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Agro-physiological and growth response to reduced water supply of somatic hybrid potato plants (*Solanum tuberosum* L.) cultivated under greenhouse conditions

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A B S T R A C T

Potato seed production from in vitro multiplication processes requires different steps, starting from greenhouse cultivation of plants and production of the first generation of tubers (minitubers). These tubers are then used as seeds in the first field culture conditions, which is followed by a second field culture using the previous tubers as seeds. The insect-proof greenhouse culture of potato plants requires regular watering and an adequate fertilization process. In this context, we focused in this report on the greenhouse culture conditions of two intraspecific somatic hybrids that previously showed improved resistance to Potato Virus Y and to *Pythium aphanidermatum* infection. Three different culture conditions were tested: a seasonal culture submitted to full irrigation (IR100) or to 50% irrigation (IR50) and off-seasonal culture. The effects of the moderate water deficit during the culture period on plant growth and tuber yield were investigated. During the different culture periods, physiological, biochemical and agronomical parameters were followed. The greenhouse experiments were applied on two commercial varieties (Nicola and Spunta) used as control in addition to the two somatic hybrid lines (CN1 and CN2). Hybrid plants exhibited better plant growth and higher yields of tubers than the commercial varieties. Under moderate water supply plants from hybrid lines showed higher tuber quality than commercial lines. Indeed, the highest levels of dry matter, starch, protein and lipid contents were measured in CN1 tubers in the full irrigation regime. Similarly, in the off-seasonal culture, the CN1 and CN2 tubers exhibited the highest dry matter content. The 50% reduction of irrigation rate slightly affected the starch, protein and lipid contents of the different plant lines. However, the CN1 hybrid tuber content in starch and reduced sugars remained higher than the other tubers.

1. Introduction

Potato (*Solanum tuberosum* L.) is a crop of high nutritive value and of considerable economic importance worldwide, being the fourth in crop production, following wheat, maize and rice. The potato's demand is increasing at a greater rate than many other food crops (Mguidich Belhaj et al., 2015). However, due to its high water consumption, potato is far more sensitive to water stress than many other crops (Porter et al., 1999, Wang et al., 2006; Iwama, 2008). Therefore, the irrigation regime is crucial in determining the ability of the plant to produce tubers especially in arid and semi-arid areas. A stable, high quality potato crop requires a uniform and high level of available water (Ierna et al., 2011; Levy et al., 2013).Water shortage reduces vegetative growth of nearly all plants, and most crops can face critical growth periods during which drought may reduce the yield (Samarah and Al-Issa, 2006; Farooq et al., 2008). Insufficient water supply may result in reduced plant growth, yield and crop quality (Al-Yahyai, 2012). Indeed, during the vegetative stage, water deficit greatly decreases the number of leaves, leaf area and stem height (Fernie and Willmitzer, 2001). In the case of the potato plant, during tuber differentiation, interrupted water supply leads to poor plant growth (Jefferies and Mackerron, 1993), whereas during tuber growth, it has a drastic effect on the total plant and marketable yield and tuber quality like shape, frequency of abnormalities, dry matter content, size distribution and chemical composition (Gregory and Simmonds, 1992; Hassanpanah et al., 2008). The frequency of irrigation and the amount of water applied to crops is a common concern of the crop producers. Water stress quite often leads to the generation of reactive oxygen species (ROS) in plant leaves

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(Munné-Bosch and Penuelas, 2003). This overproduction of ROS can induce oxidative stress that impairs the normal function of cells and the photosynthesis process (Dietz and Pfannschmidt, 2011). To alleviate these damages, plants have evolved multiple antioxidant mechanisms to detoxify ROS, including antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT).

Moreover, plants display different mechanisms to withstand drought stress. Their response to water deficit can be very different among cultivars (Hassanpanah, 2010), but also as a function of the period at which stress occurs (Kashyap and Panda, 2003). Therefore, a great attention should be paid to the optimization of irrigation management practices in different pedo-climatic conditions typical of the main growing areas (Fabeiro et al., 2001; Ierna and Mauromicale, 2006; Ierna et al., 2011). Excessive irrigation, on the other hand, may increase nutrient leaching, water-logging problems, incidence of pests and diseases and the associated cost of frequent operation and maintenance of the irrigation system (Al-Yahyai, 2012). The efficient water use for agricultural production is still one of the main challenges worldwide. Indeed, optimizing water application to plants may reduce production costs, increase plant growth, and crop yield. Thus, to improve water use efficiency in agriculture, the irrigation management must be optimized to avoid unnecessary waste of important water resources especially in those countries such as Tunisia, where water is a key limiting factor in the crop production (Saccon, 2017). In these area, the water resource is rare and random, which is an obstacle facing the economic and social development. Therefore, the amount of water provided for irrigation during the growing season must not exceed the effective crop water needs. The water requirement of a crop must be satisfied to achieve potential yields (Saccon, 2017). In Tunisia, potato plants are cultivated all the year divided into four periods. The most important production occurs during the season which starts in January (plantation) and harvesting is performed in May, and the late season which starts in August and harvesting in December.

Plant breeding using somatic hybridization can help to select new potato lines that are able to maintain crop yield under low water availability (Thieme et al., 2010). Somatic hybridization is an alternative method to sexual hybridization that allows the transfer of multigenic characters such as those responsible for the tolerance to abiotic stress (Thieme et al., 2008). In this context, intraspecific somatic hybrids, produced previously by protoplast fusion between Cardinal and Nicola di-haploid lines (CN1 and CN2) were selected and characterized (Nouri-Ellouz et al., 2006).

These hybrids showed improved resistance to *Pythium aphanidermatum* and to PVY (Nouri-Ellouz et al., 2006). Moreover, the improved quality of hybrid tubers in terms of dry matter and starch content encourage us to envisage their registration as new varieties. This step needs an evaluation of plant performance under greenhouse culture conditions used locally for the production of the first generation of seeds. To reach this goal, we investigated here the behavior of these hybrids to greenhouse culture conditions during two periods under cultivation process used by the private seed potato production company. The second objective was the evaluation of were compared to commercial (Spunta and Nicola) varieties, which are the main potato cultivars, used in Tunisia (CTPTA: Technical Center of Potato and Artichoke). The analysis of the performance of the hybrid lines with respect to growth and tuber production under greenhouse culture conditions over two irrigation regimes and two planting dates were examined in terms of some agro-physiological characters.

2. Material and methods

The study was conducted using two commercial potato varieties Spunta (Sp) and Nicola (Ni) and two intraspecific somatic hybrid lines: CN1 and CN2 resulting from protoplast fusion between Cardinal and Nicola (Nouri-Ellouz et al., 2006). The Spunta variety is the most cultivated and consumed in Tunisia (80%) because it is adapted to local climate and soil conditions. Three week-old *in vitro* cultivated plants that had developed roots in liquid MS medium (Murashige and Skoog, 1962) were transferred to a greenhouse and cultivated individually in plastic standard jars (8/8/9 cm) containing 100% Sphagnum blond peat (NPK 14.10.18) supplemented with osmocote (NPK 17.9.12) at a ratio of 1000:25 (w/w) for an adequate N fertilization. Cultures were carried out in a Tunisian agricultural development company MABROUKA according to the process used there. The greenhouse cultures were conducted over two culture periods (seasonal and off-seasonal cultures) and two irrigation regimes: The full water treatment corresponds to water saturation in jar since the plants used come from in vitro culture, while the second one corresponds to 50% reduction of water supply. Irrigation was homogenously applied on the cultivated areas using an automatically system. In the seasonal culture, the plantation started on January 29th and harvesting was performed on May 1st. During this period, 120 plants were sprinkled with 90 ml tap water per jar twice a week (corresponding approximately to 280 m^3/ha per week) to maintain adequate moisture levels. After two weeks of culture, 60 plants underwent a water shortage (reduction of 50% of irrigation water supply: IR50). In the off-seasonal culture, plantation was performed on March 16th and harvested on May 30th. The plants were irrigated twice a week with 100% water (IR100).

Phytosanitary treatments were applied every ten days by spraying diluted fungicides and insecticides. The temperature in the greenhouse ranged from 14 to 25 °C in the first culture period and from 14 to 30 °C for the second period. The reference crop evapotranspiration (ETo) in these conditions were 2.3 mm/day and 5.7 mm/day, respectively.

2.1. Growth monitoring

The growth of plants of different varieties was evaluated every week by measuring stem elongation and determining leaf number. Three plants of each variety cultivated in the different conditions (IR100 and IR50) were used to monitor the physiological parameters after 37 and 72 days of seasonal culture. All measurements were made in triplicate.

2.2. Determination of chlorophyll content

Total chlorophyll was extracted from fresh leaves using acetone (Bouaziz et al., 2012). After centrifugation at 12000 rpm for 15 min at 4 °C, the absorbance of the supernatant was measured at 645 and 663 nm. The chlorophyll a (Ca) and chlorophyll *b* (Cb) contents were determined as follows (Arnon, 1949):

Ca (mg/g FW) = [12.7* A663 − 2.69 *A645]*V/FW

Cb (mg/g FW) = [22.9* A645 − 4.68 *A663]*V/FW

FW: Fresh weight of leavesV: volume of sample

2.3. Determination of malondialdehyde (MDA) content

Lipid peroxidation was determined as thiobarbituric acid (TBA) reactive metabolites mainly malondialdehyde (MDA) content in leaves as described by Hodges et al. (1999). The amount of MDA was measured spectrophotometrically at 600 and 532 nm and calculated using a standard curve of MDA and expressed as nmol/g FW.

2.4. Determination of H_2O_2 content

The H2O2 content was measured according to Loreto and Velikova (2001). Leaves were ground in the presence of 2 ml 0.1% TCA. The extracts were centrifuged at 12,000 rpm for 15 min at 4 °C. For 0.5 ml of supernatant, 0.5 ml of potassium phosphate (10 mM) pH 7 and 1 ml of 1 M KI were added. The absorbance was measured at 390 nm. The H_2O_2 content was calculated from a standard curve and expressed as μmol/g FW.