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Fluorescence phenotyping in blueberry breeding for genotype selection under drought conditions, with or without heat stress

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ABSTRACT

Lack of water and increase in ambient temperature, caused by climate change, are already affecting agriculture worldwide. These factors will affect the physiology and development of plants in general, including blueberry plants (Vaccinium spp.). With this in mind, six cultivars of highbush blueberry (*Vaccinium corymbosum* L.) ('Star', 'Bluecrisp', 'Jewel', 'Bluegold', 'Elliott' and 'Liberty') and two rabbiteye cvs. (*Vaccinium ashei* R.) ('Bonita' and 'Powderblue') were subjected to two water treatments: continuous irrigation (Full irrigation–*FI*); and with a water deficit (only one third of the volume of water, water deficit–*WD*). Both treatments were applied in two greenhouses one of which represented ambient conditions (*At*) and the other simulated heat stress conditions ($At + 10\,^{\circ}C$). Measurements were made of chlorophyll fluorescence, stem water potential (Ψs), chlorophyll content, leaf temperature and SPAD. In *At* conditions, cultivars showed differences in most parameters of chlorophyll fluorescence, but only the quantum yield of energy conversion of non-photochemical quenching (Y(NPQ)) and Ψs were significant, along with interactions between cultivars and irrigation treatments. In addition, cultivars differed in the maximum rate of electron transport (ETR_{max}), IK and effective photochemical quantum yield of PSII (Y(II)), indicating differences in the efficiency of photosystem II (PSII). Under $At + 10\,^{\circ}C$ conditions, there were significant interactions in the minimum fluorescence in the dark-adapted state (F_0), ETR_{max}, IK, Y(II), Ψs and photochemical quenching (qP and qL). Thus indicating that when subjected to the two combined stresses ($WD - At + 10\,^{\circ}C$) the cultivars showed different responses in the efficiency and operation of PSII. The results of this study indicate that the fluorescence parameters provide a good tool for phenotyping in blueberry breeding programs and enable the detection and elimination of unwanted genotypes at the beginning of the selection process.

1. Introduction

As a result of predictions related to climate change, there has been a major emphasis in the last decade of blueberry breeding on adaptation to environmental constraints (Hancock et al., 2008). The increase in greenhouse gases has generated increased temperatures and decreased precipitation rates (Hofmann et al., 2011; Stern, 2008), which in Chile has caused reduced flow in watercourses and increased evapotranspiration; factors threatening farming (Vicuña et al., 2011). This scenario is particularly evident in areas of Chile where the highest proportion of blueberries are gown (Allen and Ingram, 2002).

There are several non-invasive and rapid methods for phenotyping physiological variables of plants, which provide information on their performance and adaptability. Among the most widely used are measurements of water potential, gas exchange and chlorophyll fluorescence (FLC) (Baker and Rosenqvist, 2004; Garriga et al., 2014; Lobos et al., 2014). The latter technique has proven to be a powerful tool for estimating the properties and efficiency of the photosynthetic apparatus (Fotosystem II–PSII) (Baker, 2008; Klughammer and Schreiber, 2008) under heat and water stress (Bacelar et al., 2007; Belkhodja et al., 1994; Dias et al., 2011; Flexas and Medrano, 2002; Molina-Bravo et al., 2011; Ogweno et al., 2009; Peck and McDonald, 2010), especially as this is one of the physiological processes that is first affected, long before other symptoms stress are evident (Lawlor and Cornic, 2002).

Despite the above, FLC has not been widely used in plant breeding. However, in the case of blueberries, this technique is especially

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interesting due to the particular characteristics of its roots. Blueberries have a fine, fibrous root system without root hairs (Eck, 1988; Eck and Childers, 1966). Hairless roots are concentrated (almost 80%) in the first 30 cm of soil (Bryla and Strik, 2007), which means that any water deficiency will rapidly impact on the plant which is likely to quickly show signs of hydric stress. The symptoms of such stress appear after a week or more, by which time the damage is irreversible. In turn, thermal stress found in blueberries, necessitates a rapid response to atmospheric demand, puts the plant and its fruit at permanent risk (Chen et al., 2012).

Similar to the light response curve (LRC) analysis using an infrared gas analyzer, the study of LRC with regards to FLC allows the evaluation of the electron transport rate (ETR). This provides information on the properties and efficiency of the PSII in less time than with the LRC, and is thus known as *rapid light curves* (RLC) (Baker, 2008; Beer and Björk, 2000). With RLC is possible to determine three curve parameter: (i) The maximum rate of electron transport (ETR_{max}), reflecting the saturation capacity of a sample; (ii) α (alpha), which is the initial slope of the light saturation curve, related to the maximum yield of PSII electron transport under conditions of limited light; and (iii) IK or light intensity at which alpha and ETR_{max} intersect, the point which is the initiation of saturation of photosynthetically active radiation (PAR) (Klughammer and Schreiber, 2008; Ralph and Gademann, 2005). Along with the aforementioned parameters, the energy used by the photosynthetic apparatus to carry out photosynthesis is called photochemical quenching (qP and qL). qP is related non-linearly to the proportion of PSII reaction centers that are open (Q_A oxidized); while qL assumes that PSII's are interconnected (Baker, 2008; Klughammer and Schreiber, 2008; Ritchie and Runcie, 2013). The energy that is not used for photosynthesis or emitted as fluorescence is released as heat, protecting the photosynthetic system (Lambrev et al., 2012), reflected as variation in qN and NPQ. The Stern–Volmer coefficient (NPQ) reflects the energy dissipated as heat at the level of the energy-harvesting antenna through the xanthophyll cycle, while qN (non-photochemical quenching) is associated with energizing levels of the thylakoid membrane (Perkins et al., 2006; Rosenqvist and van Kooten, 2003).

The maximum photochemical quantum yield of PSII (Fv/Fm), provides information on the ideal performance of the photosynthetic apparatus under conditions in which Q_A is fully oxidized, in the absence of non-photochemical quenching, and takes values of 0.823 ± 0.004 in unstressed plants (Lobos et al., 2012; Rosenqvist and van Kooten, 2003). In the case of effective photochemical quantum yield of PSII (Y(II)), information about the efficiency of PSII in the presence of non-photochemical quenching is provided (Genty et al., 1989). Together, all the parameters described above show the status and efficiency of PSII, whatever the environmental conditions may be.

In blueberries, fluorescence has been used to evaluate the performance of cultivars (cvs.) under various stress conditions, proving to be a good tool for determining the effects of stress conditions on the efficiency of photosynthesis and cultivar response. In the cultivar 'Bluecrop', under water stress conditions, a decrease in the net CO_2 assimilation rate was observed along with increased ETR, indicating processes of photorespiration (Rho et al., 2012). Chen et al. (2012) subjected the cvs. 'Brigitta', 'Misty' and 'Sharpblue' to heat stress conditions and observed that the most susceptible cvs. showed decreases in Fv/Fm and the quantum efficiency of PSII (ΦPS II) and increased F_0 parameters, together with an increase in ROS. Reyes-Díaz et al. (2010) used fluorescence measurements to determine the resistance to aluminum (Al) in 'Legacy' and 'Bluegold', and found that Fv/Fm showed no significant differences between Al treatments, while ETR and ΦPS II drastically decreased in 'Bluegold' (cv. susceptible), whereas in 'Liberty' (cv. resistant) they did not vary. For 'Liberty' NPQ increased, indicating processes for heat dissipation while there was no change in 'Bluegold'.

Blueberry breeding programs need quick and easy tools for assessing phenotypes that allow screening of large numbers and thus make it possible to discard unsuitable types early in the selection process. However, FLC or other slow measurements techniques are unsuitable for implementation in a selection program, thus creating a need to determine related variables and parameters that best reflect changes under water stress, with or without heat stress in blueberries. These subsequently need incorporation into high-throughput field phenotyping for selecting genotypes adapted to these adverse conditions. Targeting the above-mentioned elements, the aim of this work was to study the variation in efficiency of PSII (variables and parameters) for different blueberry cvs. under possible scenarios caused by climate change, in particular water stress under normal ambient condition and elevated temperature.

2. Materials and methods

2.1. Plant material and experimental conditions

The trial was carried out at the University of Talca, Lircay campus (35°24′20″S, 71°38′5″W) and was conducted in two $12 \times 9\,m^2$ greenhouses, both built of galvanized steel and alveolar polycarbonate (6 mm) sheets (86% solar transmission). The first greenhouse (with the sides open and only the roof covered) represented the ambient conditions (ambient temperature–*At*) and the second (closed sides and acclimatized) simulated conditions of heat stress (ambient temperature + 10 °C–*At* + 10 °C). Within each thermal treatment (*At* and *At* + 10 °C) there were two water treatments: (i) continuous irrigation (full irrigation–*FI*); and (ii) a third of the volume of water (water deficit–*WD*).

Experiments involved plants of Highbush blueberries, both Northern (V. corymbosum–cvs. 'Bluegold', 'Liberty' and 'Elliott') and Southern (cvs. 'Jewel', 'Bluecrisp', and 'Star'), as well as Rabbiteye blueberries (V. ashei–cvs. 'Bonita' and 'Powderblue'). All plants, 2–3 years old, were placed in 20 L flower pots with a substrate mixture of sand: peat: sawdust (20:60:20). In both greenhouses, plants were arranged on iron-framed benches at a height of 0.6 m above the ground with 0.4 m between plants, in four rows of plants with an interior pathway to facilitate access for measurements and handling. Plants were ferti-irrigated via drip irrigation, with four drippers (1.1 L/h) per pot.

2.2. Experimental design

Due to the lack of true replication (only one greenhouse per thermal condition), it was treated as two trials: (i) Trial 1 (*At*): treatment 1: *At* – *FI* and treatment 2: *At* – *WD*; and (ii) Trial 2 (*At* + 10 °C): treatment 3: (*At* + 10 °C) – *FI* and treatment 4: (*At* + 10 °C) – *WD*. The trials were a split plot design, with the main plots being irrigation and sub-plots cvs. In each trial there were three replicates of two plants.

2.3. Evaluations

All measurements were made on sunny days from 17/01/2013 to 24/01/2013 between 12:00 and 16:00 h. Fluorescence, SPAD, chlorophyll content and leaf temperature (thermocouple) were measured on the same leaf in the middle third of the plant exposed to light. On a neighboring branch, with similar leaf bud characteristics to the ones noted above, stem water potential was assessed. All these measurements were carried out as follows:

Chlorophyll Fluorescence: Chlorophyll fluorescence was measured with a portable PAM-2500 fluorometer (Walz, Germany)