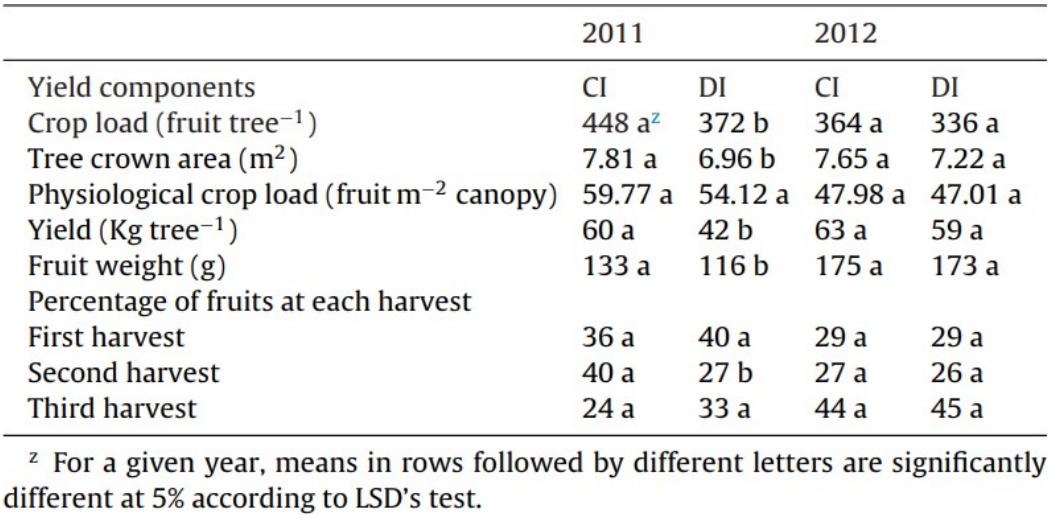
Furthermore, water stress also affects fruit growth kinetics for instance, citrus fruit on trees that have been water stressed can grow faster after re-watering than fruit on regularly watered trees (Goell et al. 1981, Huang et al. 1986, Cohen and Goell 1988).

Taking in consideration the yield, Snchez-Rodrguez et al. (2012) examined the effect of grafting on fruit yield and quality under water stress in tomato (*Solanum lycopersicum L.*). In sum, the most strongly significant effect of water stress on fruit yield is on the average fruit weight rather than on the fruit number per plant (Wei-Feng Ma et al., 2022), with a reduction in size and so in yield compared to a FULL irrigation (Minghui Cheng et al., 2021).

Gerardo Lopez at al., (2016) have evaluated the response of WD2 in nectarine (*Prunus persica*) cv. ‘Gardeta’, focussing on fruit diameter, pulp dry matter concentration, firmness, titratable acidity, soluble solids concentration, skin and pulp colour and also several sensory traits and consumer acceptance of fruit in 2 harvest years (2011, 2012). The intensity of WD2 was evaluated with midday SWP (−1.25 MPa and −1.05 MPa), while the control was going with value higher than n −0.85 MPa.

In the following table (Tab. 1) took from their article is possible to analyse the effects of WD2 comparing to control (CI):

Tab. 1: *Effects of conventional irrigation (CI) and deficit irrigation (DI) on yield components.* (Gerardo Lopez at al., 2016)



	2011		2012	
Yield components	CI	DI	CI	DI
Crop load (fruit tree^{-1})	448 a[z]	372 b	364 a	336 a
Tree crown area (m^2)	7.81 a	6.96 b	7.65 a	7.22 a
Physiological crop load (fruit m^{-2} canopy)	59.77 a	54.12 a	47.98 a	47.01 a
Yield (Kg tree^{-1})	60 a	42 b	63 a	59 a
Fruit weight (g)	133 a	116 b	175 a	173 a
Percentage of fruits at each harvest				
First harvest	36 a	40 a	29 a	29 a
Second harvest	40 a	27 b	27 a	26 a
Third harvest	24 a	33 a	44 a	45 a

[z] For a given year, means in rows followed by different letters are significantly different at 5% according to LSD’s test.

In 2011, DI reduced yield and mean fruit weight but no significant differences were observed in 2012 (Table 1). In 2011, the percentage of fruit harvested in the second pick was lower in DI trees than in CI trees. But in 2012 there were no significant differences in the percentages of fruit harvested at each pick. This is probably due to the fact that in 2011 SWP values were more negative than in 2012 and consequently the effect of water stress on fruit diameter (FD) was more evident in 2011 than in 2012 (Fig. 5). Comparing to the CI, the less yield comes from the fact that, the stress of water was imposed starting from the 3 stage of growth and Water shortages are most critical when they occur during the final stage of fruit growth (Stage III of fruit development in *Prunus persica*) and Stage III is very sensitive to water stress because cell expansion is mainly explained by an accumulation of water (Berman and DeJong, 1996).

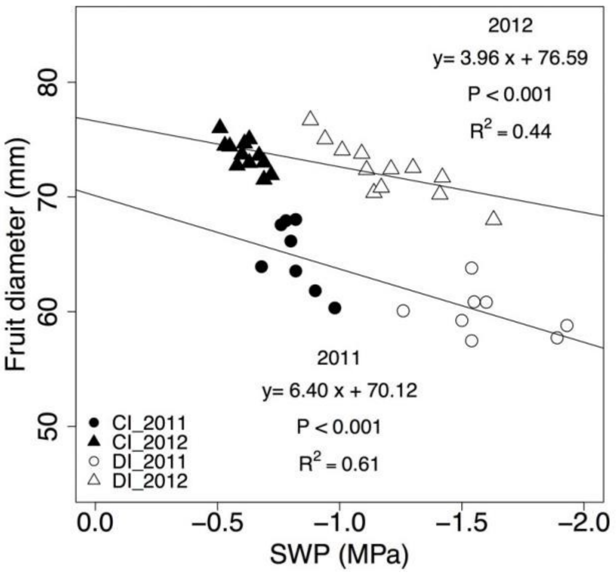


Fig. 5: *Relationship between midday stem water potential (SWP) and fruit diameter at harvest. Abbreviations: CI = conventional irrigation, DI = deficit irrigation*

(Gerardo Lopez at al., 2016)

As regards the other parameters evaluated, which affect the quality of the fruit, DI in 2011 increased pulp dry matter and soluble solids concentrations, and pulp and skin colour while in 2012, DI did not affect fruit composition. In general, this response of fruit size other quality attributes were in agreement with literature, also for other type of fruit like pear (Lopez et al., 2011b), and plum (Intrigliolo and Castel, 2010).

Another important aspect concerning water stress concerns the carbon balance and its allocation in the different tissues.

It should be underlined how the vegetative and fruits growth occur simultaneously, and therefore a water stress in this phase inevitably leads to a strong competition between the different organs, and in general from various studies it seems that in situations of stress, the growth of fruits is positive to the detriment of the vegetative one (Grossman and DeJong, 1995; Yuan et al., 2009).

Several studies have demonstrated how vegetative and reproductive growth are 2 parameters that show a different sensitivity to water stress, depending on the moment of stress and therefore on the phenological phase: according to Mitchell and Chalmers (1982), mild water stress applied during the intermediate developmental period of slow fruit growth has no effect on crop yields but significantly reduces vegetative growth in peach. The plant therefore seems to respond in favour of a better growth of the fruits to the detriment of the vegetative one, thus allocating a greater quantity of assimilates towards the fruits. In fact, according to some studies conducted on peaches, cropping trees are often more water stressed than de-fruited trees (Chalmers and Wilson 1978), while DeJong

1986, noticed how trees with fruits induce a stomatal conductance greater than 30%, causing a greater photosynthesis ratio equal to 11-15%, during the final growth phase, when assimilate demand by fruit is at a maximum, compared to non-fruiting peach trees.

According to the study taken over by N. Cui et al. (2009) and Li et al. (1989), showed that water deficit at early growth stage can inhibit the vegetative growth, reduce shoot and leaf area growth of pear-jujube tree and peach, while for pear-jujube tree, N. Cui et al. (2009), noted that fruit volume tended to be greater than controls, as water stress first reduced the vegetative development, then subsequently, through a subsequent irrigation, during the fruit growth phase, they showed beneficial effects for the development and volume of the fruit, probably due to the fact that water deficit and re-irrigation has a compensatory effect on fruit growth (Caspari et al., 1994).

This relationship between vegetative and fruit growth was also studied by Han et al. (2005), verified that direct control over vegetative growth has a strong influence on the final fruit yield.

In terms of yield, and therefore in quantity, how do fruits respond to different levels of water stress?

Taking these first 2 studies into consideration, there is agreement in the fact that in the case of mild water stress levels, the fruit does not seem to undergo changes in terms of yield and therefore quantity, if compared to the control, however the qualitative parameters are influenced such as primary metabolites, such as sugars and more.

the cells need water to elongate them self,

However, referring to the yield, we can state that it does not undergo changes when a slight stress threshold is applied, but in this case the 2 studies show how the moment in which this stress emerges, and therefore the phenological phase of the fruit, has a crucial role in determining whether or not to decrease yield. In fact, stresses applied in the first phase of fruit formation (fruit set), have an impact that penalizes the yield, in terms of quantity, with an effect mainly on the diameter of the fruits, which are found to be reduced, and the greater the stress to which they are subjected, the smaller the diameter and therefore the lower the final yield (data not shown). This is due, according to some studies, to the fact that in stage 1, and being in a phase of stress they are unable to elongate and therefore lose volume and consequently size and so weight (Yildirim et al., 2015). While if the stress is applied after the cell elongation phase (phase 2), the yield does not undergo significant reductions. This may be because fruit cells had been basically completed inflated growth at this time, light soil water stress did not negatively affect the elongation of fruit cells. However, also in this case, the greater the slight stresses, the greater the difference in terms of weight, compared to the control, which therefore can go to highlight a quantitative difference in the yield that can be significant. In conclusion\, the parameters to be taken into consideration are certainly the moment in which water stress is applied and its level, understood as stress intensity. Furthermore, if we evaluate the results of the last study (fig. 5), the fruits subjected to water stress show greater heterogeneity in terms of size, compared to the control, where the size of the fruits tend to be much more homogeneous with differences in diameter less significant.

As regards the primary metabolites, mainly sugar, how does water stress affect fruit?

Basing ourselves on the results of previous articles, we can point out that water stress influences both positively and negatively the accumulation of sugars inside the fruit, this depends, as well as for the previously described yield, on the intensity of water stress, predominantly, but also by the phenology of the fruit and therefore when stress is imposed. Obviously, differences also emerged according to the genotype and the species investigated, highlighting that there are differences also due to the type of plant and that therefore each fruit responds differently to water stress.

In general, moderate water stress levels can contribute positively to fruit quality, with an increase in the sugar content, if applied during the fruit growth phase or near fruit ripening, where the cell division phases are completed, and therefore the cell elongation phase gets involved in stress. Therefore, the increase of these primary metabolites is due to a lower concentration of water which is therefore able to dilute the sugar concentration to a lesser extent. However, water stress also appears to induce higher sugar production than the control (Yakushiji et al., 1998; Bordonaba & Terry, 2010). Taking into consideration Fig.1, it can also be seen that more marked levels of water stress lead to a decrease in the sugar content, which for some molecules can be lower than the control. While the results taken into consideration in Fig. 4.1 and 4.2 do not seem to agree. In fact, in most cases, however, there is a concentration of sugar which is higher in cases of medium-high water stress, with a decrease of some sugars during the harvest phase. The higher levels of glucose in the fruit with DI 58 and DI 33 treatments over CI in 'Summer Bright' may also be due to higher sorbitol levels which may be is metabolized into glucose by sorbitol oxidase as suggested previously by Moriguchi et al. (1990), while the decrease in the concentrations of glucose and fructose and increase in sucrose levels during fruit maturation may be due to reduction in sucrose hydrolysis. Therefore, as we note, different fruits respond in different ways to water stress, mainly as regards the primary metabolites. This also depends on the intensity of the stress, but also on the phenological phase, even if it seems to emerge from these results that intensity of stress and genetic factors seem to be the variables that best differentiate the responses of the plant and therefore of the fruit.

Take Home Message:

Through these observations, we can therefore understand that the importance of water is more related to the yield than to the quality of the fruit. If it is true that light loads of water stress affect the quality of the fruit (understood as an increase in soluble solids), it is also true that this leads to a reduction in yield, due to a reduction in volume or in size. This provides a great challenge, because of the constant increase in demand, in terms of quantity, due to the growing number of consumers. Therefore, a better understanding of water stress effects is crucial to cope with climate change and consequently to global water reduction.

In fact, from the various studies it emerged that WD generally induces a loss of fruit size compared to the control, thus resulting in a loss of yield, while for some qualitative traits, if water stress is moderate, there may be an increase in these parameters. However, in the case of medium-high density water stress, even these qualitative traits are reduced, in terms of concentration, with respect to the controls.

It should also be underlined that periods of water stress often coincide with the fruit growth phase, and from the various studies taken into consideration, this could increase the qualitative parameters described above. However, we have seen that some fruits have shown different responses also in terms of yield, which mainly depend on the genotype and therefore generalizing the answers as an increase in sugar content could be misleading.

We well understand that in a constantly evolving world, also from a climatic point of view, which leads to a reduction in the availability of water, the challenge of water stress becomes a crucial point to take into consideration.

1.3 Water stress in grapevine

Viticulture plays a considerable socioeconomic role in many countries (Torregrosa et al., 2019; Alston and Sambucci, 2019), is estimated that world area under vines is around 7.3 mHa in 2020, including young vines not yet ready for the production (OIV, 2020). Water is a critical factor for viticulture sustainability because grape production, quality and economic viability is strongly dependent on water availability (Medrano et al., 2015). In this sense, viticultural management has experienced a series of modifications due to climate change, which has a direct effect on the grapevine physiology, with a restrict in vigour and consequently on yield and berry compounds and so on wine quality (Van Leeuwen & Darriet, 2016, Webb et al., 2008, Coipel et al., 2006).

These qualitative differences are expressions of how the vine is adapting to the environment, in fact as a function of these abiotic stresses, the vine responds with a series of actions, dependent on the stress gradient, but also on the timing, and so when this stress is applied according to the phenology of the vine (Ojeda et al., 2002; Mirás-Avalos and Intrigliolo, 2017; Girona et al. 2009; Intrigliolo and Castel, 2009).

In general, water stress on the vine reduces productivity and, if well controlled, can have positive effects in improving the quality characteristics of the grape. This last effect is determined by the reduced vigor of the shoots and by the competition for carbon resources, in this sense it changes the intensity and the sink/source ratio. In the event of excess water, the vine undergoes greater vegetative growth and greater productivity, in terms of yield, however the bunches will turn out to be, qualitatively speaking, worse, as there will be a dilution effect exerted by the strong excess of water. In situations of water stress, the berries will accumulate a greater quantity of secondary metabolites, as defense metabolites against the damage that cells may suffer.

However, it should be mentioned that a marked or severe water stress causes a decrease or total cancellation of the photosynthetic activity, due to the strong stomatal closure, with negative effects on the growth and development of the plant and therefore also of its fruits. (Ferrandino and Lovisolo, 2014)

The phenological stage in which the plant is when it is hit by stress has various repercussions on its growth, development and physiology not only of the plant but also of the fruit:

- Except in particular production areas, there is hardly a stress condition during the budding phase, however if it occurs in this phenological phase it can cause a reduction in bud opening and shoot growth (Wample and Smithyman, 2002; Fregoni, 2005).
  if this stress is allowed, there may also be a poor development of flower clusters, a reduction in the vitality of pollen and pistils, and therefore a reduction in fruit setting.
- During flowering, water stress can cause floral abortion and inflorescence abscission, probably associated with hormonal changes. The reduction of the elongation of the shoots can instead lead to an insufficient development of the leaf area which is not able to support the development and ripening of the fruits. Furthermore, the differentiation of flower buds for the following year, which begins two weeks before flowering and continues for another two weeks, can be negatively influenced by water scarcity through a reduction in potential fertility (Wample and Smithyman, 2002; Fregoni, 2005).
- Immediately after fruit set, there may be a reduction in cell division and therefore in the size of the berries, while in the latent phase of the growth curve the fruit is less sensitive.
  In any phenological stage they can have due to stress, senescence, with yellowing and subsequent abscission of the lower leaves. High levels of stress can cause necrotization of the shoot apex and, if followed by rehydration, can cause the emission of female shoots which determine competition phenomena and cause an alteration of the maturation processes (Wample and Smithyman, 2002).
  In this phase excessive stress significantly worsens the quality of the product and, in the most serious cases, leads to an arrest of ripening and dehydration of the berry. In general, however, the vegetative development is more sensitive to water stress than that of the berry (McCarthy et al., 2002).
- After the harvest, the lack of water leads to a reduction in the development of the root system, with a consequent reduction in the absorption of mineral elements in the following season.

1.3.1 Effects on photosynthesis and stomatal conductance

Many studies have shown that one of the first responses to water stress is stomatal conductance, in this sense, the stomata, tiny pores on the leaf surface (Darwin, 1898) begin to close a few minutes after the onset of stress due to hydraulic and chemical signals, this translates into a decrease in the photosynthesis ratio, with a consequent decrease in photo-assimilates that can be used by the plant as a form of energy (Gambetta et al. 2020; Flexas et al., 2002; Buckley, 2019).

However, it has been demonstrated that different genotypes respond with different stomatal conductances, in fact certain genotypes of vines tend to close their stomata earlier than other cultivars (Lavoie-Lamoureux et al., 2017; Dayer et al., 2019). Schultz (2003) observed how different cultivars (Grenache and Syrah) respond differently to water stress, in particular how the stomata close, thus distinguishing isohydric and anisohydric varieties. Anisohydric tend to accept strong variations in water potential, limiting stomatal closure only partially, while isohydric cultivars limit stomatal opening at the first signs of stress, thus containing an excessive lowering of water potential (CONAVI, 2012). However, this differentiation has not yet been fully accepted since it has been seen that the environment often strongly conditions the genotypic response, which therefore does not depend only on the water content in the soil and therefore on the plant (Hochberg et al., 2018; Villalobos-González et al., 2019).

The reduction of photosynthesis is a response determined by a physiological change of the stomata, mainly determined by the stomatal conductance (g_s), understood as the quantity or mole of water that passes through the pores and therefore the stomata in a given period of time. However, one of the factors that mostly governs stomatal conductance is abscisic acid (ABA), a plant hormone and key water stress response signal, is produced and combines with turgor loss of guard cell to initiate stomatal closure under light drought stress (S. Dayer ae al., 2019; Martorell et al., 2014). However, ABA does not seem to be the only molecule that controls g_s, in fact other theories have been advanced in this regard, such as xylem sap pH (Wilkinson and Davies, 2008), electrical signals (Grams et al., 2007) or hydraulic signals (Hubbard et al. al., 2001), which seem to show some relationship with g_s.

It is interesting that according to the study conducted by Socìas et al. (1997), the stomata tend to close before the actual water shortage, due to the activity of the ABA which is translocated from the roots to the leaf level, acting as a signal for the stomatal closure (Davies and Zhang, 1991).

Flexas e Medrano (2002) demonstrated a model of plant response, in terms of photosynthesis, which suggests a sort of adaptation of the plant to drought conditions. According to these authors, for values of light or moderate water stress, the closure of the stomata is the first adaptive response and the main constraint to the photosynthetic process; in parallel, however, at levels of severe water shortage, the progressive gene "down-regulation" or the inhibition of metabolic processes determine a reduction of ATP synthesis and therefore a limited regeneration of RuBP (ribulose-1,5-bisphosphate), with consequent lowering of the rate of photosynthesis, moreover under conditions of severe stress, photoinhibition phenomena are frequent which lead to the almost complete closure of the stomata.

Using g_s as a parameter for defining the degree of water stress, it is possible to distinguish three phases of photosynthetic response to a gradual decrease in water availability (Cifre et al., 2005):

1) Phase of light water stress, with a range of g_s: 0,5-0,7 to 0,15 mol H_2O / (m^2 s): there is a reduction in net photosynthesis, mainly due to the closure of the stomata, with a general increase of water use efficiency (WUE);
2) Phase of light moderate stress, with a range of g_s: 0,15 to 0,05 mol H_2O / (m^2 s): net photosynthesis which is further reduced with a decrease in photosynthetic efficiency (Ɛ) and electron transport rate (ETR), in this phase, depending on the cultivar, there may be an increase or a decrease in the WUE;
3) Phase of severe water stress, with gs $\leq$ 0.05 mol H_2O / (m^2 s): a sharp decrease in net photosynthesis, water use efficiency, ETR and ε characterize this stage.

1.3.2 Water Use Efficiency

The limitation of the stomatal conductance and therefore the closure of the stomata, with consequent reduction of transpiration is a response implemented by the plant to safeguard the WUE (Water-Use-Efficiency) (Dong et al. 2015; Varga et al. 2017). WUE it is a parameter that can be identified as the ratio between the assimilated carbon (and therefore the number of micromoles of CO2 which are used by photosynthesis) on the quantity of water lost through transpiration (Flexas et al., 2010), it can therefore be considered a key parameter to reduce irrigation (Condon et al., 2004) or to simplify, the WUE can be defined as the productivity of the plant, in relation to the water consumption (Hatfield and Dold, 2019). This value can be obtained by analysing leaf photosynthesis, thus analysing the amount of net CO_2 assimilated by the leaf, and so photosynthesis (A_N), comparing it to the stomatal conductance g_s, thus obtaining intrinsic water use efficiency (WUE_i), or relate the ratio of photosynthesis (A_N) to leaf transpiration (E), obtaining in this case instantaneous water use efficiency (WUE_{inst}) (Fischer and Turner, 1978). Furthermore, it is also possible to estimate the WUE of the whole plant (WUE_{wp}), determined by the ratio between the production (in dry weight) of the whole plant and the total water consumption (M. Tomás et al., 2014).

From this, we can think that WUE, more precisely WUE_i can be an indicator that allows us to determine in abstract if a plant is able to perform well under water stress or not, however according to the studies carried out by Tomás et al. (2014) it was seen that the WUE of the single leaf does not reflect the WUE of the whole plant, calculated with the WUE_{wp}. The reason behind this according to the study, may be related to the inadequacy of single-leaf based sampling to represent variability encountered at the whole canopy level, due also to the fact that often for the calculation of photosynthesis or stomatal conductance the best exposed leaves are taken, in fact Escalona et al. (2003) showed that the inner canopy leaves, which represent 35–40% of its total leaf area, only contribute to less than 5% of the total net carbon gain with a much higher percentage of total water losses. Additionally, the carbon losses breathed by roots, stems, and leaves will affect the carbon balance of plants (Tomás et al., 2014).

1.3.3 Effects on carbohydrates reserves

The different allocation of carbon between sinks, and therefore short-term or long-term investments, could have a large impact on the carbon balance in the different sinks, in this reason drought favors the growth of roots to the detriment of that of the shoot, with the aim of promoting water absorption and exploring the deeper layers of the soil in search of water (Anjum et al., 2017).

In general, moderate water stress induces a reallocation of available carbohydrates away from vegetative production and towards root and berry development, this finding a relationship with water potential (Gambetta et al., 2020). In fact, the impact of water deficit on vegetative growth is noted after flowering, where the development of the first lateral shoot is inhibited, as well as that of the primary shoot which is slowed down and finally stopped completely when the apex tips wither and fall (Levin et al., 2020).

Since vegetative growth is slowed before photosynthesis declines, the vine is able to redirect carbohydrates to carbon sinks such as fruit and roots, thus promoting the synthesis of metabolites associated with grape quality (Scholasch and Riensch, 2019).

At the radical level, the sugars are then sent, in the form of starch, which also acts as a reserve molecule, capable of being converted into soluble sugar if necessary (MacNeill et al., 2017), therefore when the source/sink relationship is unbalanced and therefore internal demand exceeds photosynthetic activity, the plant will be able to use the carbon sources in reserve for replenishment, such as for example during the spring recovery where sugars are recalled starting from the root level (Rossouw et al., 2017; Martínez-Lüscher e Kurtural, 2021). The restoration of the reserve starches takes place to a greater extent after the ripening phase of the berry, where the assimilates are involved at the root level, due to the accumulation in the form of starch, but it has in any case been demonstrated that it begins at flowering, depending on the cultivars, soil and climatic conditions and yield load (Pellegrino et al, 2014).

Water stress, therefore, reduces photosynthetic activity, which therefore has 2 main effects on the carbon balance and the accumulation of reserve substances:

- on the one hand, water stress reduces photosynthetic activity, which is therefore unable by itself to compensate for the internal demand of the plant, which is forced to use reserve substances:

- moreover, this photosynthetic reduction is not used to restore the reserve substances which are used directly by the plant.

It is therefore clear that reserves play an important role in the survival of the vine, since a scarce presence of these metabolites at the time of recovery limits the survival of the vine, as reported by Falchi et al. (2021), carbon starvation is the leading cause of grapevine death during drunk, along with hydraulic failure.

1.3.4 Effects on berry growth and metabolism

The grape is a fleshy fruit, whose growth can be represented with a double sigmoidal ripening curve characterized by two distinct periods of growth in which a latency phase is interposed (Coombe, 1976). The first phase, a phase characterized by intense green growth cytokinetic activity, begins soon after fruit set and is mainly driven by cell division and expansion by accumulation of organic acids (mainly malic and tartaric) but also other metabolites. During this phase the berry has a strong hydraulic connection with the vine, and these two acids, which are responsible for most of the osmotic pressure driving the uptake of water from the xylem (Chaves et al., 2010).

After a brief latent phase which corresponds to the phenological veraison phase, maturation begins, characterized by a general softening of the berries, followed by variations in the color of the skin (due to the accumulation of anthocyanins in the red and pink cultivars) and accumulation of sugars. Grape growth following veraison is governed by cell expansion as water and solutes are imported apoplastically through the phloem (Scholasch and Rienth, 2019).

The relationships between water stress and berry composition strongly depend on the cultivar and can be equally influenced by other factors such as the source/sink balance and the timing of the water deficit (Chaves et al., 2010). Precisely, the “sink strength”, interpreted as the capacity of a plant part to predominately attract sugars (Minchin and Thorpe, 1996), often results because of a transition from passive to active phloem carbon unloading. Pre-veraison water deficits that inhibit cell division have been shown to have a greater impact on berry size, sugar accumulation and overall berry quality than deficits during the ripening phase (Chaves et al., 2010 Poni et al., 2018). The different sensitivity of the berry to water deficits, which are lower in post-veraison, is probably the result of the shift of water uptake which occurs mainly through the xylem towards the phloem.

Water deficits occurring during ripening have been shown to have less impact on berry size than pre-veraison deficits, probably due to the switch from symplastic xylem water transport to osmotically induced phloem import (Scholasch and Riensch, 2019). However, severe water stress that impairs plant photosynthesis and decreases hydraulic conductance can block ripening, affecting both berry quality and size at harvest (Wang et al., 2003).

Water deficit during maturation also has an important impact on secondary metabolites.

Mild water stress is known to improve berry quality, particularly for red wine production, by increasing the concentration of quality-associated compounds, including flavonoids and volatile aroma compounds (van Leeuwen et al., 2009; Keller 2010; Zufferey et al., 2017). Flavonoid compounds (anthocyanins, tannins and flavonols) are found in the skin and seeds of the berry, and some of this effect can be attributed to the increased skin to juice ratio resulting from the smaller berry size. However, there is an additional effect of water stress on the biosynthesis of phenolic compounds, which can be positive or negative depending on timing and severity (Ojeda et al., 2002). In red grapes, both the rate of anthocyanin synthesis and the compositional changes are positively associated with a slight water stress. The primary anthocyanins that are formed in conditions of water scarcity are methoxylated peonidin 3-O b-glucoside and malvidin 3-O-b-glucoside, which give rise to wines that tend to be more pigmented and bluer in tone (Chaves et al ., 2010; Gambetta et al., 2020).

2. Materials and Methods

2.1 Location and plant material

The experiment was carried out with potted plants (2022) in greenhouse, at the l'Institut Agro-INRAe campus, located at Montpellier, France (43°37’03’’ North| 3°51’27’’ East) (Figure 1).

Fig.1: location of the Research Center SupAgro in Montpellier (Google Earth® map), the red square in the figure represents the greenhouse where the experiment was carried out

The panel of genotypes considered for this work, belonged to a wider panel of 6 INRAE resistant varieties (*V. vinifera L. x M. rotundifolia* hybrids) and the *V. vinifera* var. Syrah, as control.

Of all the varieties present, the study was carried out only on 4 varieties: 4 hybrids, shown in Fig. 2. Some of this hybrids (Figure 2), are already certified and therefore in possession of the requisites to be planted, while other varieties are undergoing certification, in more detail we have that all this varieties, plus Syrah as control have been grafted into 140RU, and this hybrids are :

- Floreal: white berried hybrid, pyramided with 4 resistance genes, 3 years old, in possession of certification for the installation;
- 3159B: white berried hybrid, 3 years old, which is in the verification phase for acceptance of the certification;
- G5: white berried hybrid, 3 years old, which is in the verification phase for acceptance of the certification;

- G14: red berried hybrid, 3 years old, which is in the verification phase for acceptance of the certification;

As regards to G5 and G14, it should also be mentioned that, according to various studies, these varieties show, at harvest, a moderate amount of hexoses in the berry, with a reduction from 20% to 30% compared to the other traditional cultivars. These features make these varieties able to be processed for the production of wines with a low alcohol content, defined as: VDQA, "***V****ins* ***D****e* ***Q****ualité à teneur modérée en* ***A****lcool*" (Ojeda et al. 2017, Bigard et al. 2022).

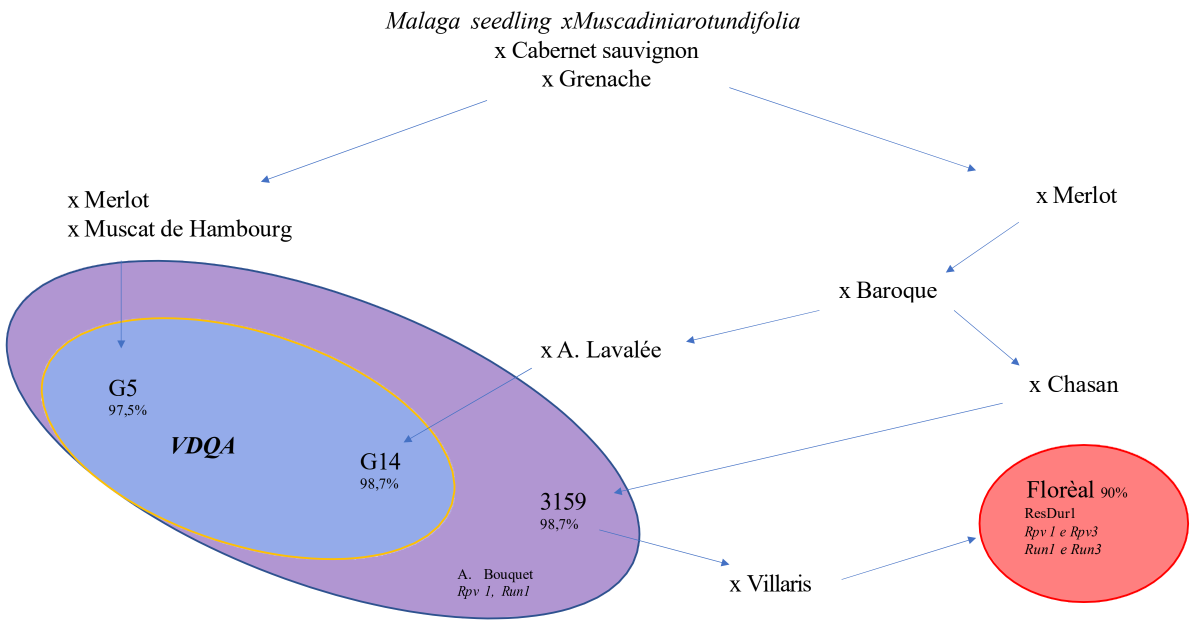


Fig. 2: scheme concerns the crosses to obtain the various hybrids, with the relative parents for back-cross and relative percentages of kinship with the parental

2.2 Growing conditions

The experiment started in the field, where potted plants, were irrigated and fertigated in conditions of optimal water status. Subsequently, at veraison, each genotype was brought into the greenhouse. In detail, when a single plant showed signs of veraison, described by Antoine Bigard et al.(2019), such as initial softening of the berry, the whole pool of plants belonging to the genotype were brought into the greenhouse, where moderate drought stress treatment started.

The levels of water stress were determined as a function of the water content in the soil (SWC).

In this sense a treatment with moderate water stress (60% of SWC, WD1), and another treatment with more severe water stress (30% of SWC, WD2), were applied. For reasons related to plant health status an initial selection was made, reducing to14 plants for genotype the panel to be tested.

Each plant was placed on a scale (Figure 3), useful for determining the weight which was recorded and saved every 15 minutes throughout the day, for every day through a software and connected to an irrigation system. , which would allow the plant and the soil to maintain a quantity of water and therefore a constant water status. Irrigation was programmed to water each plant 4 times a day, at 7:00am, 10:30am, 1.30pm, 4.30pm, with the aim to maintain a certain specific weight for each plant, depending on the quantity of water present in the soil, which therefore determined the level of water stress for each plant.

All the plants were equipped with a support, which allowed to give verticality to the shoots. Each pot was equipped with a mulching fabric that limited the soil from losing water through evaporation, thus permitting to directly infer the plant water loss through transpiration from the variations of pot weights.

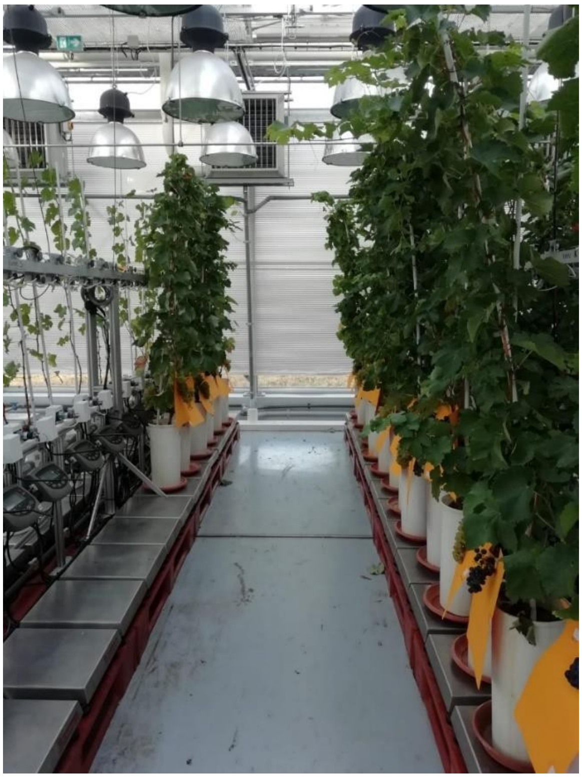
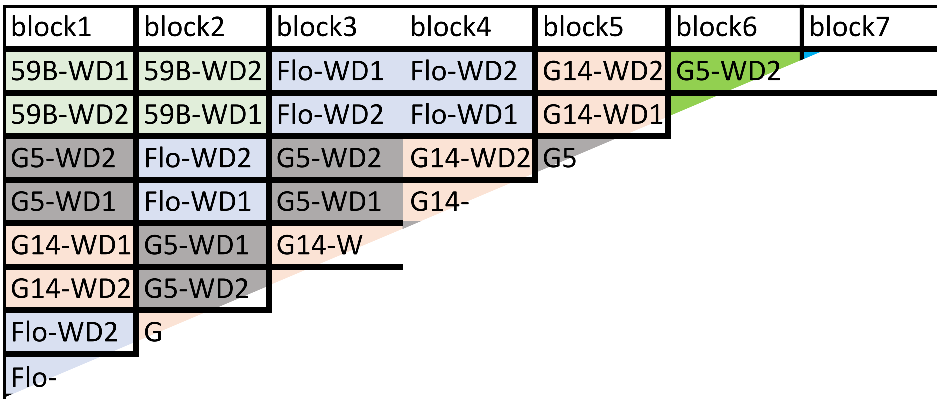


Fig 3: image of the SupAgro greenhouse. Each plant was positioned on a scale and connected to a self-irrigation system, allowing the weight to be maintained.

The plants were distributed in the greenhouse according to the scheme reported in Table 1, alternately among the varieties, with the aim of reducing the variability within experimental conditions.



block1	block2	block3	block4	block5	block6	block7
59B-WD1	59B-WD2	Flo-WD1	Flo-WD2	G14-WD2	G5-WD2	
59B-WD2	59B-WD1	Flo-WD2	Flo-WD1	G14-WD1		
G5-WD2	Flo-WD2	G5-WD2	G14-WD2	G5		
G5-WD1	Flo-WD1	G5-WD1	G14-			
G14-WD1	G5-WD1	G14-W				
G14-WD2	G5-WD2					
Flo-WD2	G					
Flo-						

Tab.1: Arrangement of the varieties in greenhouse.

For the duration of the entire experiment in the greenhouse, no phytosanitary treatments were carried out, while additions of Iron in solution, 2ml Fe/100ml of water for each plant and an addition of 2 g of Osmocote, as a slow-burning granular fertilizer, were made. release of macronutrients.

2.3 Measurement of physiological parameters

Net leaf photosynthesis (An), stomatal conductance (g_s) and transpiration (E), were parameters evaluated with the Licor-6800 Portable Photosynthesis System (PPS) with a Multiphase Flash™ Fluorometer (6800-01A) for CO_2 and H_2O gas exchange measurements. Measurements were performed on fully expanded leaves (one per plant). To this aim, the plants were moved to an area well exposed to sunlight, remaining inside the greenhouse, for a minimum of 30 minutes before the measurements, with the purpose of avoiding interference due, due to the structure of the greenhouse, which intercepted the sunlight, causing areas of shade (Figure 4).

The PPS consists of two major components: the console, which includes the digital interface and the chemical columns for control of air composition, and the head, which contains the 6 cm^2 leaf chamber and controls leaf temperature. The LI-6800 PPS controls environmental conditions at the leaf level, including temperature, humidity, light intensity and wavelength, and CO2.

The PPS also controls airflow and fan speed. The PPS uses IRGAs to detect gas concentrations of CO2 and water from before (reference, REF) and after (sample, SAM) the leaf chamber. At the time of use we always proceeded with an initial phase of machine calibration, in an experimental environment, to then proceed with the necessary analyses.

The environmental parameters set were the Vapor-pressure deficit (VPD) for a value between 1.9kPa and 2.1kPa, the temperature of the room where the leaf was present, for an average of 28°C, but with a range between 26 °C and 30°C, the concentration, or flux of CO_2 equal to 400 µmol * mol^{-1}.

Fig. 4: Licor 6800 which analyzes the photosynthetic and stomatal response.

2.4 Berry maturity assessment

The harvest time was determined based on the maximum volume that the bunch could reach, due to the phenological phase called phenological maturity, in which the phloem stops pumping sugars at the level of the berry, which therefore stops expanding further (Bigard et al., 2020).

To determine the final volume of the berry, a daily monitoring was carried out by taking pictures of one bunch chosen for each plant, with calibrated background, allowing to determine the progressive increase in the bunch volume, and to ensure proper conditions for software functioning. The images obtained were processed and the pixel count analysed using Image J ver. 1.53e software (Lopes & Cadima, 2021).

The images were taken with a camera, on a fixed support, where one bunch per plant was used as a candidate for measurement (Figure 5). The aim therefore was to measure the increase in surface area on a weekly basis, always on the same bunch for each plant. Once the results of the photos and the software showed no increase, we proceeded with the harvest, with the assumption that clusters had reached their maximum volume.

However, the analysis of the Brix degrees was performed, at harvest, to verify differences in sugar concentration between treatments.

Fig.5: Bunch picture for volume evaluation and harvest date determination.

2.5 Dry matter allocation

The carbon allocation to vegetative organs was estimated as a function of pruning weight per plant, using the fresh and dry weight of shoot and leaves. To determine the carbon content inside the bunch, an approximation was made: a water content inside the berry of about 85% was assumed as proposed by the study by Keller (2005) and as can be seen from the search by Deloire et al. (2009) (Figure 6), where not the harvest date is taken into account, but the moment in which the berries are at their maximum volume (Vmax) (red circle in Figure 6).

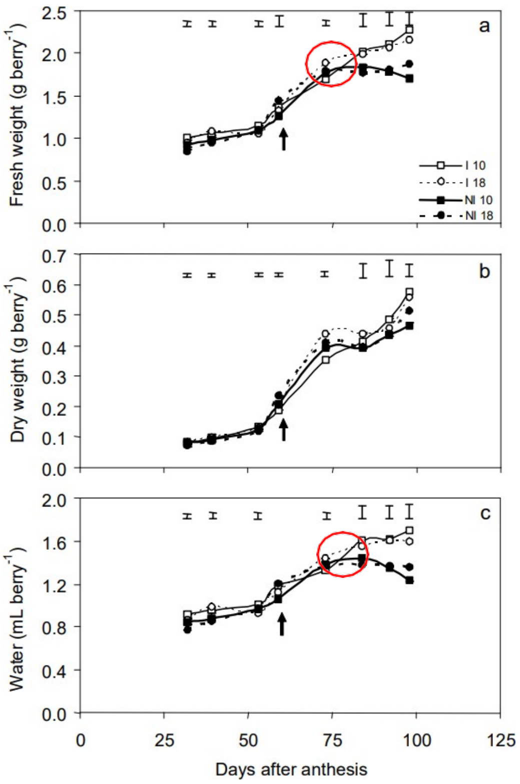


Fig. 6: graphic representation of the fresh weight (a), dry weight (b) and the amount of water (c) of a berry. (Etchebarne, et al., 2009

2.6 Analysis of Water Use Efficiency (WUE)

To calculate the values of instantaneous (WUE_i), the ratio between the values of photosynthesis (An) and leaf transpiration (E) were used (Fischer and Turner, 1978; Flexas et al., 2010).

Statistical analysis

All graphical processing and statistical tests, Student's t, were performed using Microsoft Excel®

3 Results

3.1 Photosynthesis, stomatal conductance and transpiration

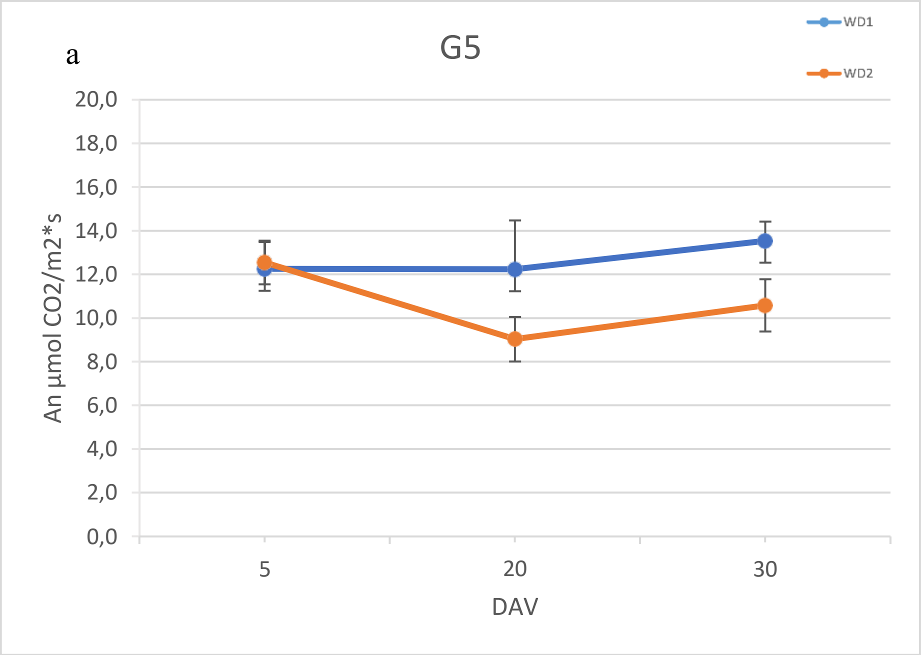
The various photosynthesis analyses were carried out at different times, starting from veraison.

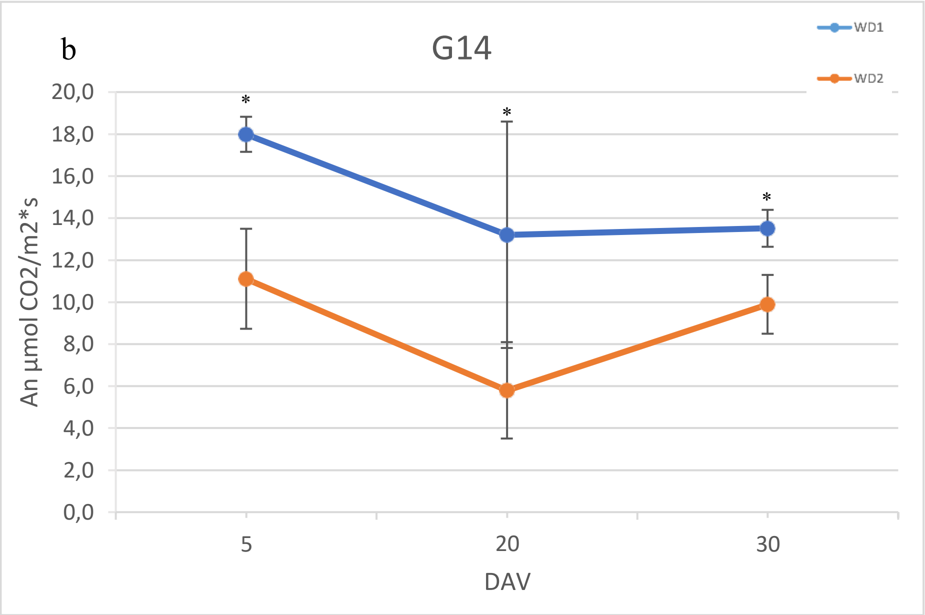
In detail, 3 measurements of photosynthetic response and stomatal conductance were collected: 5 Days After Veraison (DAV), 20 DAV and 30 DAV.

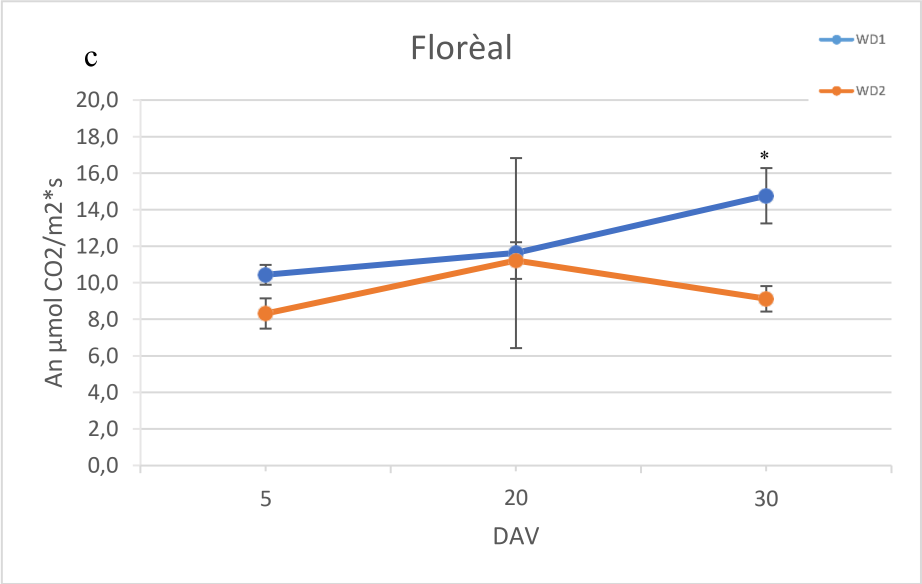
The responses between the varieties were different both in quantitative and qualitative terms, where some plants, with the same treatment, underwent a continuous decrease in photosynthesis and stomatal conductance, while others adapted immediately to the stress, showing an increase of both parameters.

In terms of photosynthetic response, in the case of the WD1 treatment (blue line), G5, Florèal and 3159B showed a continuous growth of photosynthesis, while the G14, it immediately showed a strong decrease in photosynthesis. Therefore, the experimental conditions were successful in obtaining higher photosynthetic rates in the G14 variety which showed a photosynthesis value at 5DAV equal to 17.988 µmol CO_2/m^2s, compared to the 3159B variety, which instead had the lowest value, 7.114 µmol CO_2/m^2 s, while Florèal and G5 showed intermediates, 10.438 µmol CO_2/m^2 and 12.253 µmol CO_2/m^2 respectively. At 20 DAV the varieties tend instead to have similar photosynthetic values, with values ranging between 13.204 µmol CO_2/m^2 (G14) and 11.629 (Florèal) µmol CO_2/m^2. While at 30 DAV, there was a slight increase for all strains with average values around 13.8 µmol CO_2/m^2

As expected, WD2 treated plants had lower photosynthetic rates than the WD1 ones (orange line). WD2 plants showed, in general, a trend mirroring that of WD1, with a lower photosynthetic rate (Figure 7).


a
G5
WD1
WD2
An µmol CO2/m2*s
20,0
18,0
16,0
14,0
12,0
10,0
8,0
6,0
4,0
2,0
0,0
5
20
30
DAV


b
G14
WD1
WD2
An µmol CO2/m2*s
20,0
18,0
16,0
14,0
12,0
10,0
8,0
6,0
4,0
2,0
0,0
*
*
*
5
20
30
DAV


c
Florèal
WD1
WD2
An µmol CO2/m2*s
20,0
18,0
16,0
14,0
12,0
10,0
8,0
6,0
4,0
2,0
0,0
*
5
20
30
DAV

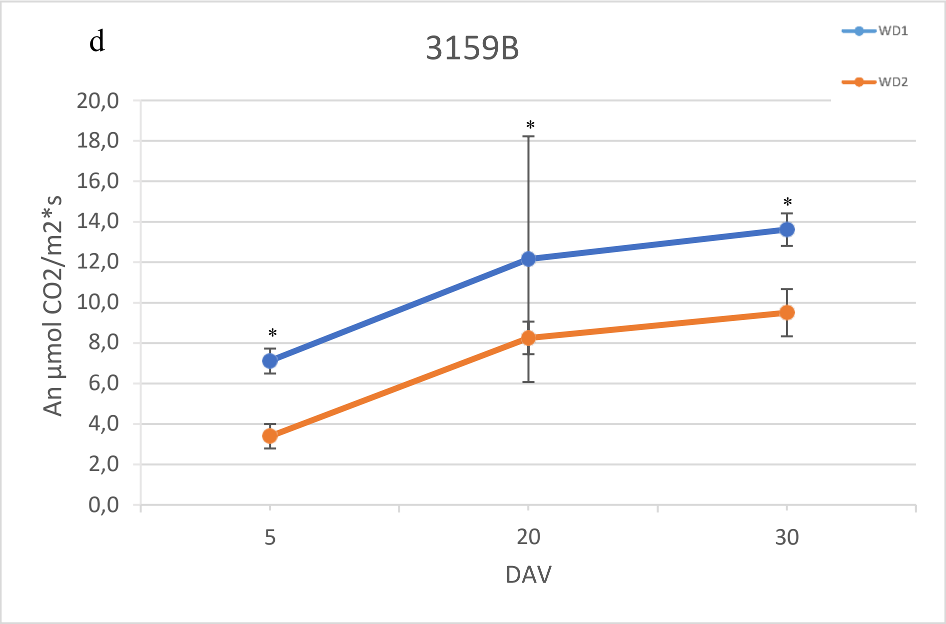
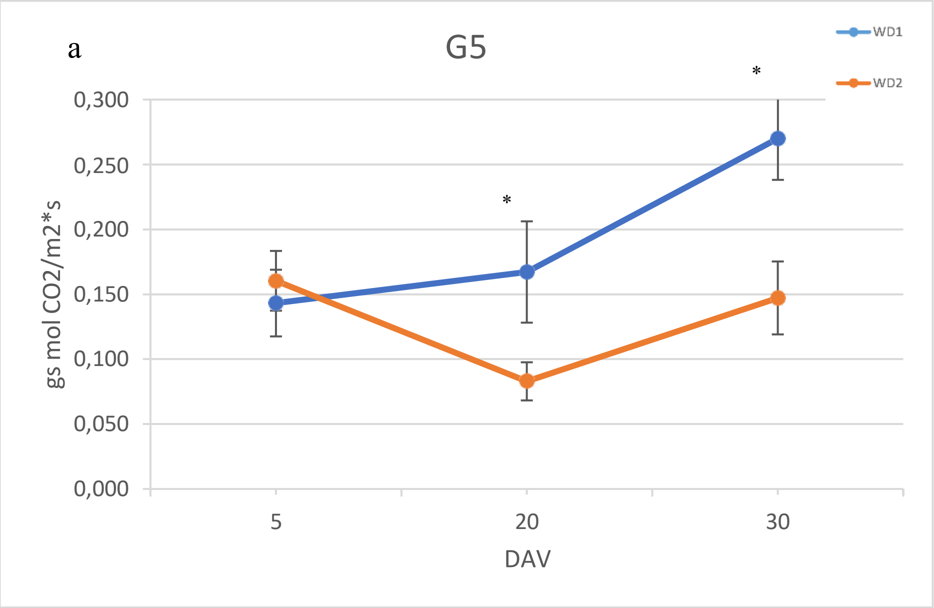


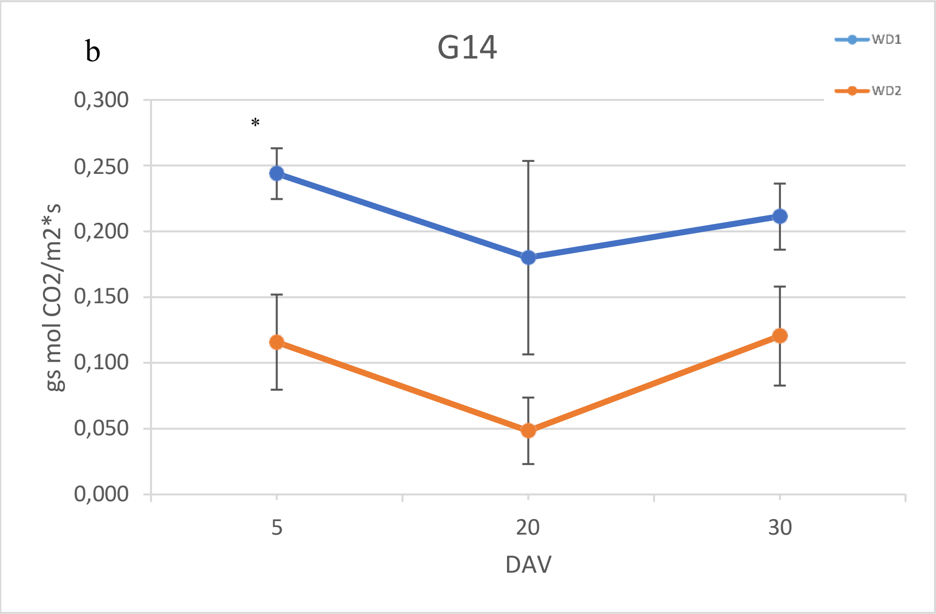
Fig.7: Photosynthesis (µmol m−2s−1) trends in the different varieties and Days After Veraison (DAV).

a) G5; b) G14; c) Florèal; d) 3159B. Asterisks (*) means that there were statistical differences (p-value < 0,05) between the 2 treatments for that point (DAV), Blu= WD1, Red = WD2; while the bars indicate the standard error

The analysis of the statistical differences has revealed how in the case of photosynthesis, the G5, did not present significant differences, for the whole duration of the experiment, while G14 has shown a similarity of the photosynthesis data, only at 30 DAV, therefore as the of the phenological stage of maturation. On the contrary, the Florèal, where for the first 20 DAV, there were no differences between the treatments, while in proximity of the phenological maturation, there are important and significant differences between the treatments. The impact of treatment was well noticeable in 3159B in terms of photosynthetic response, in fact despite a similar trend, the photosynthetic capacity reduction was statistically different in all the time points.

A subsequent comparison with the different rates of stomatal conductance of the varieties according to the treatment was carried out to verify the differences and relationships with the different photosynthetic increases and decreases. Also in this case, differences in plants’ response were detected, due to both genotype and treatment. As expected, the stomatal conductance patterns mirrored the photosynthetic rate, with a greater difference (and statistical significance) related to the water regime (Figure 8).


a
G5
WD1
WD2
gs mol CO2/m2*s
0,300
0,250
0,200
0,150
0,100
0,050
0,000
5
20
30
DAV
*
*


b
G14
WD1
WD2
gs mol CO2/m2*s
0,300
0,250
0,200
0,150
0,100
0,050
0,000
5
20
30
DAV
*

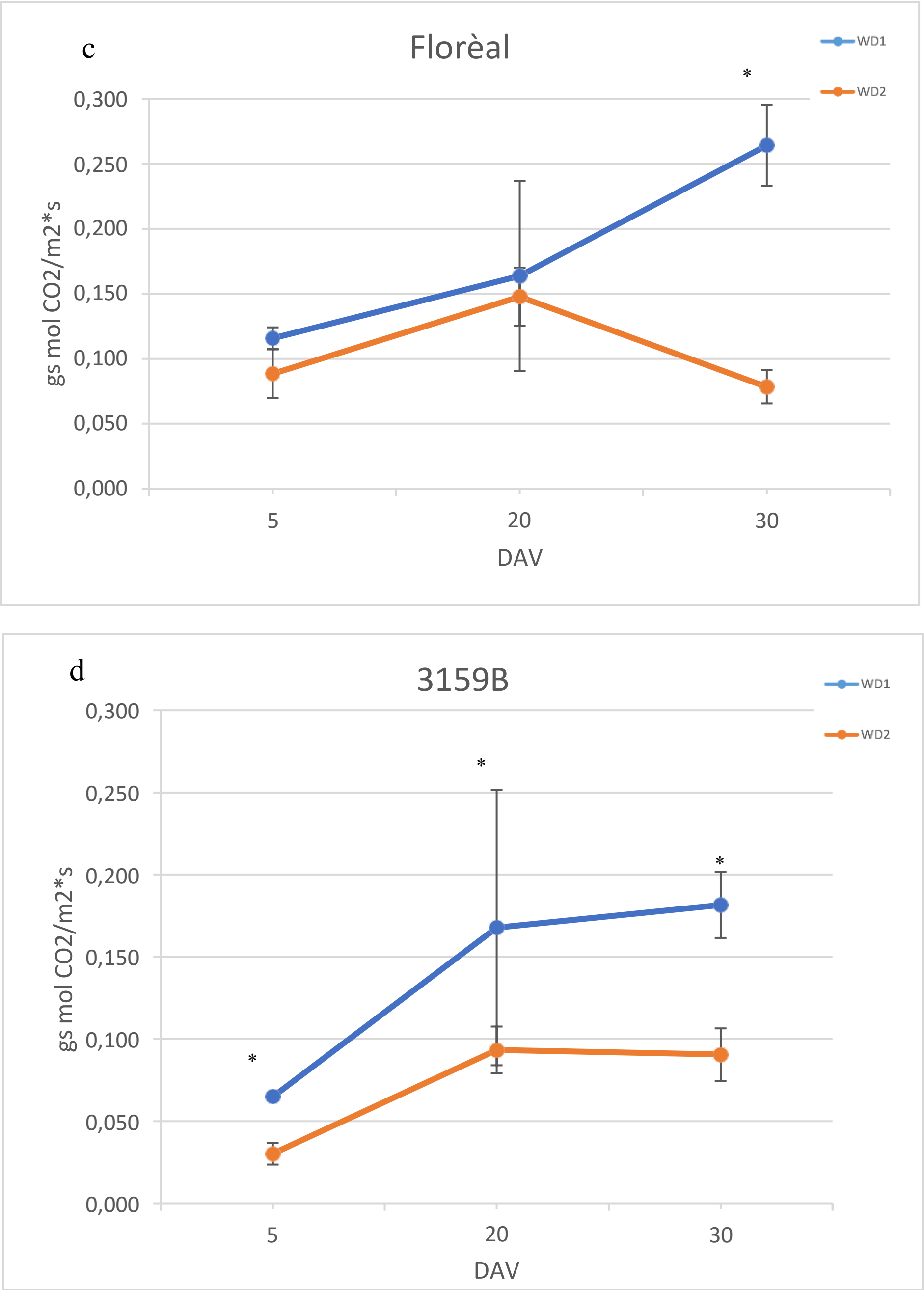
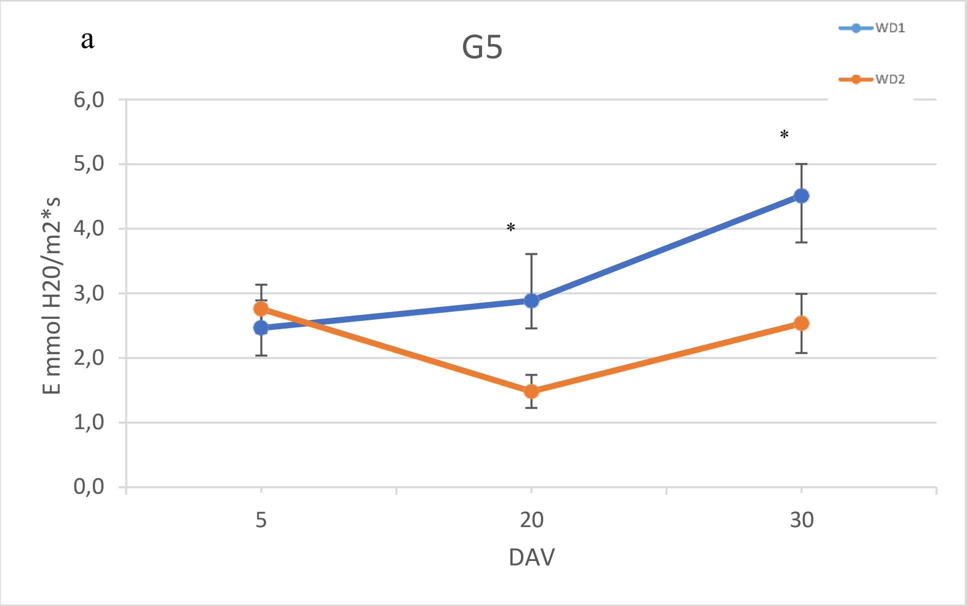
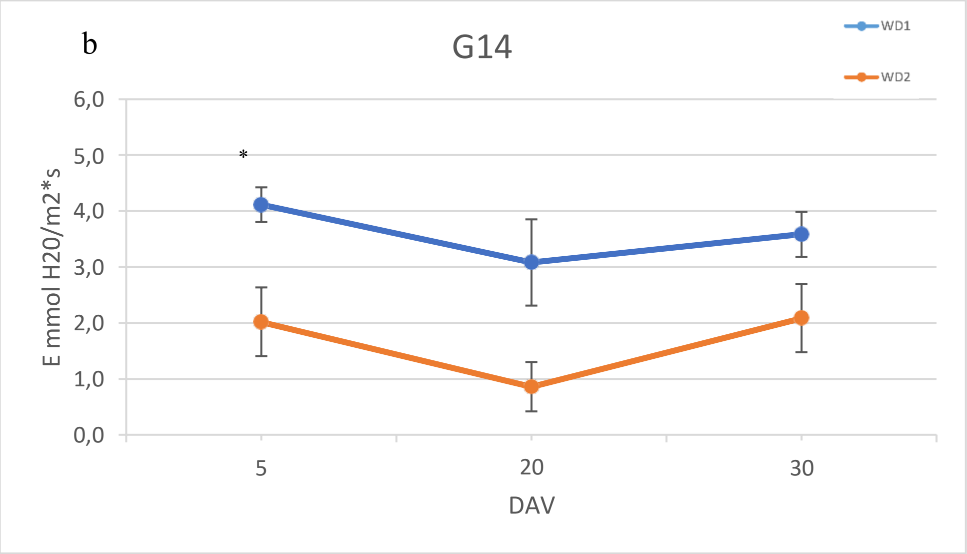


*Fig.8: trend of the stomatal conductance of the different varieties, in relation to the water treatment: a) G5; b) G14; c) Florèal; d) 3159B. * means that there were statistical differences (p-value < 0,05) between the 2 treatments for that point (DAV), Blu= WD1, Red = WD2;while the bars indicate the standard error*

It is noteworthy that the WD2 treatment leaded to a different stomatal response in the non-VDQA and VDQA varieties, as happened for photosynthesis, where the stomatal conductance showed two different behaviours.

The results obtained from the analysis of leaf transpiration gave results consistent with the ones coming from photosynthesis and stomatal conductance. In fact from Figure 10 it is possible to notice an increase of leaf transpiration, even if not of the same intensity, for the variety of G5, Florèal and 3159B, also in line, with the stomatal opening. The G14 had a diversified behaviour in the different time points, in fact, if from 5 to 20 DAV there was a decrease in transpiration, from 20 to 30 DAV instead there was a small increase in transpiration, in line with the increase in stomatal conductance, recorded at this point.





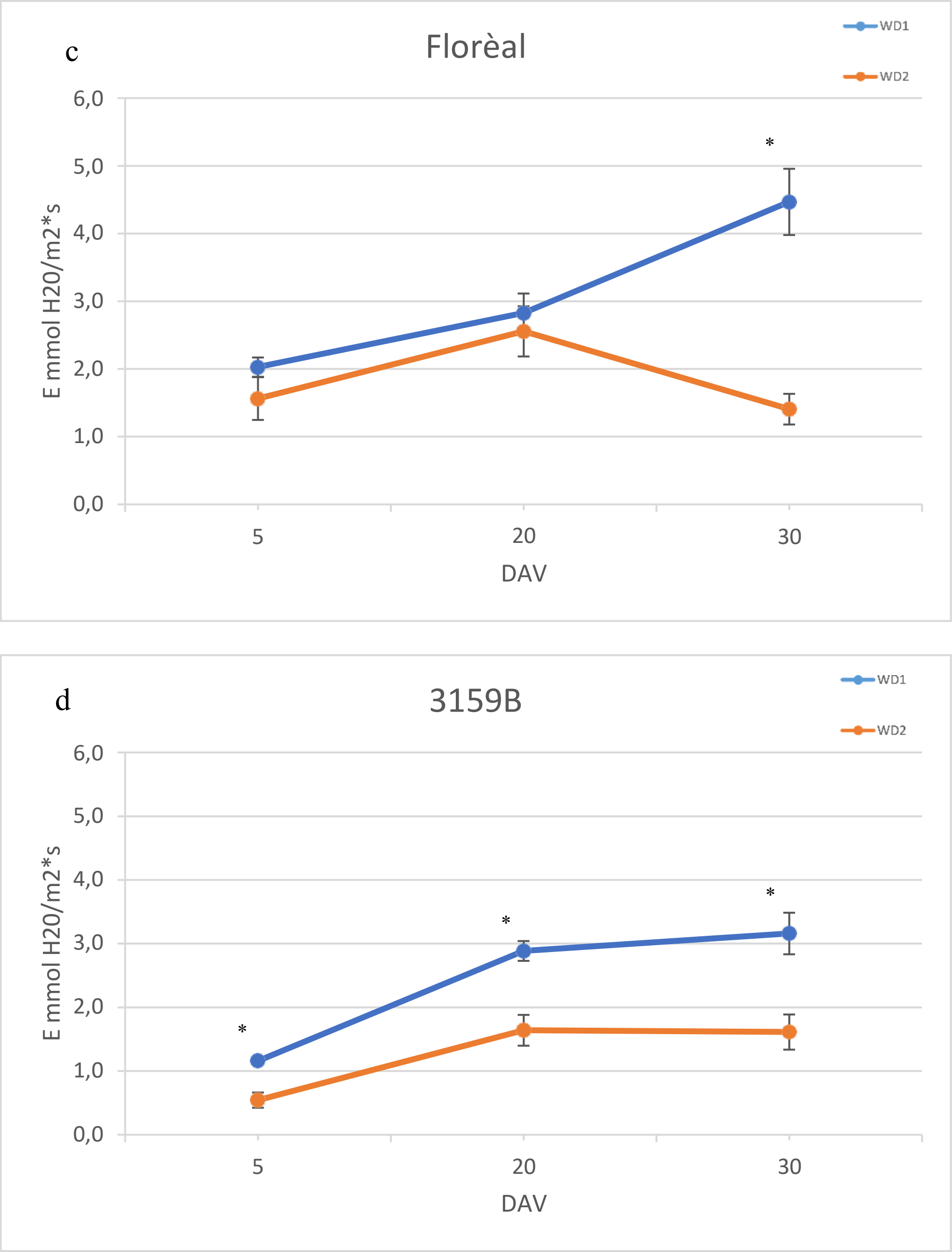


*Fig 10 : trend of the leaf transpiration rate (E) of the different varieties, in relation to the water treatment:a) G5; b) G14; c) Florèal; d) 3159B. * means that there were statistical differences (p-value < 0,05) between the 2 treatments for that point (DAV), Blu= WD1, Red = WD2; while the bars indicate the standard error*

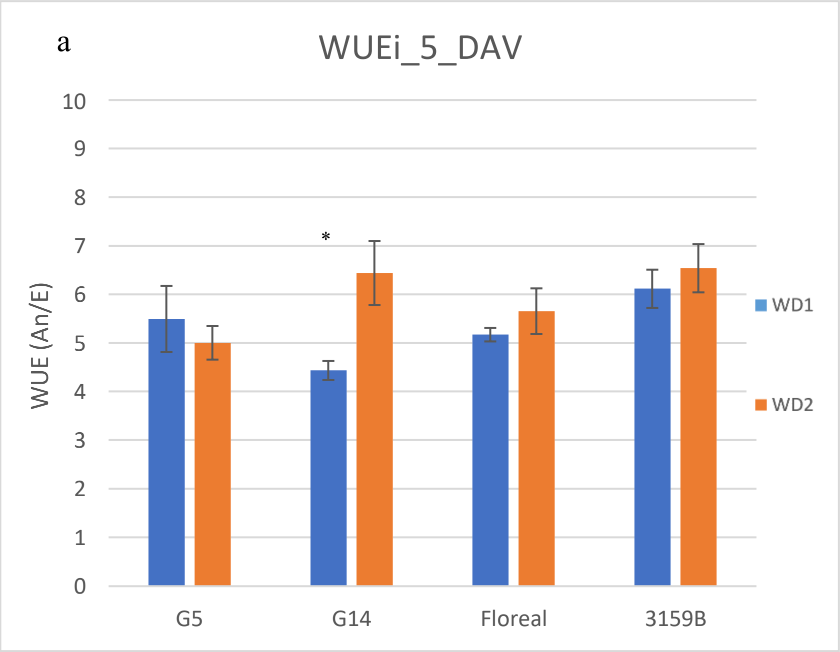
In relation to stomatal conductance, the statistical differences between the treatments of a variety were the same, with 3159B which differs statistically for the treatments, in the survey of the transpiration demand.

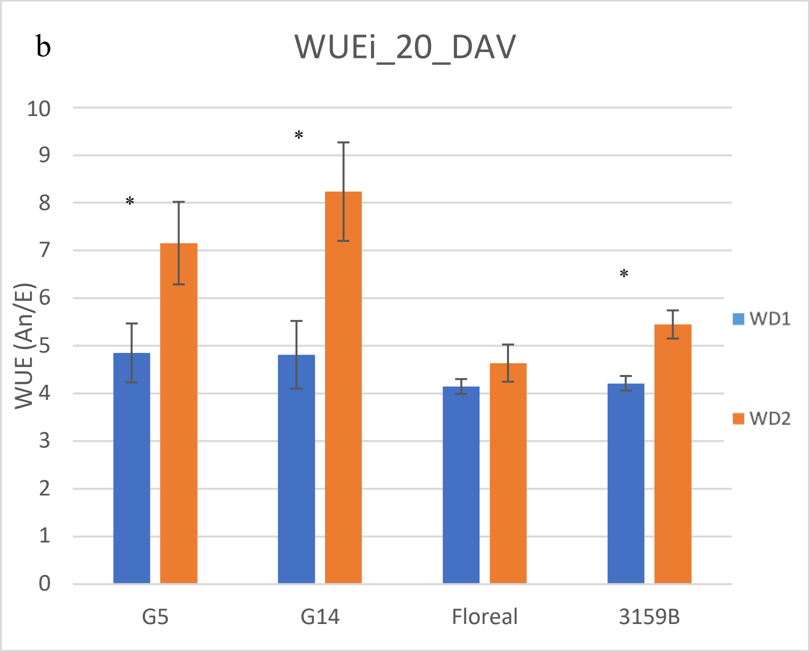
Florèal, which for the point measured at 30 DAV, has always shown statistical differences, also visible in the graphs, where WD1 tended to increase, while WD2 tended to decrease.

G14, showed a difference in the first survey, while the other *VDQA* variety, G5, on the contrary showed differences only in the last 2 points.

3.2 Water Use Efficiency

The instantaneous Water Use Efficiency (WUE_i) was evaluated in the 3 dates (DAV), using the mean value obtained from the different replicates. Therefore, 3 graphs representing the WUE_i in the 3 measurement dates were constructed (Figure 11)





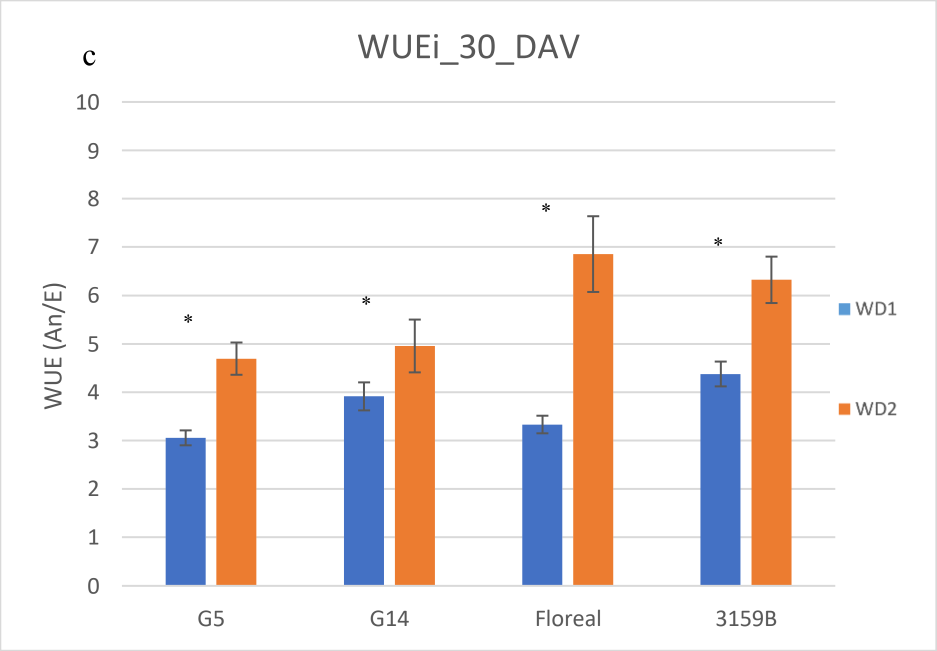


*Fig 11. WUEi of the varieties, according to the treatments, * means that there is statistically significant difference between treatments (p-value < 0.05), while the bars indicate the standard error*

The results obtained show that the difference between the two treatments tends to increase for all the varieties as the season progresses. In fact, at 5 DAV only the G14 shows statistical difference between treatments, where the WUEi is higher in the WD2 treatment, in line with the statistical differences found in photosynthesis and transpiration, at the same time point (Figure 7 and Figure 9). For the other varieties, however, there is no significant difference and 3159B is the variety with the highest WUEi for both treatments. Conversely, at 20 DAV, the G14 in the WD2 treatment appears to be the one with the highest WUEi, as well as for the WD1 treatment very similar to that of G5. In addition, at 20 DAV, the varieties statistically different for the two treatments are all, with the exception of Florèal.

At 30 DAV, all the varieties exhibit a statistical difference, in terms of treatments, in which Florèal increases the difference between treatments, with a higher WUEi in the WD2 treatment, as well as the one with the highest value compared to the others.

3.3 Yield

The harvest was carried out at different times, as evidenced in Table 4. In fact, as expected, each variety showed different times of phenological maturation, and within the same cultivar, even the treatments showed significant differences. In general, WD1 treatments were collected earlier than WD2 treatments, with a mean difference of 7 days, except for G14, whit treatments collected on consecutive days.

The differences between treatments were detected in the average fresh weights of the grape clusters (Figure 12), but no statistical significance was found in the comparison of the treatments for the different varieties.

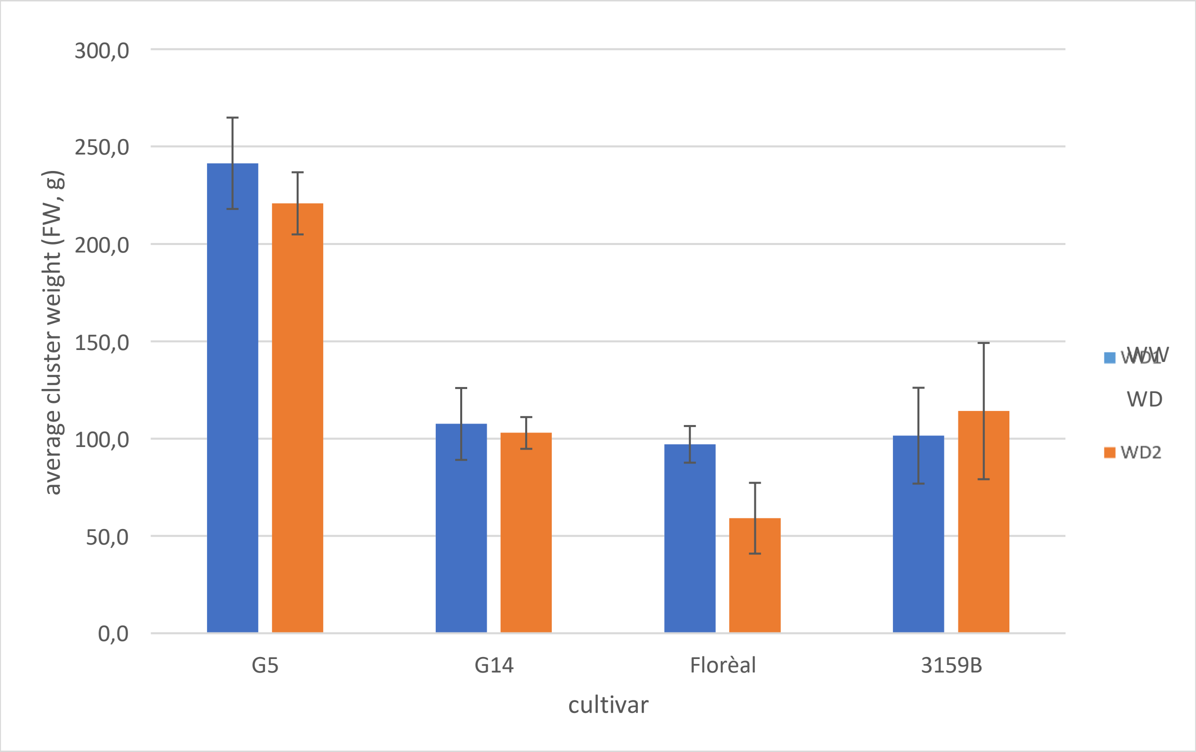


Fig. 12: average fresh weight of the clusters of each variety and the treatments, bars indicate the standard error

However, the genotypic differences are still important, in fact the G5 is the variety that on average displayed the greatest fresh weight of the clusters, while the Florèal is the variety with the lowest fresh weight of the clusters, especially in case of WD2, although there is no effective statistical difference.

With the harvest, the difference in the ripening times of the varieties was then highlighted, in fact Table 4 highlights the experiment starting dates, as well as the beginning of veraison. The difference between the days therefore indicates the time that the varieties have employed for the bunches to reach phenological ripeness.

Entrance	G5	G14	Floréal	3159B
	11/07/2022	18/07/2022	06/07/2022	06/07/2022
Harvest	G5	G14	Floréal	3159B
WD1	23/08/2022	24/08/2022	18/08/2022	24/08/2022
WD2	29/08/2022	25/08/2022	23/08/2022	30/08/2022
ΔWD1	43	37	43	49
ΔWD2	49	38	48	55

Tab.4: summary table of the experiment starting dates (transfer into the greenhouse) of the different varieties, with differences in maturation times (Δ) for Treatment WD1 and Treatment WD2

It is possible to identify that G14 has reached the phenological ripening phase faster, while the variety that has needed the highest number of days to reach this phenological phase is the 3159B, for both treatments.

At the harvest a study was also carried out on the Brix degrees. Table 5, summarizes the different sugar content, expressed as Brix concentration, of the different treatments and cultivars.

	3159B	Floreal (*)	G14 (*)	G5
WD1	18,70	20,05	19,28	18,46
WD2	20,50	22,20	18,32	18,86
Δ	1,80	2,15	-0,97	0,40

*Tab.5: Table of Brix degrees, of different varieties and different treatments with relative variation in percentage, * means that there is statistically significant difference between treatments (p-value < 0.05),*

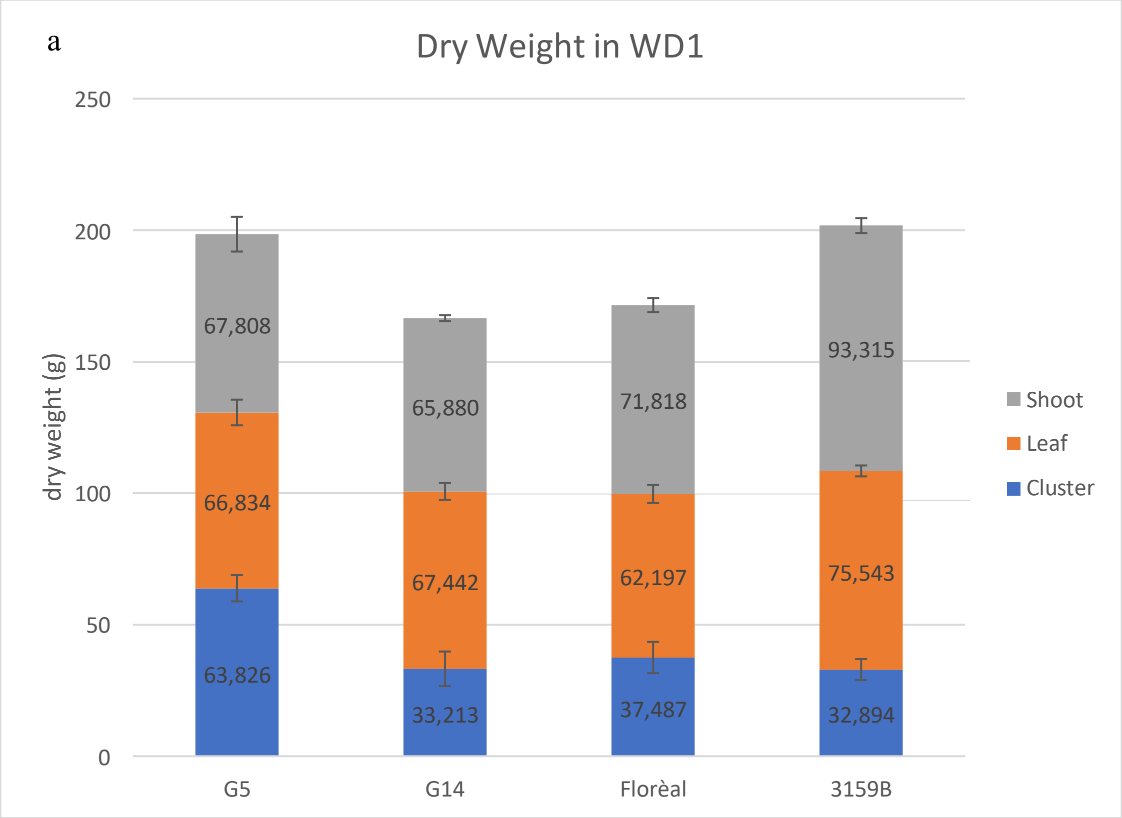
The general trend can be described as an increase in the Brix degree in the WD2 treatments, with the exception of G14, which instead showed a decrease in the Brix degrees in the WD2 treatment.

Moreover, Florèal is the variety that showed the highest Brix degree in both treatments, while the sugaress varieties, as expected, exhibited the lower average value than the other varieties.

3.4 Biomass production and carbon allocation

Dry weights were obtained to identify the distribution of carbon among the different organs: shoot, leaves and bunches, for each variety and for each treatment (Figure 13). Water stress clearly reduced the total biomass accumulated, and the difference between cultivars and irrigation treatments in non-permanent and permanent organs was significant.

Compared to the treatments, WD1 has a higher dry content in the different varieties than the WD2 treatment. Furthermore, a statistical difference was identified between the different treatments for the shoots of the G14, Florèal and 3159B varieties, while as regards the dry content of the leaves, the statistical differences were present only in 3159B. No statistical differences were identified between treatments for the cluster.



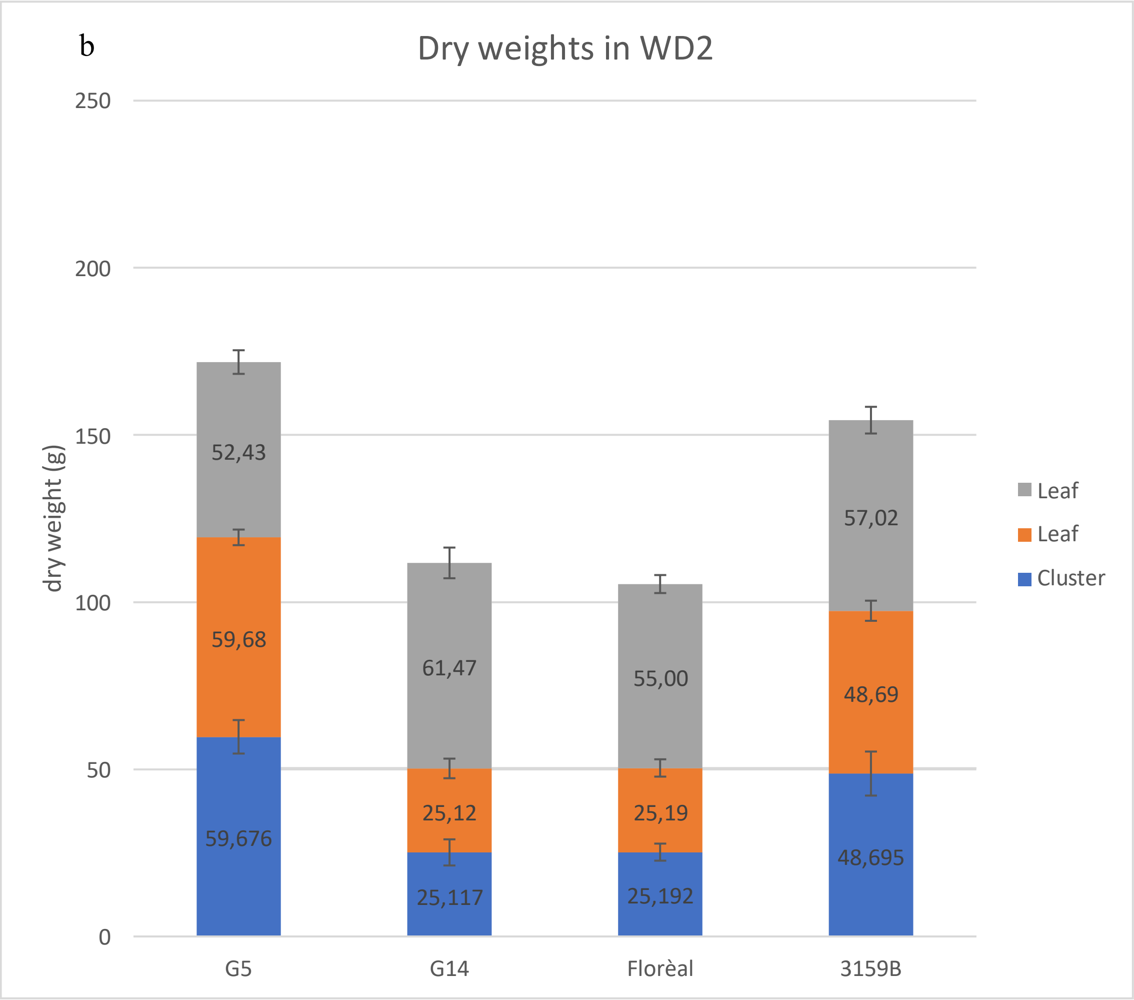
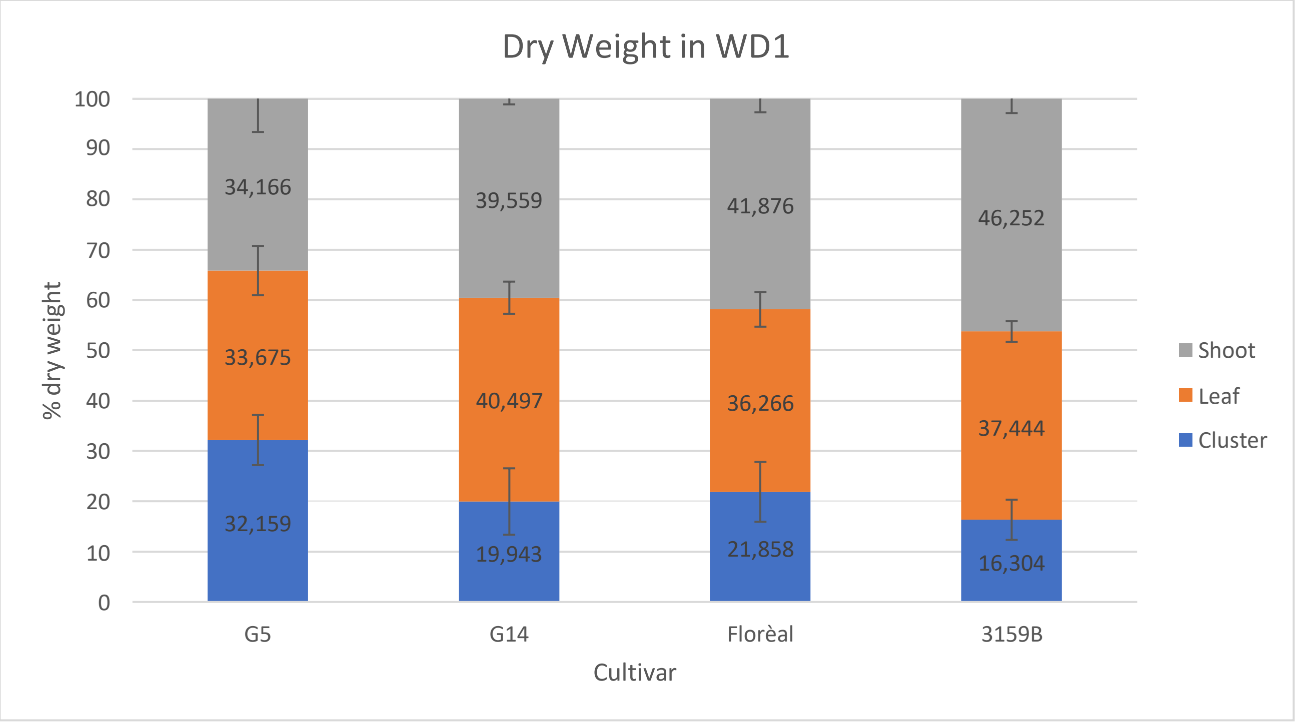


Fig. 13: distribution of dry mass between the different organs (shoots, leaves, bunches) for the different varieties and different treatment, with their respective quantity. a: WD1; b: WD2.

Concerning the cluster, the difference between the treatments showed an atypical dry biomass distribution in 3159B, where the dry biomass content was higher than in the WD1 treatment.

G5 and 3159B are the varieties that produced the most in terms of dry mass, in the WD1 treatment, even if the partitioning of dry mass in the different organs is very different. As regards to G14 and Florèal varieties, despite the lower biomass production, in WD1 and WD2, they showed similar biomass values.



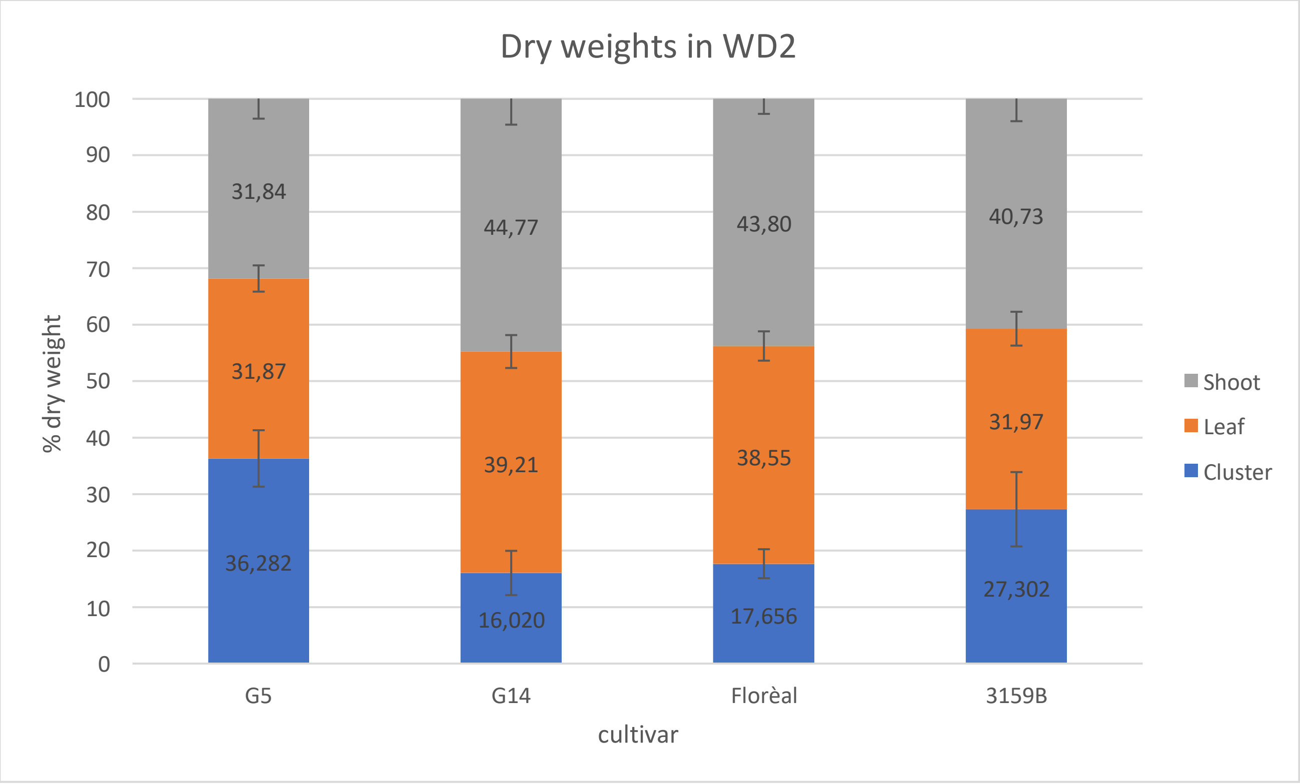


Fig. 14 Percentage distribution of dry matter among the organs: cluster, leaves, shoot in the different cultivars at harvest.

The differences in the distribution of the dry matter between the treatments led on average to increases in the percentage of the vegetative part in WD2, only for the G14 and Florèal varieties, while in the case of 3159B and G5 there was an increase in the concentration of dry matter dry for the cluster. G14 in the WD2 treatment showed a specific increase in the distribution of dry matter towards the leaves, compared to the shoot, while Florèal increased the percentage for both vegetative organs taken into consideration (Figure 14).

4 Discussion

Water stress is one of the main factors mostly influencing grapevine's yield of grapes from both the quantitative and qualitative point of view (Koundouras et al., 2006). The continuous climate change is one of the main factors in the reduction and scarcity of water in the world. Although grapevine is well adapted to arid and semi-arid climates (Chaves et al. 2010), the response to water stress remains very different among the varieties and this response is also different according to the duration of the stress to which this plant is subjected. In general, grape plants subjected to water stress increase their sugar concentration, which translates into musts that are more concentrated in sugar and therefore wines with a higher alcohol content. In this sense, the *VDQA* varieties have been selected for their ability to produce wines with a low alcohol content profile, considered an adaptation to global warming (Salmon et al., 2018). VDQA varieties ripen at lower concentrations of sugar (150-200 g/L) which allows the production of lower alcohol wines (9-11% v/v), one of the major concerns of winemaking in hotter climates (Escudier et al., 2017).

4.1 Differences in photosynthesis and stomatal conductance between treatments

As we know, one of the first responses of the plants to water shortage is stomatal closure, with therefore a decrease in photosynthesis and transpiration rate. From our experiment, this relationship appears partly evident.

We showed that the more intense is the stress, the lower is also the photosynthesis: all varieties recorded lower photosynthesis in WD2 treatment compared to WD1, due to low stomatal conductance values. This behavior was previously reported in other studies, demonstrating that the more severe is the stress, the greater is the stomatal closure and so low rate of photosynthesis (Lebon et al. 2006, Medrano et al. 2002)

In general, the major differences between the treatments emerged mainly in the stomatal conductance, making it a very sensitive parameter helpful in differentiating the treatments.

Although at the photosynthetic level the G5 does not show significant differences as a function of stress, it should be remembered that the gs differs, especially at the end of the season (20 DAV and 30 DAV), when the ripening of the berry is almost complete. However it is possible to notice that both VDQA varieties show a slightly increasing trend in the stomatal conductance, even in WD2 conditions. Based on these results, they seem to behave like an anisohydric plant, where regardless of the stress, the plant keeps the stomata open.

Floreal and 3195B exhibited a similar behavior, except for the last measurement point where Floreal seems to be more stressed, showing important decreases in both g_s and An.

These increases in photosynthesis are most likely due to the fact that the plant has undergone severe stress when entering the greenhouse, which has significantly decreased the values of photosynthesis, conductance and transpiration, which in fact start from minimum values in the measurements. Subsequently, through progressive irrigations, aimed at maintaining stable stress, there was a partial recovery of the photosynthetic activity of these varieties, also in agreement with what was shown by Pou et al. (2012).

In fact, the plants entered the greenhouse to begin the experiment, in a situation in which, before veraison, the irrigation approximated the field capacity. For this reason, the greenhouse could have created a strong, albeit momentary, stress.

On the other hand, G14 follows a trend that we expect, with a decrease in the initial stomatal conductance, which however starts from much higher average values than those recorded by the other varieties on the same day of treatment. This could be due to the possibility that G14, as soon as the stress was imposed, kept photosynthesis and the opening of the stomata constant, but then, depending on the continuous stress, started to close them, trying however not to decrease too much the accumulation of photosynthetates.

4.2 Water Use efficiency

Concerning the water use efficiency, analyzing the differences between the treatments, as expected, the results were in agreement with other studies, which demonstrate that stress increases the WUE, since, as the conductance decreases stomatal, there is an increase in WUE (Tortosa et al., 2019).

The decrease in stomatal conductance leads to a decrease in transpiration and a consequent increase in the An/E ratio.

However, this difference between the treatments becomes clearly visible between the varieties starting from 20 DAV, in fact at 5 DAV. The only variety that differs statistically from the others is the G14, which has statistically valid differences for the values of An and E respectively, with values in the WD2 treatment much lower than in the WD1 treatment.

From 20 DAV the differences begin to be visible as the WD1 treatment begins to differentiate from the WD2, in this sense the restoration of the stomatal activity, previously highlighted, has greater validity, since in the graph the Florèal remains the only variety that does not show significant differences.

4.3 Yield components

Water stress is one of the main parameters that most influences the final yield, in fact, water stress reduce berry size, and this decrease increases with increasing stress (Gambetta et al. 2020).

However, the period in which stress occurs is of paramount importance. In our case the stress was imposed at veraison, i.e. at the stage in which the water transport routes inside the berry change. In this sense, the transport of water, which switches from xylem to phloem, with a strong accumulation and transport of sugar (Greer and Rogiers, 2009; Gambetta et al., 2020)

Assuming that the fresh weight of the berry, at the harvest, and therefore its water content, in a non-stress situation is 85% (Deloire et al. 2009), it can be evident that water stress will decrease the volume and final weight of the berry .

From our results this is not so evident for all genotypes, in fact no obvious statistical differences were found for the treatments. This could be related to the fact that the treatment as a whole was not sufficiently different. Furthermore, the genotypic response, as evidenced by Mirás-Avalos and Intrigliolo (2017), was very different. G5 was the variety that produced the most compared to the others, regardless of the different levels of stress. This is also in relation to the fact that the stomatal, but mostly photosynthetic differences were not statistically different, showing that this variety has good resistance to water stress, if we refer to production.

The other varieties, on the other hand, presented a low yield, where stress could have had a strong influence, mainly on 3159B, which showed very low photosynthesis rates. Water stress actually slows down the plant's growth processes, but further experiments on this variety are needed to determine its response to stress.

On the other hand, the analysis of the Brix degrees showed an increase in the concentration of WD2 treated plants compared to WD1, due to the lower water content, with the exception of G14 that diplayed a decrease. This atypical result could be explained by the fact that G14, in the WD1 treatment, possibly passed the stage of phenological maturity at the time of harvest, showing for this reason a higher sugar concentration. In fact, we noted that both treatments for G14 were harvested in 2 days, while all the other varieties had a phase of detachment of about 7 days each for each treatment.

From the results obtained with the Florèal, we can say that the lack of difference between treatments in terms of yield is in relation to the lack of statistical difference recorded for photosynthesis for most of the duration of the experiment.

4.4 Carbon allocation

In general, when a grapevine is water-stressed, it will allocate more carbon to the shoots in an attempt to maintain their growth and survival. This means that less carbon will be available for the fruit, leading to reduced fruit growth and size. As a result, water stress can lead to a decrease in the total yield of a grapevine (Tombesi et al., 2022).

This is what also occurred in our experiment; in fact the dry weights were lower in WD2 than in WD1 for each organ analysed: cluster, leaves and shoot. However, the exception was found in 3159B, where the average dry weight of the bunch increased in the WD2 treatment.

The hypothesis that could be most validated lies in the fact that, since the stress began at veraison, a phase in which all the berries were already formed, and therefore each plant had a number of bunches and a number of berries that could differ , the increase in dry weight derives from a different quantity of berries that were present in the plants of the 2 treatments, i.e. WD2 most likely showed a higher quantity of berries and perhaps bunches compared to WD1, which is therefore higher.

For all the other organs, however, no differences with the literature emerged, with a decrease in the total quantity of dry matter of each organ analysed.

The distribution of dry matter differs in behavior between treatments and varieties, in fact the results demonstrate that G5 and 3159B increased the distribution of dry matter in the WD2 treatment to the reproductive organs. This is in agreement with what emerges from Miller et al., 1996, after veraison, fruit dry matter increased progressively on vines with more clusters, but slowed down after veraison on vines with less fruit. This explains why Florèal and G14, on the other hand, did not show this result, preferring the vegetative organs. In fact, the distribution of dry matter is greater in the organs that attract more of it. Therefore, at veraison, the bunches can exert a greater recall activity, as they are sink organs, and therefore increase the final dry substance. Since the bunches are bigger, the recall will be higher than with smaller bunches.

5 Conclusions

The purpose of the study was to highlight physiological differences in response to water stress between the different varieties, in four hybrid varieties, two of which belong to the sugarless category, suitable for the production of VDQA , while two other are non-sugarless hybrids.

Water stress has highlighted important physiological responses on all hybrids. In general, the results do not differ from the literature, demonstrating that the resistant varieties respond similarly to the *Vitis vinifera* varieties. However, the genotypic difference played a strong role in determining responses in terms of adaptation, growth and final yield.

The G5 was the variety that performed best, both in physiological terms, in terms of maintenance not significantly different, of photosynthesis and stomatal conductance, but also, perhaps as the most important parameter, in terms of yield, even if it should be emphasized that both treatments applied were both two levels of water stress and therefore there are no references in well water conditions.

The other varieties showed interesting aspects, from the point of view of adaptation to stress, without however having an important difference in yield.

The VDQA varieties, despite the G14 not being very performing, have the qualities to be more involved in the field, also given the possibility of obtaining wines with a lower alcohol content, which make them unique varieties from an oenological point of view. In fact, for both there seems to be a tendency to anisohydric behaviour, which leads them to photosynthesize at a good level even in stronger water stress conditions, which is now happening due to continuous climate change.

Further investigations on the physiological behaviour of these varieties, therefore, are of importance, not only with the aim of enhancing the variety and making them more involved in the production processes, but also as a research tool, given their resistance to powdery mildew and downy mildew.

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