Journal of King Saud University – Science 31 (2019) 336–344

Contents lists available at ScienceDirect

Journal of King Saud University – Science

journal homepage: www.sciencedirect.com

Original article

Sewage sludge fertilization alleviates drought stress and improves physiological adaptation and yield performances in Durum Wheat (*Triticum durum*): A double-edged sword

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ARTICLE INFO

Article history:
Received 8 June 2017
Accepted 19 December 2017
Available online 20 December 2017

Keywords:
Sewage sludge application
Introduced variety
Drought stress mitigation
Wheat plant growth
Yield components

ABSTRACT

Sewage sludge-based fertilization could be the most effective way to mitigate the negative effects of water stress on wheat yield in arid and semi-arid regions. The study examines morpho-physiological and yield performances of the waha variety of durum wheat (an introduced variety in North Africa) at different levels of sewage sludge-based fertilizer combined with drought stress gradient. The experiment was conducted under greenhouse in plastic pots divided into four water stress levels (100%, 80%, 50%, 30% of field capacity). Each level contained five fertilization treatments of the soil (each one replicated four times): (i) control soil (without fertilization), (ii) urea, (iii) 56.67 g DM of sewage sludge (SS)/pot, (iv) 141.67 g DM of SS/pot, (v) 283.33 g DM of SS/pot. Two-way ANOVA and Tukey tests were applied to estimate variations of morphophysiological and grain yield parameters (number of tillers, height, membrane integrity, nitrogen content, soluble sugars, grain weight, number of grain per spike). Drought stress adversely affected plant growth in height and number of tillers and therefore yield components. The application of SS mitigated the effect of water stress by improving all study growth and yield parameters. This improvement mainly resulted from the effect of SS that allowed a good root growth of plants to explore deep the soil and absorb more water, thus avoiding the effects of water deficit. Sewage sludge-based amendment is revealed as a promising solution that increases crop yields under severe environmental conditions. This biosolid alleviates stress effect, allowing the plant to survive and cope with drought conditions.

1. Introduction

The cultivation of durum wheat (*Triticum durum*) is very ancient in the semi-arid Mediterranean areas where it is based on two germplasms: (i) traditional local genotypes, characterized by low production potential and (ii) introduced genotypes that unlike local varieties are represented by a high production potential. However, the introduced varieties do not last long before disappearing due to their unstable spatial and temporal yield performances (Hazmoune, 2008). This rapid disappearance results from poor adaptation and high sensitivity to climate variability that characterizes production areas, especially in semi-arid regions where the climate is severe with frequent intense and long droughts (Bouthiba et al., 2008; Bezzalla et al., 2018; Zhang et al., 2017) with unfavorable soil conditions, soil salinity in particular (Oustani et al., 2015; Chenchouni, 2017).

As stated in the above paragraphs, it is important to sustainably exploit the introduced varieties that represent additionally to local varieties a genetic enrichment for the country's agricultural potentialities (Hazmoune, 2008). One of the processes that consistently consider these varieties is the use yield-improving techniques such as biofertilization (Antolin et al., 2005; Tammeorg et al., 2014; Debiase et al., 2016). The idea of using sewage sludge as a fertilizer has achieved promoting results in agriculture because of the good effectiveness of these biosolids compared to chemical fertilizers

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.jksus.2017.12.012
1018-3647/

(Pascual et al., 2007; Boudjabi et al., 2015). The application of sewage sludge into the soil appears to be one of the most environmentally and cost-effective options (Mingorance et al., 2014). Indeed, the agricultural application of these residues is of great agronomic value for the soil because they contain high content of organic matter that improves soil physicochemical properties such as moisture and water holding capacity (Boudjabi et al., 2015; Debiase et al., 2016). Also, it is noteworthy that chemical fertilizers are expensive, but the use of sewage sludge is very interesting and a good way of substitution towards environmentally friendly agriculture.

The implementation of new wastewater treatment plants daily creates large amounts of sewage sludge and biosolids. With the increase of the activity of these plants and quantity of the waste materials produced, management of these residues represents a real environmental problem. Because landfilling of sewage sludge, which is one of the most commonly adopted techniques, certainly helps to get rid of these biomaterials; however, this disposal route has a disadvantage for the environment since the release of leachate or heavy metals is a serious problem that can cause contamination of groundwater and thus pose an inescapable environmental issue (Latare et al., 2014; Alvarenga et al., 2016). Fortunately, the orientation of these residues to the agricultural recovery is a management strategy, as part of sustainable development. It allows first to largely solve the landfill problem that may constitute in the near future a serious environmental problem, in addition to improving crop yields without the use of chemical fertilizers, which are too expensive unlike the sludge that is free (Boudjabi et al., 2010; Mosquera-Losada et al., 2014).

However, the controversy that was established around the agricultural application of sewage sludge is the quality and safeness of food products, because in some cases the problem of heavy load accumulation of salts, heavy metals and micropollutants in the soil and crops arises (Nkoa, 2014, Alvarenga et al., 2015). These accumulations are related to a poor spreading management that is directly related to the sludge stabilization state, its chemical composition, or the application mode of this biosolid. Besides, the effects of sewage sludge application vary following several parameters such as climate, type of soil being fertilized, applied doses etc.). when this contamination occurs, it can generate long-term imbalance in the functioning of soil-plant interactions; and may subsequently impose a potential risk of contamination of the human food chain via the resulting crops (Nkoa, 2014). Nevertheless, with the use of adequate pretreatments and monitoring of pollutants and pathogens in sewage sludge, to meet safety regulations, the applications of sewage sludge as fertilizer are of benefit (Alvarenga et al., 2015; Boudjabi et al., 2015).

It is with this context that falls the present work, which aims to study the behavior of an introduced and drought-sensitive variety of durum wheat 'Waha' to the application of sewage sludge combined with different drought stresses. We test in this study if the morphophysiological parameters and yield performance of this variety will not be affected by water stress when fertilizing amendments by sewage sludge are applied. The sewage sludge-based fertilization is expected have a synergistic effect that tend to mitigate drought stress by triggering high accumulation of osmoregulators that allow the plant to cope with water stress especially when the sludge is applied with high doses (Oustani et al., 2015). We expect that morphophysiological parameters and yield will decrease with the increase of water stress (Zhang et al., 2014) and we hypothesize that the application of sewage sludge allows a positive improvement of all studied growth variables, and that this improvement will be correlated with increasing doses of sewage sludge applied (Debiase et al., 2016).

2. Materials and methods

2.1. Experiment design

Physiological and morphological performances of durum wheat variety waha to the application of sewage sludge under different water stress levels were studied under greenhouse conditions. The experiment took place during a complete growing cycle of durum wheat, from January to June 2010 at the University of Tebessa, northeastern Algeria. The study variety "waha" was introduced in Algeria in 1987, it is a short, early variety, and resistant to common fungal diseases (Bouthiba et al., 2008).

Twenty plastic pots containing 5 kg of soil taken from the region were allocated to four repetitions of the following five levels of fertilization treatments: (i) control soil without fertilization {Control}, (ii) soil treated with 0.15 g of urea per pot (equivalent to 35 kg N/ha) {Urea}, (iii) soil fertilized with 56.67 g DM of sewage sludge per pot (equivalent to 20 t/ha) {dose SS1}, (iv) fertilization with 141.67 g DM of sewage sludge per pot (=50 t/ha) {dose SS2}, and (v) fertilization with 283.33 g DM of SS per pot (=100 t/ha) {dose SS3}. Treatments of fertilization were replicated and criss-crossed with four intensities of water stresses expressed as levels of field capacity (FC): FC = 100%, FC = 80%, FC = 50% and FC = 30%. Field capacity is the amount of soil moisture held in the soil after excess water has drained away and the rate of downward movement has decreased.

Once the pots were installed in the greenhouse, ten seeds provided from the Algerian Interprofessional Office of Cereals (OAIC) of Tebessa were sown at a depth of 5 cm. After that, irrigation was weekly monitored by following soil moisture.

2.2. Characteristics of experimental soil and sewage sludge

Before conducting the experiment, physicochemical parameters of both soil and sewage sludge were determined using conventional analysis methods (Baize, 2000; Pansu and Gautheyrou, 2006; Rodier et al., 2016). Soil samples were collected from the Faculty of Exact Sciences and Natural and Life Sciences (University of Tebessa, Tebessa, Algeria). Whereas the sewage sludge used in the experiment is an urban activated sludge provided from the wastewater treatment plant of Ain Sfiha in Setif (Algeria). All samples were crushed, dried and sieved, and after that analyzed for the determination of physicochemical characteristics and heavy metal content.

Organic carbon was determined by titrimetry (Baize, 2000). For the determination of assimilable phosphorus, a filtrate of sewage sludge was treated with ammonium molybdate and tin chloride ($SnCl_2$), then the phosphorus content was deduced using UV–VIS 1250 spectroscopy from a curve of calibration (Baize, 2000). Electrical conductivity was established on the sludge filtrate (1/5 w/v) with a conductivity-meter (model WTW/LF 330), whereas pH was deduced from the electrometric reading of the sludge filtrate (2/5, w/v) using a pH-meter type V503 10 (Pansu and Gautheyrou, 2006). Nitrate ions were obtained according to Rodier et al. (2016). A sludge filtrate was evaporated to dryness in the presence of sodium salicylate. The residue obtained was taken up with a tartrate solution of sodium and potassium. Then the nitrate content was measured using UV spectroscopy. Heavy metal concentrations were determined using a Perkin Elmer AA2000 atomic absorption spectrophotometer. The results of the Physicochemical analyzes mentioned above are given in Table 1.

2.3. Measurement of height and number of tillers

The measurement of the height and number of tillers was carried out during the heading stage that coincides with April. The

Table 1
Physicochemical characteristics of soil and sewage sludge used in experiments.

Physicochemical parameters	Soil	Sewage sludge
Organic carbon [%]	1.28	28.7
Organic matter [%]	2.2	49.36
Total nitrogen [%]	0.38	7.98
Total phosphorus [%]	–	5.7
Assimilable phosphorus [μg/g]	2.64	17.44
Total $CaCO_3$ [%]	12.96	–
Active $CaCO_3$ [%]	3.12	–
Electrical conductivity [dS/cm]	0.223	1.38
pH	7.23	7.88
Nitrate [mg/kg]	13.3	42.38
Iron [ppm]	4.9	6.6
Zinc [ppm]	1.36	21.18
Copper [ppm]	7.14	12.54
Manganese [ppm]	26.6	26.6
Lead [ppm]	0.12	2.8
Cadmium [ppm]	0	0
Soil texture	Silt-clay	–

average height of all existing plants in each pot was quantified using a measuring tape. Height measurements were taken from the plant base to the ear tip. Over all the plants grown in each pot, the tallest plant was chosen to calculate the number of tillers per pot.

2.4. Estimation of physiological variables

The estimate of nitrogen content in plants and membrane cell integrity was performed at the heading stage, where a sample was taken from three different plants per pot for each treatment level and for each repetition. On each plant, all the flag leaves were used to analyze cell integrity and content of total sugars. The rest of leaves of the same plants were dried then used to estimate the nitrogen content.

Nitrogen was determined by Kjeldahl method (Sáez-Plaza et al., 2013). The organic nitrogen contained within each sample was quantitatively converted to ammonium sulphate $(NH_4)2SO_4$ by mineralization of 1 g of dry plant material using concentrated sulfuric acid, heated to boiling and in the presence of a catalyst ($CuSO_4 + K_2SO_4$). Ammonia was then moved from its salt with sodium hydroxide, and then driven by the water vapor in a sulfuric acid solution of known volume. The residual acidity was assayed using a standard solution of sodium hydroxide. The endpoint of the reaction was assessed by colorimetry. The amount of nitrogen present in the sample was deduced using the following formula: $N = n \times 0.0014 \times 100/w \times 100/(100\text{-}h)$, where: n = the volume of sodium hydroxide used to neutralize the solution ammonia; w = sample weight (g), h = water content of sample.

Soluble sugars were assayed using the method of Dubois et al. (1956). Extraction of sugars was carried out on 100 mg of plant material placed in test tubes containing 3 ml of ethanol (80%). The tubes were left at room temperature for 48 h, then they were placed in water bath at 80 °C to evaporate the alcohol. In each tube, 20 ml of distilled water was added, then 1 ml of this solution was mixed in new test tubes with 1 ml of phenol (5%) and 5 ml of sulfuric acid. After cooling, a reading of the optical density of samples was made at a wavelength of 496 nm. The content of sugars was derived from the calibration curve ($y = 0.323 + 0.0029x$; R^2 = 0.926).

The membrane integrity was assessed by a conductivity meter following the method described by Ben Salem and Vieira Da Silva (1991), which consists of measuring the electrolytes released following the partial destruction of cytoplasmic membranes. For each treatment level and replication, samples of five leaf disks of 5 mm in diameter were washed with distilled water and then soaked in 50 ml of water for 4 h at 25 °C. During this soaking, cell electrolytes pass into water, it is the free conductivity (*EC*). Thereafter, leaf discs were autoclaved for 20 min at 121 °C to destroy the leaf tissues. The solution was cooled down to 25 °C, then the second measurement with the conductivity meter corresponded to the total conductivity (*TC*). Thus, the percentage of cell membrane integrity (CMI) was determined by the formula: $CMI = (1 - EC)/TC \times 100$.

2.5. Estimation of root weight and yield components

After six months of culture, all the remaining plants were removed at the maturity stage to calculate the number of grain per spike and the average grain weight. To do this, we separated all the spikes obtained in each pot from the aerial part of plants. The number of grains per spike was averaged to the number of collected spikes. The grains obtained in each pot were weighed and then the average grain weight was obtained by diving the total weight by the number of grains. The roots were obtained by carefully emptying the pots from the soil. Roots were cleaned in bowls full of water. Once cleaned of the soil, roots were weighed with a precision balance to have the root weight per plant.

2.6. Statistical analysis

The data obtained from the experiment for each calculated parameter were reported as the mean with standard deviation (SD) for levels of drought stress and fertilizer treatments. The full dataset is accessible at: https://doi.org/10.6084/m9.figshare.4876832. Two-way ANOVA were applied to test the effects of the two factors 'water stress' and 'fertilizer treatment' and their interaction 'stress × treatment' on the variation of morpho-physiological parameters and yield components. When ANOVA was significant (*i.e.* $P < .05$), Tukey post hoc test was conducted to classify levels of the factor in question that having a significant effect. Statistical analyzes were performed using the R software (R Core Team, 2016).

3. Results

3.1. Number of tillers

Overall, plants subjected to 100% of field capacity (FC = 100%) exhibited the highest number of tillers (3.6 ± 0.6 tillers/plant), but this parameter decreased gradually as water stress increased (Fig. 1). The ANOVA showed that the number of tillers varied significantly between water stress levels ($F_{(3,60)} = 15.40$, $P < .001$). Tukey test classified stress levels following the descending order: FC = 100% > FC = 80%-FC = 50% > FC = 30% (Table 2). The interaction between the factor 'water stress' and 'fertilization' was not significant ($F_{(12,60)} = 0.55$, $P = 0.876$). Sewage sludge amendment has clearly induced a significant improvement of the number of tillers ($F_{(4,60)} = 20.93$, $P < .001$), especially with the two doses SS3 and SS2, which engendered the growth of 3.93 ± 1.00 and 3.62 ± 0.92 tillers/plant, respectively. Mineral fertilizer (2.06 ± 0.42 tillers/plant) and the dose SS1 (2.68 ± 0.74 tillers/plant) showed comparable values (Fig. 1). These two treatment levels represented the same group according to Tukey's test that classified fertilization treatments into three groups according to the following descending order: S S3,SS2 > SS1 ≥ urea ≥ control (Table 2).

3.2. Height (length of tiller)

The variation of tiller height was significant according to the effect of drought stress ($F_{(3,60)} = 26.44$, $P < .001$) and fertilization treatments ($F_{(4,60)} = 18.66$, $P < .001$). However, the effect of the

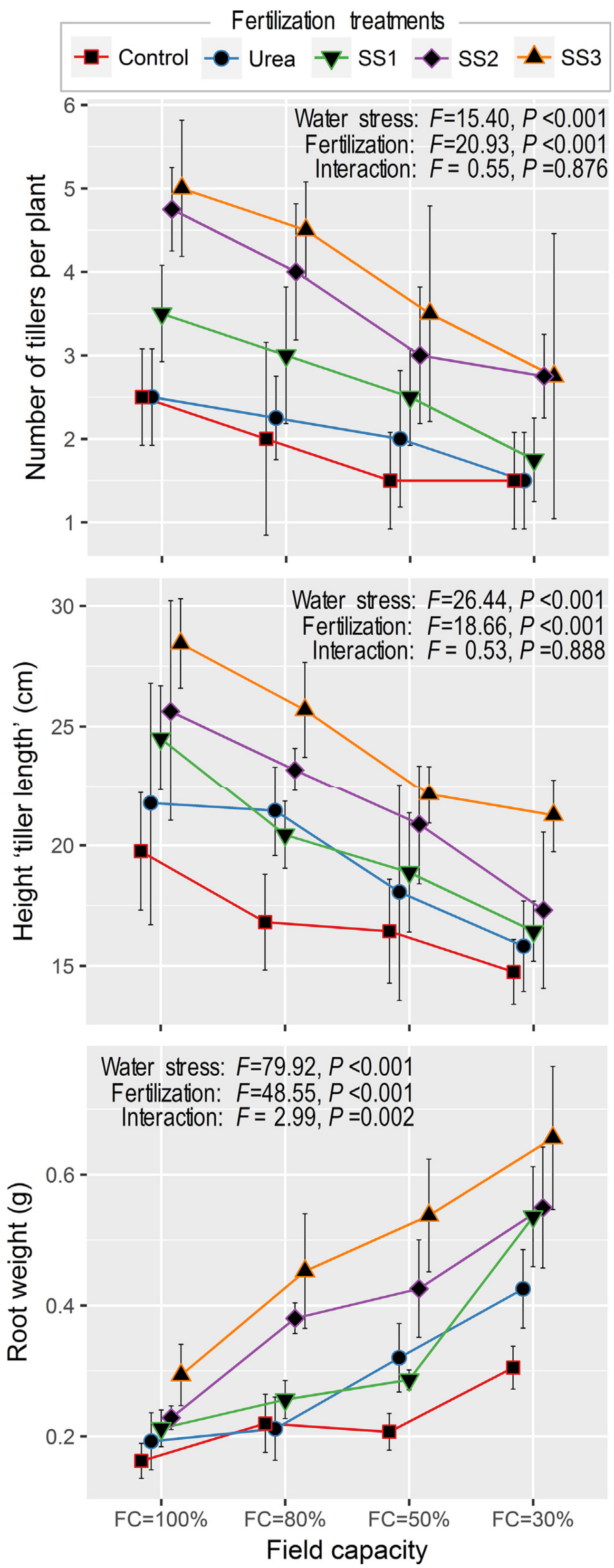

Fig. 1. Effect of different fertilization treatments including sewage sludge (SS) on morphological growth variables (number of tillers, height and root weight) of Durum Wheat (*Triticum durum* var. waha) grown under different water stress (field capacity). Vertical bars associated with means (symbols) are standard deviations (±SD). Test statistics (*F* and *P*-values are two-way ANOVA testing the effects of water stress, fertilization and their interaction. (Control: no fertilization, urea: 35 kg N/ha, SS1: 20 t/ha of SS, SS2: 50 t/ha of SS, SS3: 100 t/ha of SS).

interaction 'stress × fertilization' was not significant ($F_{(12,60)}$ = 0.53, P = 0.888). According to the Tukey test, water stress identified four groups of tiller heights that decreased according to the increase of drought level. The first was the level FC = 100% averaging 24.0 ± 3.0 cm, followed by FC = 80% with a height of 21.5 ± 1.6 cm, then FC = 50% with 19.3 ± 2.6 cm and finally FC = 30% with 17.1 ± 1.8 cm (Fig. 1, Table 2). Whatever the level of water stress, the application of sewage sludge improved the growth of plants in height in contrast to mineral fertilizer (19.26 ± 2.84 cm) which showed no significant difference compared to control plants (16.93 ± 2.07 cm). Tukey's test indicated that the improvement in plant height was very important for the fertilizing dose SS3 (24.37 ± 3.31 cm) which was significantly higher that to the dose SS2 (21.75 ± 3.53 cm) and SS1 (20.06 ± 3.38 cm) (Fig. 1, Table 2).

3.3. Weight of roots

The trend if root weight changes significantly increased with the increase of the severity of water stress. The ANOVA showed a significant effect of water stress ($F_{(3,60)}$ = 79.91, P < .001) and Tukey test indicated that the highest root weight was allocated to water level FC = 30% with 0,49 ± 0.40 g/plant, followed by FC = 50% with 0.36 ± 0.05 g/plant and FC = 80% with 0.30 ± 0.05 g/plant. The control level (FC = 100%) was ranked last with 0.22 ± 0.03 g/plant (Fig. 1, Table 2). Furthermore, the effect of fertilization on the variation of the root weights was significant ($F_{(4,60)}$ = 48.54, P < .001), and it is the same for the interaction of two factors of the study ' Stress × fertilization' ($F_{(12,60)}$ = 2,92, P = .002). The application of sewage sludge induced an improvement of root weight compared to control plants and those treated with urea. Tukey's test grouped root production depending on fertilization treatments according to the following decreasing profile: SS3 > SS2 > SS1 > urea, control (Table 2). Indeed, plant fertilized with the dose SS3 produced roots heavier (0.48 ± 0.15 g/plant) than those treated with SS2 (0.40 ± 0.13 g/plant) and SS1 (0.32 ± 0.15 g/plant). Mineral fertilizer slightly increased root weight (0.29 ± 0.11 g/plant), but which is not statistically different from the control plants (0.22 ± 0.06 g/plant) (Fig. 1).

3.4. Total sugars

The effect of drought stress induced an accumulation of sugars in wheat leaves, which was proportional with the level of water stress. The variation of sugar contents was significant between water stress levels ($F_{(3,60)}$ = 49.12, P < .001). According to Tukey's test, total sugars obtained for each level of drought was significantly different from the other. The highest sugar content (1.46 ± 0.16 µg/g DM) was obtained with FC = 30%, followed by the value 1.27 ± 0.13 µg/g DM for the level FC = 50%, then FC = 80% with 1.08 ± 0.15 µg/g DM, and finally the control 'FC = 100%' with 0.93 ± 0.09 µg/g DM (Fig. 2, Table 2). The amount of total sugars varied significantly between fertilization treatments ($F_{(4,60)}$=3.35, P = .015). However, Tukey test classified the different fertilizations as a homogeneous group. It is noteworthy that that the highest sugar contents were observed with control plants with 1.25 ± 0.29 g/g DM and the dose SS3 with 1.26 ± 0.18 g/g DM (Fig. 2, Table 2). The interactive effect 'stress × fertilization' was not significant ($F_{(12,60)}$ = 0.97, P = .487).

3.5. Total nitrogen

The content of nitrogen elements in plants significantly decreased with increasing water stress ($F_{(3,60)}$ = 11.52, P < .001). The total nitrogen decreased from 2.88 ± 0.63% for the drought level FC = 100% to the lowest content of 1.73 ± 0.46% for FC = 30%

Table 2
Results of Tukey's post hoc tests. The same letters of each fertilization treatment and water stress level are significantly not different.

	Morphological, physiological and yield parameters							
	Number of tillers	Tiller length	Root weight	Total sugars	Total nitrogen	Membrane integrity	Grain weight	Nbr. grains per spike
Water stress								
FC = 100%	C	D	A	A	B	C	C	D
FC = 80%	BC	C	B	B	A	BC	B	C
FC = 50%	AB	B	C	C	A	B	A	B
FC = 30%	A	A	D	D	A	A	A	A
Fertilization								
Control	A	A	A	A	A	A	A	B
Urea (35 kg/ha)	AB	AB	A	A	C	B	A	A
SS1 (20 t/ha)	B	B	B	A	B	BC	B	B
SS2 (50 t/ha)	C	B	C	A	B	C	C	C
SS3 (100 t/ha)	C	C	D	A	C	D	D	D

(Fig. 2). Tukey test revealed two homogeneous groups: FC = 100% > FC = 80%-FC = 50%-FC = 30% (Table 2). ANOVA showed that the effect of fertilization on the variation of N was significant ($F_{(4,60)}$ = 34.76, $P < .001$). According to Tukey test, the application of mineral fertilizer 'urea' and the dose SS3 formed the first group with a significant improvement of total nitrogen in plants with 3.30 ± 0.64% and 3.02 ± 0.70%, respectively. The second group included the dose SS2 with 2.07 ± 0.76% and SS1 with 1.66 ± 0.72%. The control plants held the lowest value of nitrogen with 0.95 ± 0.05%, they form the last group (Fig. 2, Table 2).

3.6. Cell membrane integrity

The statistical study showed significant effects of water stress ($F_{(3,60)}$ = 27.66, $P < .001$) and fertilization ($F_{(4,60)}$ = 89.10, $P < .001$) on the variation of membrane integrity, whereas the interaction of these two factors was not significant ($F_{(12,60)}$ = 1.56, $P = .126$). The increase of drought stress induced a significant decrease in the integrity of cell membranes. Multiple comparison of means stressed three groups: the first formed by the level FC = 100% with 23.45 ± 1.83% and FC = 80% with 22.25 ± 2.19%), followed by the second group containing FC = 30%, FC = 50% (20.75 ± 1.42%) and the third group included FC = 30% with 17.90 ± 1.53% (Fig. 2, Table 2). The application of sewage sludge and urea promoted the maintenance of the cell membrane integrity. The variation of average values showed that cell integrity increased with increasing doses of fertilizers. Tukey test revealed four groups: The first formed by the highest dose SS3 with 28.87 ± 3.75%, followed by the second group including SS2 (21.68 ± 1.43%) and SS1 (19.87 ± 2.10%); the third group comprised SS1 and urea (18.93 ± 2.72%); while the last was represented by control with 16.06 ± 2.67%.

3.7. Average grain weight (AGW)

ANOVA showed very highly significant effect ($P < .001$) of the factors 'water stress', 'fertilization' and their interaction on the variation of weights of wheat grains. For the different stress levels, the increase of AGW was dependent on sewage sludge-based fertilizations. Tukey's test highlighted four distinct groups: SS3 > SS2 > SS1 > control, urea (Table 2). The average weight (0.23 ± 0.12 g) obtained with fertilization dose SS3 was significantly higher than SS2 (0.17 ± 0.09 g) and SS1 (0.10 ± 0.07 g). The mineral fertilizer 'urea' did not show any improvement of grain weight (0.04 ± 0.03 g) compared to control plants that weighted 0.07 ± 0.05 g.

The plants irrigated with 100% field capacity showed the highest grain weight values with 0.20 ± 0.02 g. This parameter decreased with increasing drought to reach 0.08 ± 0.03 g with the stress FC = 50% and then 0.05 ± 0.01 g with the stress FC = 30%. AGW of plants treated with FC = 80% was 0.17 ± 0.03 g (Fig. 3). The order of groups following Tukey test was FC = 100% > FC = 80% > FC = 50%, FC = 30% (Table 2).

3.8. Number of grains per spike

Water stress caused a significant decrease in the number of grains per spike. According to ANOVA test, this parameter significantly varied between water stress levels ($F_{(3,60)}$ = 46.94, $P < .001$). Tukey's test confirmed that means of the number of grains were ranked into four FC groups: FC = 100% with the highest values (6.40 ± 0.78 grains/spike) > FC = 80% (5.15 ± 0.95) > FC = 50% (4.15 ± 0.71) > FC = 30% (3.25 ± 0.82) (Fig. 3, Table 2). The application of sewage sludge induced a significant improvement ($F_{(4,60)}$ = 66.56, $P < .001$) in the number of grains per spike unlike the mineral fertilizer that presented a number of grains (2.50 ± 1.06) significantly lower than control plants (3.81 ± 1.06 grains/spike). The largest number of grains/spike was achieved with the dose SS3 (7.31 ± 1.79) followed by the second SS2 group (5.50 ± 1.33). the dose SS1 had a value (4.56 ± 1.66) similar to that of the control (Fig. 3). Thus, the groups obtained by Tukey test were: SS3 > SS2 > SS1, control > urea (Table 2).

3.9. Relationships between growth and yield parameters

All correlations performed between the morpho-physiological and yield parameters of durum wheat were statistically significant, except those with root weight that showed only a positive and significant relationship with total sugars ($r = 0.6$, $P < 0001$), the rest of correlations of this parameter were not significant ($P < .05$). The amount of total sugars was negatively correlated with the rest of the six growth and yield parameters. These latter were all positively correlated with each other (Fig. 4).

4. Discussion

The results of this study show that under drought deficit wheat plants adjust their sizes by reducing their ground biomass with the decrease in the number of tillers and tiller height. This morphological adaptation mechanism is implemented in plants to avoid tissue dehydration through transpiration effect (Aroca et al., 2012) and provide maintenance and a balanced water distribution between different aerial parts of the plant (Moshelion et al., 2015). These observations are consistent with those of Ullah et al. (2014) and Albouchi et al. (2003) that reported decrease in the number of leaves associated with a loss in height and total dry biomass in species exposed to water stress. The significant correlation between number of tillers and tiller height reveals the positive effect of sewage sludge on the development of these morphological parameters. This effect results from the composi-

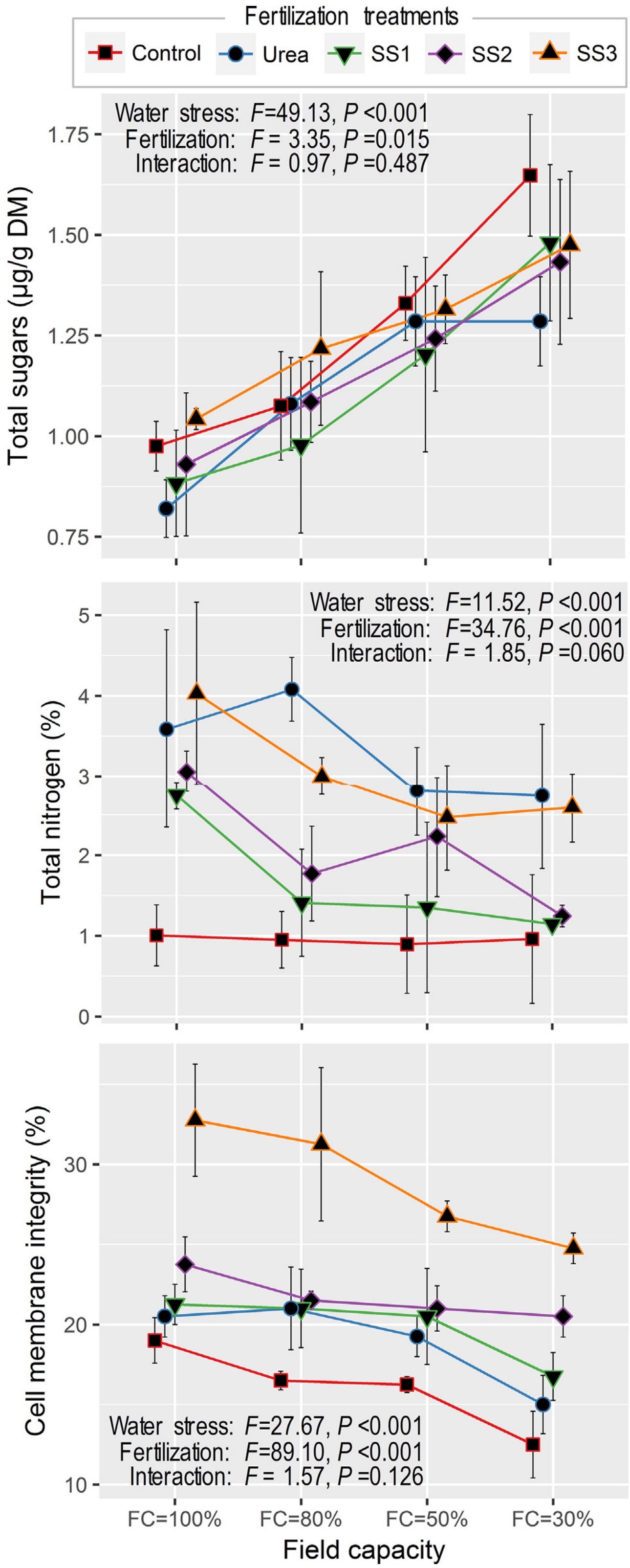

Fig. 2. Effect of different fertilization treatments including sewage sludge 'SS' on physiological parameters of Durum Wheat (*Triticum durum* var. waha) grown under different water stress (field capacity). Vertical bars associated with means (symbols) are standard deviations (±SD). Test statistics (*F* and *P*-values are two-way ANOVA testing the effects of water stress, fertilization and their interaction. (Control: no fertilization, urea: 35 kg N/ha, SS1: 20 t/ha of SS, SS2: 50 t/ha of SS, SS3: 100 t/ha of SS).

tion of the biosolid, which besides trace elements, it also contains an important and large stock of mineral and organic matter that improves the nutritional capacity of the soil and allows nutrient release that are made available for plants and soil microorganisms. Thus, this good nutrition positively influences the growth of aboveground biomass of wheat (Rughoonundun et al., 2012; Mattana et al., 2014).

It is also clear that this growth of wheat plants is sharper with the two high doses of sewage sludge used, especially for the level SS3 (100 t/ha). Similar results were reported by Boudjabi et al. (2015) that highlighted a linear increase in aboveground biomass of durum wheat with increasing doses of sewage sludge. This effect is explained by the high concentration of nutrients existing in this biosolid. Indeed, the physicochemical analysis of sewage sludge revealed that it richer than soil in terms of organic carbon, organic matter, total nitrogen, total and assimilable phosphorus, and other minerals. Results of pH indicated an alkaline environment, which may be explained by the effect of liming applied to sludge during its treatment in the treatment plant (Baize, 2000). The high electrical conductivity is due to the large load of the various existing salts (Chenchouni 2017), in the case of sewage sludge, these are nitrates, heavy metals, assimilable phosphorus, etc.

As water is the solvent of the sol solution, the water status of the biosolid strongly determines the availability of nutrients in plants, in particular nitrogen (Manzoni, et al., 2012; Erice et al., 2014). Through these results, the nitrogen nutrition of plants is negatively affected by the water shortage. Therefore, there is a decrease in the concentration of nitrogen in plant tissues (Aroca et al., 2012; Manzoni et al., 2012). Our results are consistent with other studies that reported that drought is a determinant factor that decreases the passage of soil nitrogen into wheat seedlings (Larsson, 1992). Moreover, Erice et al. (2014) demonstrated that water stress induces a decrease of about 90% of nitrate contents in the aerial part of durum wheat. Roiloa et al. (2014) reached similar results with a variety of strawberry (*Fragaria vesca*).

A dehydration-tolerant plant can be described as a plant having a high stability in its membrane structure (Rivero et al., 2014). The results regarding cell integrity show that drought stress negatively affects membrane stability of wheat cells. This effect is due to a response of plant to overcome this lack of water; where plants synthesize an osmotic regulator which is the proline. Proline is formed from a membrane proteolysis that allows the release of proteins as source of nitrogen, which is an essential element in the synthesis of this amino acid (Albert et al., 2012; McKiernan et al., 2014). However, the application of sewage sludge improves membrane integrity and limits its degradation through the improvement of nitrogen nutrition. Our results indicated that the effect of fertilizing treatments is decisive on nitrogen nutrition of plants. Thus, the supply of nitrogen via sewage sludge causes the increase of nitrogen in plant tissues which subsequently sustains the cellular integrity of the plant and its aerial growth. Accordingly, this improvement of cell membrane integrity and aerial growth has a positive influence on the growth of tillers and stubble length.

The significant correlations detected between the cellular integrity and the last two morphological parameters confirm these relationships. The sewage sludge is regarded as a source of high nitrogen content (Mosquera-Losada et al., 2014). Indeed, the dosage of our sludge at the laboratory shows that it is rich with this element (Boudjabi & Kribaa, 2012). It is documented that soils amended with this biosolid showed an improved enzymatic activity, mainly proteases that release nitrogen from the breakdown of organic matter (Mingorance et al., 2014; Tammeorg et al., 2014); thus resulting in an increase of this mineral element in plant tissues (Pascual et al., 2007; Manzoni et al., 2012) and therefore

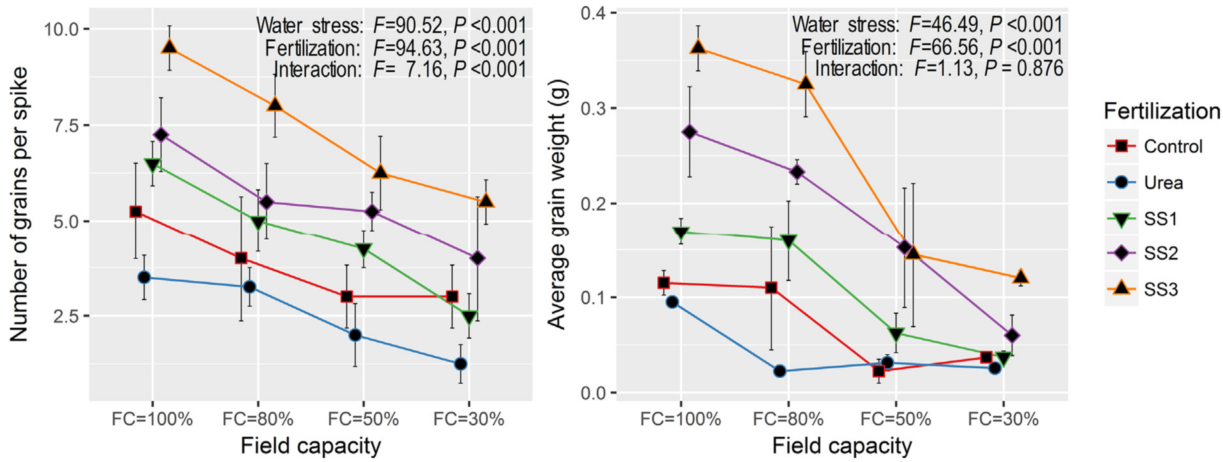

Fig. 3. Effect of different fertilization treatments including sewage sludge levels (SS) on yield parameters (grain weight and number of grains per spike) of Durum Wheat (*Triticum durum* var. waha) grown under different water stress (field capacity). Vertical bars associated with means (symbols) are standard deviations (±SD). Test statistics (*F* and *P*-values are two-way ANOVA testing the effects of water stress, fertilization and their interaction. (Control: no fertilization, urea: 35 kg N/ha, SS1: 20 t/ha of SS, SS2: 50 t/ha of SS, SS3: 100 t/ha of SS).

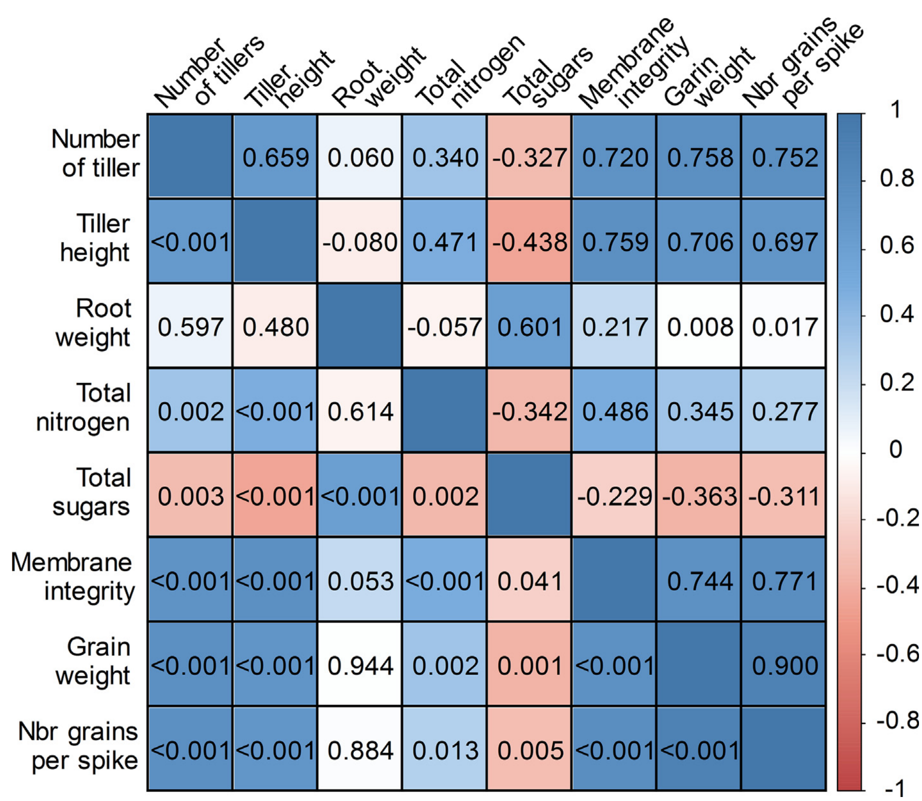

Fig. 4. Correlation matrix between growth parameters of Durum Wheat variety waha grown under nursery conditions. Pearson test values are given as the correlation coefficient (above diagonal) and P-value (under diagonal).

a good growth of plants. Pascual et al. (2007) reported that the spreading of anaerobic sewage sludge enhances nitrogen concentration in tissues of rapeseed.

Physiologically, the study shows that sugar contents increase considerably in plants subjected to different levels of water stress (Ramalho et al., 2014). The dosage of total sugars extracted from wheat leaves indicates a proportional increase between the amount of accumulated sugars and the level of drought stress applied to the plants. This effect reflects the role of this solute in the osmotic adjustment through decreasing water potential that becomes more negative and ensuring thus a sustaining movement of water to the leaves and accordingly their turgor. This physiological mechanism is considered as an acclimatization strategy adopted by plants to cope with environmental stresses in particular droughts (McKiernan et al., 2014; Bezzalla et al., 2018). Our results concerning the accumulation of this solute are in agreement with the study of Cabuslay et al. (2002) that observed an accumulation in the content of soluble sugars in several rice genotypes, where sugar contents were higher in stressed plants compared to the control. For the effect of fertilizing treatments, the sewage sludge similarly has a very significant effect on the accumulation of this solute, where it reduces sugar concentrations in particular with high water stress.

Besides, the correlation obtained between the content of sugars in plants and root biomass reflects the role of sugar as energetic materials in the root development. Actually, the development of roots resulted from the combined effect of both factors 'water stress' and 'biofertilization'. Indeed, the results obtained show that the stress effect induced a biomass allocation into the root section at the expense of the aerial parts. This extension in root biomass contributes to further absorb water from the soil, thus avoiding the effects of water deficit at the surface (Mori and Inagaki, 2012; McKiernan et al., 2014; Bezzalla et al., 2018). Furthermore, the sewage sludge has been instrumental in the development of root biomass in this present study. This is explained by their structuring effect on the physical properties of soil as well as the improvement of soil chemical characteristics (Latare et al., 2014). The organic materials contained in the biosolid allow the creation of new pathways for roots which makes the soil more permeable and its depths easily accessible to roots (Shaheen et al., 2014). Since the sludge used is rich in phosphorus (see Boudjabi and Kribaa, 2012), and as this mineral element is provided in sufficient quantities, it stimulates the growth of leaf area and increases the production of carbonated materials. Which coincides with an increase in contents of soluble sugars in the apex, and promotes the extension of primary and lateral roots (Johannessen et al., 2012; Prosser et al., 2015).

Our findings corroborate with those of Korboulewsky et al. (2002) in which an improvement in root length of *Diplotaxis erucoides* was observed under the effect of sludge-based fertilization. Regarding wheat yield components, this study evidenced that water stress induces a reduction in the flow of minerals into the roots, which results in a decrease in yield components essentially the average grain weight and number of grain per spike (Zhang et al., 2014). It is known that wheat yield is often diminished by the lack of water (Boudjabi et al., 2015). According to O'Leary et al. (2014), the lower limit of grain production in durum wheat is determined at a level of water consumption ranging between 200 and 210 mm/year.

The positive correlations between average grain weight (AGW) and the number of tillers and between AGW and tiller height shows that there has been a good grain filling through the improved growth of aerial parts of the plant. This improvement in AGW may be explained by the influence by the effects of sewage sludge that holds longer water in soil through the organic material it contains. Sewage sludge is known to play a major role of a reservoir and a holder of soil moisture (Tammeorg et al., 2014), which ensures proper water supply and thereby allows a good transfer of minerals to the treated plants in contrast to control plants and those amended by urea.

Our findings are also similar for the number of grains per spike which the good growth of tiller height and the number of tillers positively affect this yield component. It is known that this parameter of fertility closely depends on the amount of phosphorus in the soil (Johannessen et al., 2012). Indeed, the sludge used in this study are rich in this element and its enduring mineralization allows to provide a significant amount of this mineral for plants and subsequently ensure a gradual growth in the number of grain per spike (Mingorance et al., 2014; Prosser et al., 2015). The current study indicated that the effect of mineral fertilizers on wheat yield components and also on all morphological and physiological parameters shows a slight improvement which is significantly lower compared with sewage sludge performances. We believe that the exploitation of this mineral has not entirely occurred in the plant. Our results on the yield are in agreement with the study of Antolin et al. (2005), which indicates that the amendment with sewage sludge is more beneficial for increasing yield than nitrogenous mineral fertilization for the cultivation of barley.

5. Conclusion

The application of sewage sludge as soil fertilizer improves yield and productivity of Durum Wheat. Fertilization with sewage sludge gives better results than mineral-based fertilizers. Under water stress, wheat plants fertilized with sewage sludge show high physiological adaptations that allow them to cope with drought and improve yield component compared to stressed plants. These adaptations and yield parameters increase with the increase of sewage sludge doses applied. The application of sewage sludge for agronomic purposes must be conducted under adequate control and monitoring of all the possible risks that can arise. The use of effective tools and bioindicators is a necessity to monitor and diagnose fertilized soils and crops grown on it. When sewage sludge is applied, the distribution of heavy metals and micro-pollutants in soil profiles should be evaluated to prevent their diffuse into natural vegetation and crops.

Funding

This study was not funded by any sources.

Conflict of interest

The authors declare no conflict of interest.

Authors' contributions

SB and MK conceived and designed the study. SB conducted the experiment and laboratory work. HC and SB analysed data and drafted the manuscript. All authors read and approved the manuscript.

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