EFFECT OF USING DIFFERENT FORMULATIONS OF FERTILIZERS ON STOMATAL CONDUCTANCE, LEAF CHLOROPHYLL FLUORESCENCE, GROWTH AND YIELD OF TABLE GRAPES CROPS GROWN IN THE NORTHEAST OF MOROCCO
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ABSTRACT

The aim of this research study was to investigate the impact of using fertilizers based on organic matter and amino acid on leaf stomatal conductance, chlorophyll a fluorescence, leaf relative content and stress index and growth and yield of the table grapes. Experiments were conducted on a commercial production scale of 35 ha located in the region of "El GARET", northeast of Morocco. Variety of Regal was used with plant density of 2000 plants/ha. Three commercial liquid fertilizer formulations were used: Fertilizer-1 (Total Nitrogen: 6,77%, Total Organic Nitrogen: 6,41%, Organic Carbon: 27,6%, Total Amino Acid: 36,01%, Free Amino Acid: 7,06%, Organic Matter: 47,5%), Fertilizer-2 (Total Nitrogen: 4%, Ureic Nitrogen: 4%, Oxide of potassium: 15%, Organic Matter: 30%, Boron: 0,5% and Molybdenum: 0,2%) and Fertilizer-3 (Total Nitrogen: 3,42%, Organic Nitrogen, 3,38%, Calcium: 0,20%, Iron: 78ppm). Three treatments were applied in this experiment: T1 (control), T2 (Fertilizer-1 and Fertilizer-2) and T3 (Fertilizers-3). An amount of 3L, 4L and 4L of irrigation water was injected above the root system and under the dripper for each tree. A conventional fertilization program was used by the grower in T1, T2 and T3 plots; except for the prototype T2 and T3 treatments plots where the fertilizers 1, 2 and 3 were applied. Fertilizers were applied 4 times and every 15-20 days. Results showed a significant increase in plant yield and stem growth for T2. Stomatal conductance was decreased for T2. The relative water content (RWC) was significantly increased with T3 and no differences between treatments were recorded for the stress index; F_v/F_m, DI_0/RC and ET_0/RC.

INTRODUCTION

Table grapes (*Vitis vinefera*) present an important crop production in Morocco. The surface area of the plantations is 49 000 ha, where 38 000 ha is for table grapes and 11 000 ha for wine production (Agrimaroc, 2021). The main varieties produced are: Doukkala, Muscat of Italy, Valency, Abbou, Boukhanzir and Muscat of Alexandria, which present about 77% of the total area of table grapes production. The practice of tree fertilization is based on nitrogen, phosphorus, potassium, calcium, magnesium, and micronutrients. Previous research demonstrated that using mixed fertilizers with microelements, organic matter, and amino acid 4-5 times during the phonological stage improved growth and yield of different crops (Martinez et al, 2018). Organic matter enhances plant growth and development, and it plays a vital role for improving photosynthesis (Ye et al., 2022). Amino acid is considered also as an element that increases production and improves plant stress status; It involves respiration; assimilation of chlorophyll transport and storage of carbohydrates (Popko et al., 2018).

Chlorophyll a fluorescence measurement presents a suitable way to evaluate photosynthesis efficiency and stress index of the crops (Schreiber, 2004). This method can provide data on the ability of plants to respond and tolerate environmental stresses (Maxwell

and Johnson, 2000). The relationship between chlorophyll a fluorescence and nutrient status was evaluated in several studies on different species (Strand and Lundmark, 1995). Chlorophyll fluorescence usually indicates the transfer of electrons during the light phase of photosynthesis from the excitation of chlorophyll by light energy to the transfer of electrons for the dark phase (Tsimilli-Michael and Strasser, 2001). The ratio F_v/F_m varies between 0.75 and 0.85 in non-stressed plants and it is a good indicator for stress level status. Moreover, the parameter ET_0 indicates the number of electrons transferred for the dark reaction of photosynthesis to fix the CO_2 during the Calvin cycle (Cahize et al., 2018). The more plants are stressed, the electrons chain is getting interrupted, and the number of electrons transferred to the Calvin cycle decrease (Murata et al., 2007). The DI_0 is also a good indicator of plant stress and indicates the dissipation of energy as heat; the more plants are stressed the more the value of DI_0 increases (Waraiach et al., 2012).

The objective of this work is to evaluate the effect of different formulations of mixed fertilizer based on organic matter and amino acid on leaf stomatal conductance, chlorophyll a fluorescence and stress index, leaves relative content and plant growth and yield of table grapes grown under Mediterranean climate conditions of the northeast of Morocco.

MATERIALS AND METHODS

Experimental site

This study was carried out in the region of "El GARET" (34°59'51.0"N 3°03'50.9"W), northeast of Morocco, on a production of 8-year-old table grapes (Fig. 1a). This region is characterized by Mediterranean climate, with an average precipitation rate of 250-300 mm/ year. The planting density was 2000 plants/ha. Each row was spaced at 3m and the distance between two plants of the same row was 1,5 m. Variety of "Regal" was used during this experiment. A dripper of 4 L/h was used for irrigation. The crops have been managed according to the good practices of the commercial production of table grapes in Morocco.

Treatments

Three commercial liquid fertilizer formulations were used: Fertilizer-1 (Total Nitrogen: 6,77%, Total Organic Nitrogen: 6,41%, Organic Carbon: 27,6%, Total Amino Acid: 36,01%, Free Amino Acid: 7,06%, Organic Matter: 47,5%), Fertilizer-2 (Total Nitrogen: 4%, Ureic Nitrogen: 4%, Oxide of potassium: 15%, Organic Matter: 30%, Boron: 0,5% and Molybdenum: 0,2%) and Fertilizer-3 (Total Nitrogen: 3,42%, Organic Nitrogen, 3,38%, Calcium: 0,20%, Iron: 78ppm and). Three treatments were applied in this experiment: T1 (control), T2 (Fertilizer-1 and Fertilizer-2) and T3 (Fertilizers-3). A conventional fertilization program was used by the grower in T1, T2 and T3 plots; except for the prototype T2 and T3 treatments plots where the fertilizers 1, 2 and 3 were applied. For T2, the Fertilizer-1 was applied on April 11th, May 11th, June 9th and July 7th, 2022, and the Fertilizer-2 was applied on April 29th, May 25th, June 23rd, and July 21st, 2022, respectively. For T3, the Fertilizer-3 was applied only, on April 11th, May 11th, June 9th, and July 7th, respectively. According to the commercial recommendation of three products, an amount of 3L, 4L and 4L of irrigation water was injected above the root system and under the dripper for each tree with 1ml (or 2 L/ha), 5ml (10 L/ha) and 5ml (10 L/ha) of the concentrate solution for fertilizers 1, 2 and 3, respectively. Twenty-four trees were selected for each treatment to quantify the physiological and productivity measurements.

Measurements

Plant growth: parameters were measured every 2 weeks from April until July. Measurements were recorded on 12 plants for each treatment. Plant height and number of sticks

were recorded. One stick was selected for each tree to measure it length, number of nodes. Then, the fifth shoot from the apex of the stick was selected to measures its length.

Relative water content (RWC): twelve samples were taken twice (July 17th), early in the morning, from each treatment. Top-most fully expanded leaves were sampled; the fifth leaf from the apex of the fifth selected shoot of the selected stick. Fresh samples were weighted (FW) and then were immediately hydrated in distilled water to full turgidity and then moved to the laboratory for 48 h under normal room light and temperature conditions. After hydration, samples were weighted (TW) and then moved to steaming room at 80°C for 48h and weighed to determine dry weight (DW). The formulate of the calculation of RWC is presented as following (Equation 1):

$$RWC\ (\%) = [(FW-DW) / (TW-DW)] \times 100 \quad \text{(Equation 1)}$$

Stomatal conductance (gs) was measured by & Porometer (SC-1 Porometer, Meter Group Inc., USA) and chlorophyll a fluorescence with Handey PEA (Hansatech, UK). Measurements were recorded on a hot and sunny day, (July 13th) from 11:00 until 13:00. Measurements were taken on the fifth leaf from the apex of the fifth selected shoot of the selected stick. For the stress fluorescence measurements, leaves were adapted to the dark for 30 min using a clip. Then, a light flash of 3000 µmol/m2/s (650 nm) was applied for 1 s (gain = x1) on the leaf adapted to darkness. The measurements were taken on 6 plants for each treatment. Parameters of F_v/F_m, DI_0/RC, ET_0/RC were measured, and each has a specific physiological indication (F_v: variable fluorescence; F_m: maximum fluorescence; DI_0: dissipated heat; RC: reaction center, ET_0: number of electrons transferred for the dark reaction of photosynthesis to fix the CO_2 on the Calvin cycle):

Fruit yield: at the end of the experiment; October 23rd, number of clusters were measured on 12 plants for each treatment. The cluster weight was recorded on 8 plants for each treatment.

Data analysis

Statistical analysis was performed using IBM-SPSS (version 21). For each evaluated parameter, replicates were taken for treatments. Means with standard deviations were used to determine the differences. The mean values obtained were compared by analysis of variance (ANOVA). The test of Duncan was used, and the significance level was $P < 0.05$.

RESULTS AND DISCUSSION

No significant difference was recorded for plants growth parameters between treatments (Table 1). Those parameters were: principal stem height, secondary stem number and the number of nodes taken at the level of the selected stem. However, the length of the third stem taken at the level of the sixth node was significantly reduced for T3 compared to the control and to T3. Regarding the values of the relative water content recorded on July 17th (Table 2), a significant decrease in RWC was recorded for T3 compared to T1 and T2. This means that water content in the leaves was increased (Soltys-Kalina et al., 2016) by the fertilizer 3 treatment.

Moreover, the stomatal conductance was significantly decreased for T2 compared to the control and to T3. Sheng et al., 2020 reported that amino acid and organic matter increased the stomatal conductance and in this case the opposite was occurred. Regarding the plants stress parameters, the ratios F_v/F_m, DI_0/RC, ET_0/RC didn't change with treatments during sunny and hot conditions day conditions (July 17th, at mid-day). In this case the ratio ET_0/RC which indicates that the number of electrons transferred from the light reaction from thylakoid membrane to stroma for the dark reaction and to fix the CO_2 was the same for all treatments.

Also, the parameter DI_0/RC shows that plants in all the treatments dissipated the same heat energy which indicate that and theirs stress status were the same. Contrary, previous work (Badiane et al., 2012; Gunes et al., 1994) showed that plant stress was reduced by the application of amino acid and organic fertilizer.

Regarding plant yield, the fruit weight was significantly increased for T2 compared to the control treatments (data not shown). This increase in T2 were supported by Mostafa et al., 2008 and Nikiforova et al. 2006 who showed that organic matter and amino acid increased plants yield.

Table 1. Parameters of plant growth measured on table grapes grown under the control (T1) and prototype treatments (T2 & T3) (recorded on July 7th, 2022).

Treatments/Parameters	Control (T1)	T2	T3
Principal stem height (cm)	1,27 ±0,07 a	1,33 ±0,09 a	1,30 ±0,08 a
Secondary stem number	6 ±1 a	6 ±1 a	7 ±1,7 a
Length of 3rd stem (at 6th node) (cm)	55,8 ±18 b	86,3 ±16 a*	60,1 ±16 b
Number of nodes/ selected stem	12 ±2,4 a	12 ±3,8 a	12 ±2,1 a

T1= without fertilizers 1, 2 & 3; T2= with fertilizers 1 & 2; T3= with fertilizer 3.
Data are mean of 12 repetitions ±standard deviation.
*Significant data; the Duncan test was used for comparisons between averages, with α=0,05.

Table 2. Leaf relative water content (RWC) of table grapes plants grown under the control (T1) and prototype treatments (T2 & T3) (measured on July 17th, 2022).

Treatments/Parameter	Control (T1)	T2	T3
RWC (%)	74 ±9 b	74 ±7 b*	84 ±4 a

T1= without fertilizers 1, 2 & 3; T2= with fertilizers 1 & 2; T3= with fertilizer 3.
Data are mean of 12 repetitions ±standard deviation.
*Significant data; the Duncan test was used for comparisons between averages, with α=0,05.
The 5th leaf from the apex of the 5th selected shoot of the selected stick was used as a sample.

Table 3. Stomatal conductance (g_s) and the stress index F_v/Fm, DI_0/RC, ET_0/RC, measured on leaves of the table grapes during a hot and sunny day (July 13th, 2022) for the control (T1) and prototype treatments (T2 & T3) (11:00-13:00).

Treatments/Parameters	Control (T1)	T2	T3
g_s (mmol/m^2/s)	316 ±71 a	226 ±58 b*	280 ±100 a
F_v/F_m	0,79 ±0,04 a	0,79 ±0,03 a	0,80 ±0,03 a
DI_0/RC	0,43 ±0,08 a	0,49 ±0,07 a	0,38 ±0,08 a
ET_0/RC	0,73 ±0,03 a	0,73 ±0,08 a	0,73 ±0,09 a

T1= without fertilizers 1, 2 & 3; T2= with fertilizers 1 & 2; T3= with fertilizer 3.
F_v= variable fluorescence; F_m= maximum fluorescence; DI_0= dissipated heat= RC= reaction center; ET_0= number of electrons transferred for the dark reaction of photosynthesis to fix the CO_2 on the Calvin cycle.
Data are mean of 6 repetitions ±standard deviation.
*Significant data; the Duncan test was used for comparisons between averages, with α=0,05.
The 5th leaf from the apex of the 5th selected shoot of the selected stick was used as a sample.

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