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Date palm waste compost promotes plant growth and nutrient transporter genes expression in barley (*Hordeum vulgare* L.)

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ABSTRACT

Organic fertilizers have been used in agriculture to improve soil fertility, promote plant growth and protection which improves crop productivity. A field experiment was conducted in order to study the effect of organic fertilizers (compost and its water extract) on growth and yield of organic barley production. The results achieved showed that either compost (T3) or dual application with compost water extract (T4) as biofertilizers increased most yield parameters including straw yield as well as biological yield, number of grains per spike and grain income. With respect to photosynthesizing pigments, the application of T3 and T4 increased chlorophyll a, chlorophyll b, total chlorophyll as well as carotenoid content compared to control plants (T1). Nutrient uptake of barley plant, but not grain, also increased under T3 and T4 treatment. This suggests a promoting effect of compost on barley plant growth, development and productivity, as well as nutrient content. Overall findings indicate the effectiveness of compost and its extract application in significantly improving yield performances of barley crop under a field organic farming system. This indicates that compost and its extract could be used as organic fertilizer for barley cultivation under organic farming condition. Real-time PCR analysis showed differential expression of 24 candidate genes such as *NRT1, NRT2, AMT* and *PHT* involved in N and P metabolism in barley leaves and roots. This suggests that these genes may play various functions and control the uptake, transport, reduction, assimilation and translocation of nitrate, ammonium and phosphorus in barley vegetative organs.

1. Introduction

The application of organic amendments such as compost could be considered as a good strategy to environmentally sustainable management. Therefore, several studies reported that manure compost is used as an alternative fertilizer to increase soil fertility and crop production in organic farming for maize production (Geng et al., 2019). Farm compost (FC) is made of varying ingredients available on the farm such as wood chips and bark, slurry, manure, straw and crop residues (D'Hose et al., 2012). Convincing evidence indicates that application of FC can improve the chemical (like soil pH, cation exchange capacity, organic matter and nutrient levels), physical (including bulk density and soil aggregation) and biological soil (mainly beneficial soil organisms) characteristics (Scotti et al., 2015). Moreover, application of FC has been proposed as a strategy for reducing plant diseases caused by soil borne pathogens which are the major factors limiting the productivity of agro-ecosystems (Panth et al., 2020). Interestingly, the beneficial effect of FC on the growth and yield of various crops such as wheat (Ibrahim et al., 2008), maize (Mucheru-Muna et al., 2007), tomato (Ghorbani et al., 2008), cucumber (Mahmoud et al., 2009) and vegetable crops like cauliflower (Simarmata et al., 2016) has been demonstrated.

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According to Al-Bataina et al. (2016) compost addition to the soil serves as a basis for the nutrition of soil microbes, and therefore increases their activities leading to increased soil nutrient (such as nitrogen, phosphorus and sulfur) availability and thereby nutrient uptake by the plants.

In Tunisia, crop yields in semiarid and arid regions have significantly declined as a result of the loss of organic matter in soil that is strongly linked to soil degradation and desertification. In these regions, soil organic matter contents are less than 1%, because the Tunisian climate results in rapid decomposition of organic matter and also because very little organic matter is added to soils during cultivation (Dridi and Arfaoui, 2017). The use of organic fertilizer by farmers can play a beneficial effect in improving the physical, chemical and biological properties of the soil and increasing the availability of nutrients for plant growth. In general, the organic fertilizers that are widely used by Tunisian farmers are animal manures from cows, chickens, goats and sheep. However, in the arid regions of southern Tunisia the application of a mixture of animal manures (especially cow manure) and date palm (*Phoenix dactylifera* L.) wastes, which are valorized as organic fertilizer (through composting) is considered the best practice that is commonly used by farmers. The application of date palm compost obtained by co-composted dry date palm leaves with cow manure in a 3:1 ratio at a moderate dose (30 t ha^{-1}) significantly increased fresh biomass production of alfalfa (*Medicago sativa* L.) which may be related to better minerals uptake (Benabderrahim et al., 2018). Similar data were reported in spinach (*Spinacia oleracea* L.) plants grown in soils amended with cow manure after a co-composting with poplar leaf litter at the rate of 20 t ha^{-1} (Anwar et al., 2017). In this context, Abid et al. (2018) reported that the use of compost based on date palm leaves waste and goat manure significantly improved soil quality by increasing its organic matter content. Furthermore, date palm compost supply increased tomato seeds germination rate and seedling growth under greenhouse conditions. More recently, Yakubu et al. (2020) reported that application of date palm compost obtained by co-composted date palm leaves with poultry manure at a 4:1 ratio significantly enhances growth and physiological performance of date palm seedlings. In faba bean (*Vicia faba* L.), the application of date palm leaves compost significantly reduced faba bean root rot disease caused by *Rhizoctonia solani* and *Fusarium solani* as well as improved plant growth and yield parameters under greenhouse and field conditions (Abdel-Monaim et al., 2017).

Therefore, application of compost as organic matter can reduce negative effects and increase soil quality including carbon content which might lead to a beneficial effect on plant growth and yield (Adugna, 2016).

Recently, compost production for agriculture received considerable attention in socioeconomic conditions in Tunisia, especially in organic farming because it replaces fertilizers and improves soil global fertility. Today, Tunisia is ranked the second largest exporter of organic products in Africa after Uganda and 24th in the world in terms of certified organic agricultural land area. Interestingly, the organic sector contributed about 10% of the total Tunisian exports and in 2018; the exported quantities of organic products were estimated at 48,500 tons for a value of 186 million US dollars (Mtimet et al., 2020). An exponential growth in international demand promoted the development of local organic farming in Tunisia which leads the government to make organic production a national priority. This could contribute to sustainable farming and consequently promote sustainable development of agriculture in Tunisia. Moreover, another use of compost regards the production of compost tea, a liquid extract, which can be active in plant protection against phytopathogenic fungi and bacteria and in plant growth and yield promotion. Stimulatory effects occur on plants through microorganisms including PGRs (Plant Growth Regulators), nutrients, humic substances and hormone-like molecules secreted by microbes present in compost tea (Hegazy et al., 2015). Several studies reported that foliar and soil application of compost tea considerably improved plant performance traits and increased yield of various plant species such as pepper (Zaccardelli et al., 2018), lettuce (Pane et al. 2014), cucumber (Santiago-López et al., 2016), navel orange (Omar et al., 2012), tomato (Pane et al., 2016), soybean and sweet corn (Kim et al., 2015), cowpea (Hegazi and Algharib, 2014) and water spinach (Bethe et al., 2017). Recently, the interest in organic farming showed an increased interest in Tunisia, and the use of FC became popular in organic farming and there is a growing interest to use it in low-input agricultural systems. However, there is a lack of information on the agronomic value and environmental impact of FC.

The objective of this study was to investigate the effectiveness of FC and its extract as an organic biostimulant on growth and yield of barley in an arid region in Tunisia. The gene expression profiles of different genes involved in N and P uptake and translocation were examined under different biofertilizer regimes using qRT-PCR. The results may provide new insights into the parts FC play in plant N and P uptake capacity.

2. Material and methods

2.1. Experimental design

An open field experiment was carried out from December 2020 to June 2021 at the production station of ASOC (Association for Saving Oasis of Chenini, Gabes, Tunisia). The climate is Mediterranean arid, with an annual temperature of 20.06°C, and an annual average precipitation of 160.20 mm. The experimental treatments were arranged in a randomized block design (each experimental plot size was 3 m length *x* 2 m width) with three replicates as the following: (T1) unamended biological soil (control); (T2) unamended biological soil and compost tea-spray plant treatment at tillering, stem extension and heading stage; (T3) biological soil amended with 30 t ha^{-1} of date palm leaves compost and (T4) biological soil amended with 30 t ha^{-1} of date palm leaves compost and compost tea-spray plant treatment at tillering, stem extension and heading stage. Physico-chemical analyses of the experimental soil are presented in Table 1. The plots were spaced 1 m within and between rows, and were irrigated regularly with conventional intervals of 15 days. The compost was mixed in the top 15 cm of soil to simulate the common field conditions. The local barley (*Hordeum vulgare* L.) cultivar Sahli, which is the most commonly grown cultivar in Tunisia organic farming, was used in this study. The seeds were obtained from the Technical Centre of Organic Agriculture (TCOA, Tunisia) and were sown on 16 December (2020) using seed rate of 120 kg ha^{-1}. The crop was harvested during the 3rd week of June (2021) and individual samples

Table 1
Physico-chemical properties of the experimental soil before treatments.

Parameters	Value
Clay (%)	5.50
Silt (%)	8.30
Sand (%)	84.40
Soil texture	Sandy
pH	7.50
EC (dS m^{-1})	4.02
Organic matter (%)	0.905
Total organic carbon (%)	0.525
Total N (g kg^{-1} soil)	0.28
Available P (mg kg^{-1} soil)	4.92
Exchange K (mg kg^{-1} soil)	292
Total coliforms (MPN g DW^{-1} soil)	24 × 10^2
Faecal coliforms (MPN g DW^{-1} soil)	< 0.3
Escherichia coli (MPN g DW^{-1} soil)	< 0.3
Faecal Streptococci (MPN g DW^{-1} soil)	39 × 10^2

were threshed manually. At this stage grain yield and yield components were determined. For some physiological and growth associated parameters; all samples were collected before flowering. For molecular analyses, leaf and root samples were snap frozen in liquid N_2, and stored at -80 °C prior to further analysis. Data on yield, yield components and seed quality were determined at harvest time.

2.2. Preparation of date palm leaves compost and compost tea

Date palm leaves compost was mechanically produced at a composting station of ASOC and two raw materials were used in the composting process. The first raw material was cow manure and it was collected from farmers in the area of Chenini, Gabes, Tunisia. The second raw material represented date palm leaves and it was obtained from farmers in the same area. The date palm leaves were air-dried and chopped into small pieces, about 0.2–0.5 cm in length. These raw materials were mixed in a windrow of 5 t, having the following composition: 50% cow manure + 50% date palm leaves. During the composting process, moisture was maintained around 50% (w/w). The windrow was turned once every week during the first two months and once a month for the remaining period of composting (4 month). The temperature was measured on a daily basis at different positions in the core of the windrow and the average of all the measurements was recorded. Physical, chemical and biological properties of the date palm leaves compost used in this study are presented in Table 2. Date palm leaves compost was used for the production of aerated compost tea.

The compost was mixed with tap water in a ratio of 1:5 (v/v) in polyethylene non-degradable 50 l containers at room temperature and with daily mechanical aeration. The duration of the fermentation process was 5 days and, at the end, the compost tea was filtered through double layered cheesecloth and 5 L were used in treatments of barley plants in each plot at tillering stage and 10 L at stem extension and heading stage. The chemical characteristics of the prepared compost tea are shown in Table 3.

2.3. Growth traits and photosynthetic pigments

At the tillering stage, ten random barley seedlings from each treatment were harvested to score shoot length (SL) and root length (RL). Moreover, root and shoot samples of each treatment were dried at 65 °C for 72 h and weighed in order to determine dry matter of each plant part. An average value was calculated for the determined shoot dry weight (SDW) and root dry weight (RDW) trait (Boudiar et al., 2020). For photosynthetic pigments characterization, leaves were immediately wrapped in aluminum envelopes, stored in thermally isolated recipients containing dry ice and taken to the laboratory. Leaf samples (0.2 g) were used for chlorophyll content determination using cold acetone 80% (v/v). The content of chlorophyll a (Chla), chlorophyll b (Chlb), total chlorophyll (Chlt) and carotenoids were determined spectrophotometrically according to the method of Lichtenthaler (1987).

Table 2
Characteristics of experimental palm compost.

Parameters	Value
Total organic carbon (%)	18.58
Total N (%)	1.21
C/N	15.36
P (%)	0.54
K (%)	0.95
Ca (%)	8.18
Mg (%)	1.05
Na (%)	0.42
Alkalinity (% CaCO3)	11.50
Zn (mg kg^{-1} DW compost)	70.10
Fe (g kg^{-1} DW compost)	70
Mn (mg kg^{-1} DW compost)	130
Cu (mg kg^{-1} DW compost)	11.60
Cd (mg kg^{-1} DW compost)	0.20
Pb (mg kg^{-1} DW compost)	4.15
Cr (mg kg^{-1} DW compost)	11.50
Ni (mg kg^{-1} DW compost)	5.88
Total coliforms (MPN g DW^{-1} compost)	143.33 ± 5.77
Faecal coliforms (MPN g DW^{-1} compost)	120 ± 17.32
Escherichia coli (MPN g DW^{-1} compost)	114 ± 23.79
Faecal Streptococci (MPN g DW^{-1} compost)	114.33 ± 23.8
Salmonella spp. (MPN g DW^{-1} compost)	< 0.3
Shigella spp. (MPN g DW^{-1} compost)	< 0.3

Table 3
Chemical characterization of the used compost extract.

Parameters	Value
pH	7.80
EC (dS m^{-1})	8.69
Organic C (mg L^{-1})	900
N (mg L^{-1})	130
P (mg L^{-1})	33.40
K (mg L^{-1})	795
Ca (g L^{-1})	1.53
Mg (mg L^{-1})	224
Potassium permanganate oxidizable carbon (POX-C, mg L^{-1})	2600
Faecal coliforms (MPN 100 ml^{-1})	670 ± 51.96
Escherichia coli (MPN 100 ml^{-1})	416 ± 23.09
Salmonella spp. (MPN 100 ml^{-1})	< 0.3
Shigella spp. (MPN 100 ml^{-1})	< 0.3

2.4. Grain yield and yield components estimation

At harvest time, barley plants were collected and the grain yield and yield components characters were determined (Dyulgerova and Dyulgerov, 2020; Mekonnen, 2018; Mohamed et al., 2019). For plant height (PH), ten random plants per plot measured at a distance from the bases of the culms to the tips of the spikes. Spike length (SL) expressed as average of ten spikes length from each plot. Spikes number per plant (SP) expressed as average of spikes number of ten random plants from each plot. Grain number per spike (GNS) was determined as average of grains number of ten random spikes from each plot. Thousand seed weight (TSW), estimated as the weight of 1000 cleaned kernels in grams for each plot. Grain yield (GY) was measured as the weight of all grains harvested from the entire plot. Biological yield (BY) was determined as biomass in terms of dry-matter yield of straw and grain yield from the entire plot. GY and BY in each plot was determined and then converted into t ha^{-1}. Harvest index (HI) was calculated as GY/BY into 100. Crude protein content was determined by the Kjeldahl method (Kjeldahl, 1883) applying a factor of N × 6.25 using a Kjeltec 1035 Analyzer instrument.

2.5. Mineral analysis

To determine grain nutrient constituents (N, P, K, Ca, Mg, Na, Fe, Mn, Zn and Cu), representative samples from ground grains (0.1 g) were digested using a mixture of nitric and perchloric acids (4:1) at 100 °C until complete evaporation. Then, 5 ml of nitric acid solution (0.5%) was added to the samples and the mixture was filtered using Whatman filter paper (Zaier et al., 2010). Total nitrogen content was determined by Kjeldahl method (Kjeldahl, 1883). K, Ca and Na were determined using flame photometer (Corning 400 uk). P was determined by colorimetric method with spectrophotometer (Pradhan and Pokhrel, 2013). The Mg and micronutrients (Fe, Mn, Zn and Cu) were determined using atomic absorption spectrophotometer (Shimadzu AA 6800).

2.6. Total RNA extraction and analysis of gene expression by qRT-PCR

Total RNA was extracted from leaf and root samples (0.2 g) according to the protocol of Chang et al. (1993). The quality of total RNA was determined by Nanodrop spectrophotometer and using gel electrophoresis (1.2%). First strand cDNA was synthesized by First Strand cDNA Synthesis Kit (AMV) from Biomatik following the manufacturer's protocol. The qRT-PCR was carried out in a 7300 Real-Time PCR Detection System (Applied Biosystems, Foster City, USA) and performed in a 25 μl reaction volume tube using the Maxima SYBR Green/ROX qPCR Master Mix (Biomatik) according to the method described previously by Abid et al. (2020). Specific primers for the genes studied, and the internal *actin* gene control (Table 4) were designed using the Primer3 Input (version 0.4.0) software (Rozen and Skaletsky, 2000) (http://frodo.wi.mit.edu/primer3/). To confirm that only one PCR product was amplified and detected, a melting curve analysis of amplification products was performed at the end of each PCR by slow heating from 65 °C to 95 °C at 0.5 °C/s and continuous monitoring of the fluorescence signal. Gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method (Schmittgen and Livak, 2008) and the *actin* as a reference. The heat maps are generated using R package (http://www.r-project.org/) to compare the expression profiling of the transcriptome in different treatments.

2.7. Statistical analysis

For data analysis at least ten plants were randomly selected in each plot and each treatment. The differences between treatments were determined using analysis of variance (ANOVA) and treatments means were separated by Duncan's multiple range test ($P < 0.05$) using SPSS 19.0 program.

3. Results

3.1. Effect of date palm leaves compost on photosynthetic pigments and plant growth

The application of compost and its extract as organic fertilizer showed significant effects on photosynthetic pigments and plant

Table 4
List of gene-specific primers used for real-time RT-PCR analysis.

Gene name	GenBank accession ID	Primer pair sequence (5′→3′)	Tm (°C)	Product size (bp)
HvNRT1.1	AK361245	F: CCTCGTCGTCTACCTCTTCG	63	145
		R: AGAAACACGAACGGACGAAC	59	
HvNRT1.2	AK360067	F: TCAGCGCACTCAACTACGTC	61	118
		R: TGCACCGACACCCTACATTA	59	
HvNRT1.3	AK367366	F: TTCAGTCTCTCCGGCTTGAT	59	85
		R: GGAGCATCGTCTCCGAGTAG	63	
HvNRT1.4	AK359624	F: TCACCGTGTTCATGCTGATT	57	144
		R: GAGAGCGTGGTGAGGAAGAG	63	
HvNRT1.5	AK248673	F: CGGAGGTGATGATGTACGTG	61	115
		R: AAGTAGTTGCCGAGCGACAT	59	
HvNRT1.6	AK362653	F: GGAACGAGACGTTCGAGAAG	61	121
		R: CCGGAGAATACGTTGAGGAG	61	
HvNRT1.7	AK373468	F: CACCAAGGAAGAGGCAGAAG	61	81
		R: GGACAAATTTCCCTCCCAAT	57	
HvNRT1.8	AK375888	F: ACTGGTCTGGCACCACCTAC	63	105
		R: CGAGGAAGATGAGCTGGAAG	63	
HvNRT1.9	AK376903	F: CATGTTCTTCCTGACGCTGA	59	120
		R: GCGAAGGAGATGAAGAGCAC	61	
HvNRT1.10	AK353903	F: CTAGTTTTGCGCAGGTCCTC	61	121
		R: GTGTAGGGCAGCTTGGAGAC	63	
HvNRT2.1	U34198	F: GTTTTCTGCATGGCCGTTAT	57	94
		R: ATTGGCATGACACGAACGTA	57	
HvNRT2.2	U34290	F: TCCTTCTTCACCTGCTTCGT	58	80
		R: TTGGCGAGGTTTAGGTTGTC	57	
HvNRT2.3	AF091115	F: ATGGCGTATTGCCTACTTCG	58	90
		R: TTCCCATCAGGGAGATCTTG	58	
HvNRT2.4	AF091116	F: ACATGGCCAAGGACAAGTTC	58	122
		R: AATGACATTGCCAGTGGTGA	57	
HVNAR2.1	AY253448	F: TACCTCTCCAAGCTGCCTGT	61	140
		R: TTCTTGTAGTCGGCGTCCTT	59	
HVNAR2.2	AY253449	F: ATCGAGAAGCGCAAGAAGAA	57	145
		R: CCCCGAAAAGAGAACATCAA	57	
HVNAR2.3	AY253450	F: TACCTCTCCAAGCTGCCTGT	61	143
		R: TTCTTGTAGTCGGCGTCCTT	59	
HvPHT1.1	AK371267	F: GAAGCAAGCCACATCAGACA	59	99
		R: GAGAAGAGGCCCCAGGTATC	63	
HvPHT1.2	AY220455	F: GAAGCAAGCCACATCAGACA	59	102
		R: GAGAAGAGGCCCCAGGTATC	63	
HvPHT1.6	FM866444	F: ACGAAACCAAGGACAACGAC	59	142
		R: TAGAAGGCGATGTCGAGGAG	61	
HvPHT3	AK355175	F: AAAGGAGCAAGGAGCTAGGG	61	129
		R: GGCCCAGCCATATCTGAGTA	61	
HvPHT4	AK358207	F: GTCGTAAGCTTCCTGGCAAC	61	105
		R: CAGCCATACAGAGAGCCACA	61	
HvAMT1	AK354234	F: GAACATCATGCTCACCAACG	59	119
		R: CGAAGAAGTGCTTCCCGATA	59	
HvAMT2	AK252569	F: TTCCTTCCGGTCACAAACTC	59	141
		R: TAGCCGGATGACGACACATA	59	
HvActin	AY145451	F: CGACAATGGAACCGGAATG	58	56
		R: CCCTTGGCGCATCATCTC	59	

growth parameters (Table 5). ANOVA analysis showed significant differences among the treatments on the average of chlorophyll and carotenoids content, shoot length, shoot and root dry weight at the tillering stage. But there was no significant difference on the average root length. T4 treatment recorded the maximum SL (57.16 cm) among other treatments. T3 recorded 53.50 cm SL (Table 5). Compost is more effective when applied in combination than alone. Both compost and its extract improved barley growth parameters. However, SDW showed significant differences for T4 (632.70 ± 30.38 mg) as compared to T3, T2 and T1, suggesting that the maximum and significantly improved SDW was observed, when the compost was used in combination with its extract. Similarly, RDW showed significant differences for T4 as compared to T1 and T2. However, there were no significant differences between compost treatments with means of 247.63 ± 15.49 mg and 254.33±10.20 mg for T4 and T3, respectively.

Application of compost and its extract revealed significant increases in the concentrations of photosynthetic pigments such as Chla, Chlb, Chlt and total carotenoids (Table 5). Indeed, the Chla, Chlb, Chlt and total carotenoids content increased with the compost and its extract over the control (T1). Moreover, the compost (T3) and combination of compost and its extract (T4) remained more effective than the compost extract (T2) when used alone. Chlb content of T2, T3 and T4 increased by 39.08%, 411.03% and 423.44%, respectively, compared with T1. On the other hand, only mixture application of compost and its extract (T4) exhibited significant differences in the content of Chla when compared with the control treatment (T1). Application of compost extract alone (T2) had no significant effect on accumulation of Chlt content compared with control (T1), while Chlt concentration of T3 and T4 increased by 31.93% and 39.21%, respectively. Table 5 shows that Chla/b ratio is the highest in T1 and T2 and is the lowest in T3 and T4. The ANOVA data revealed significant differences between the effects of treatments on carotenoids concentration, which is the lowest in T1 compared to other treatments.

3.2. Effect of date palm leaves compost on barley yield and yield components

The data presented in Table 6 revealed that compost treatment (T3 and T4) significantly increased the plant height and most yield and yield components such as spike length and number, grain number per a spike and 1000-grain weight over untreated (T1 and T2). Moreover, grain yield as well as straw yield and consequently biological yield also showed high records in T3 and T4 in comparison with T1 and T2. In general, the application of compost (T3 and T4) resulted in taller plants compared to those obtained with compost extract (T2) and untreated (T1). A comparison of treated plant height with the control revealed an increase of 30.27, 36.69 and 8.25% in T4, T3 and T2, respectively. The same table (Table 6) also showed that application of compost induced a similar trend for other yield and yield component traits**.** The barley grown gave a significant grain yield of 5.11, 5.53 and 3.43 t ha^{-1} in T4, T3 and T2, respectively. This resulted 61.70, 75 and 8.54% more grains over control (T1). It is evident from the data that the maximum number of spikes per plant, spike length and grain number per spike was recorded in T4 and T3 followed by T2 and T1. Statistical analysis of the data revealed that applied compost significantly improved the biological yield of barley crop. Maximum biological yield of 15.53 t ha^{-1} was observed in T4 treatment followed by T3 with 13.43 t ha^{-1} biological yield; the least were T2 and T1 with 9.97 and 9.02 t ha^{-1}, respectively. Similar to the biological yield the straw yield of barley was also significantly affected by applied compost, the maximum straw yield of 10.41 t ha^{-1} was produced by T4 followed by T3 with 8.89 t ha^{-1} and the minimum straw yield was obtained in T2 (6.54 t ha^{-1}) and T1 (5.86 t ha^{-1}) where no compost was added. Data regarding the harvest index could not exhibit a significant difference between treatments. Indeed, T1, T2, T3 and T4 were found statistically similar to each other (Table 6).

3.3. Effect of date palm leaves compost on leaf mineral concentration and organic matter

The application of compost (T3 and T4) has resulted in significant improvement in uptake of nitrogen (N), phosphorus (P) and potassium (K) in barley plants compared to T1 (Table 7). However, the addition of compost (T3 and T4) and compost extract (T2) seems not to have a positive impact on organic matter (OM) of the barley plants.

3.4. Effect of date palm leaves compost on quality of barley grain

The effect of organic biofertilizers on quality of barley grain is presented in Table 8. Data revealed that compost and its extract (T2, T3 and T4) applied improved the nutrient content of most elements in barley grain compared to untreated (T1). In general, application of compost and its extract enhanced the concentrations of N, P, K, Ca, Mg, Na, Fe, Zn, Mn and Cu in barley grain, whereas the concentrations of Cd, Cr, Ni and Pb were all at low levels and still much lower than the limit. Application of compost (T3 and T4) significantly increased Na, Ca and Mn composition of grain compared to T1 which showed the lowest increase. Indeed, Ca content of T4, T3 and T2 increased by 14.46%, 10.41% and 5.75%, respectively, compared to T1. Table 7, clearly depicts an increase by 43.47%, 35.07% and 0.28% in Na content of T4, T3 and T2, respectively, compared to T1. Mn content increased by 83.20%, 41.01% and 26.20% in T4, T3 and T2, respectively compared to T1. On the other hand, despite a maximum N, P, K, Mg, Fe, Zn, and Cu concentration being recorded in T4, application of compost and its extract slightly but not significantly increased the N, P, K, Mg, Fe, Zn, and Cu content of barley grain compared to T1. The same trend was also observed with the protein composition of barley grain (Table 6).

Table 5
Effect of different treatments on chlorophyll a (Chla), chlorophyll b (Chlb), total chlorophyll (Chlt) and carotenoids content, shoot length (SL), root length (RL), shoot (SDW) and root dry weight (RDW) at the tillering stage. All values are means of 10 replicates (±SE). Different letters indicate significant difference at $P < 0.05$ (Duncan's test) probability level.

Parameters	T1	T2	T3	T4
Chla (mg g^{-1} FW)	14.53 ± 0.19^{b}	15.35 ± 0.85^{ab}	15.80 ± 0.66^{ab}	16.61 ± 0.30^{a}
Chlb (mg g^{-1} FW)	4.35 ± 0.29^{c}	6.05 ± 0.56^{b}	22.23 ± 0.27^{a}	22.77 ± 0.72^{a}
Chlt (mg g^{-1} FW)	20.45 ± 0.50^{b}	21.90 ± 2.13^{b}	26.98 ± 0.27^{a}	28.47 ± 1.68^{a}
Chla/b	2.84 ± 0.89^{a}	2.53 ± 0.09^{a}	0.70 ± 0.02^{b}	$0,72 \pm 0.01^{b}$
Carotenoids (mg g^{-1} FW)	0.47 ± 0.02^{b}	0.84 ± 0.14^{a}	1.04 ± 0.09^{a}	0.95 ± 0.18^{a}
SL (cm)	40.83 ± 3.48^{c}	45.66 ± 4.13^{b}	53.50 ± 2.88^{a}	57.16 ± 4.49^{a}
RL (cm)	15.37 ± 2.19^{a}	14.37 ± 1.76^{a}	15.37 ± 1.84^{a}	16.25 ± 2.18^{a}
SDW (mg $plant^{-1}$)	377.36 ± 12.96^{d}	428.76 ± 17.12^{c}	555.93 ± 15.52^{b}	632.70 ± 30.38^{a}
RDW (mg $plant^{-1}$)	149.80 ± 3.31^{c}	210.76 ± 17.20^{b}	254.33 ± 10.20^{a}	247.63 ± 15.49^{a}

Table 6
Effect of different treatments on yield, yield components and crude protein content of barley plants. All values are means of 10 replicates (±SE). Different letters indicate significant difference at P<0.05 (Duncan's test) probability level.

Parameters	T1	T2	T3	T4
Plant height (cm)	54.50 ± 3.53^{b}	59 ± 2.82^{b}	74.50 ± 4.95^{a}	71 ± 4.24^{a}
Number of spikes per a plant	3.75 ± 0.50^{b}	3.80 ± 0.83^{b}	5.37 ± 0.51^{a}	5.87 ± 0.99^{a}
Spike length (cm)	6.46 ± 0.70^{b}	6.22 ± 0.61^{b}	7.74 ± 0.57^{a}	8.02 ± 0.48^{a}
Grain number per a spike	26.20 ± 2.16^{b}	23.80 ± 2.58^{b}	31.60 ± 2.70^{a}	34.20 ± 2.16^{a}
Grain yield (t ha^{-1})	3.16 ± 0.20^{b}	3.43 ± 0.36^{b}	5.53 ± 0.33^{a}	5.11 ± 0.37^{a}
Straw yield (t ha^{-1})	5.86 ± 0.29^{c}	6.54 ± 0.15^{c}	8.89 ± 0.22^{b}	10.41 ± 0.27^{a}
Biological yield (t ha^{-1})	9.02 ± 0.50^{c}	9.97 ± 0.51^{c}	13.43 ± 0.56^{b}	15.53 ± 0.65^{a}
Harvest index (%)	34.99 ± 0.33^{a}	34.35 ± 0.18^{a}	33.76 ± 0.10^{a}	32.91 ± 0.11^{a}
1000 grains weight (g)	49.40 ± 1.01^{b}	50.26 ± 1.12^{ab}	50.88 ± 1.04^{ab}	52.87 ± 0.62^{a}
Crude protein (% DM)	12.27	12.41	12.67	13.12

3.5. Effect on gene expression of nitrogen and phosphate metabolism genes

An *in silico* analysis was performed in order to identify genes involved in barley nitrogen and phosphate metabolism. A total of 24 genes expressed in barley showed significant homology with their homolog in other plant species including *Arabidopsis thaliana* (Table 4). The expression pattern of these genes in barley leaves (Fig. 1) and roots (Fig. 2) was investigated using quantitative real-time PCR in response to T1, T2, T3 and T4 in order to gain more insight into their roles in nitrogen and phosphate use efficiency in organic barley crop production. The genes expression is shown as log2 signal intensities and data were visualized using heat maps. The selected genes expression was differential under the various treatments. For leaves, these genes are grouped into 7 clusters (Fig. 1). Cluster 1 contains 2 members (*HvNRT1.3* and *HvNRT1.7*, 8.3%) of 24 studied nitrogen and phosphate metabolism related genes, which were downregulated in T3 and T4 by application of compost compared to untreated control (T1) and T2. Cluster 2 mainly consists of 2 genes (*HvPHT3*, and *HvAMT2*, 8.3%) which were widely downregulated in T3 compared to T1. Cluster 3 contains 4 members (*HvNRT1.6, HvNRT1.8, HvPHT1.6* and *HvPHT4*, 16.6%) with upregulation in T2, T3 and T4 compared to T1. The 2 members (*HvNRT1.2*, and *HvNRT1.4*, 8.3%) of cluster 4 exhibited the highest expression in T3 and T4 in comparison to T1. Indeed, the expression of these genes was widely upregulated in T3 and T4 but almost unchanged in T2.

Cluster 5 had also 2 genes (*HvNRT1.9*, and *HvNRT1.10*, 8.3%) with the highest expression in T3. Moreover, a similar expression level of *HvNRT1.9* was recorded in T1 and T2, while reported in T3 and T4 for *HvNRT1.10*). The 2 members (*HvNRT1.1* and *HvNRT1.5*, 8.3%) of cluster 6 disclosed the lowest expression in T1 compared to other treatments. Moreover, *HvNRT1.5* showed a similar expression level in T2 and T4, while *HvNRT1.1* showed the highest expression level in T4. Cluster 7 mainly consists of 10 genes (*HvNRT2.1, HvNRT2.2, HvNRT2.3, HvNRT2.4, HvPHT1.1, HvPHT1.2, HvAMT1, HvNAR2.1, HvNAR2.2* and *HvNAR2.3*, 41.9%) with no expression or very weakly expressed in leaves.

For roots, the heat map clustered the genes analyzed in 9 clusters (Fig. 2). Cluster 1 contains 4 members (*HvNRT1.5, HvNRT1.9, HvNAR2.2* and *HvPHT1.2*, 16.5%) with a significantly downregulation in T3 and T4 compared to T1. Moreover, the expression of *HvNRT1.5, HvNRT1.9* and *HvNAR2.2* was widely downregulated in T2 compared to T1; however, the expression of *HvPHT1.2* exhibited no change in T2. Cluster 2 contains 2 genes (*HvNAR2.3* and *HvPHT4*, 8.3%), which was mainly downregulated in T2, but upregulated in T3 in comparison to T1. In T4, the expression level of *HvNAR2.3* was almost unchanged compared to T1. Nevertheless, *HvPHT4* was significantly downregulated. Cluster 3 had only 1 member (*HvPHT1.6*, 4.2%), which was mainly upregulated in T2, T3 and T4 compared to T1. Indeed, the highest expression was recorded in T2. Cluster 4 mainly consists of 5 genes (*HvNRT1.3, HvNRT1.4, HvNRT1.6, HvNRT1.7* and *HvPHT.3*, 20%) with no expression in roots. Cluster 5 had also only 1 transcript (*HvAMT2*, 4.2%), which was significantly and weakly upregulated in T3 and T4, respectively but nearly unchanged in T2 compared to T1. Cluster 6 has 6 members (*HvNRT1.2, HvNRT1.8, HvNRT1.10, HvNRT2.2, HvNRT2.4* and *HvNAR2.1*. 25%) and displayed significantly upregulated in T3 and T4 compared to T1. In T2, the expression of *HvNRT1.2, HvNRT1.8, HvNRT1.10* and *HvNAR2.1* showed nearly unchanged; however *HvNRT2.2* and *HvNRT2.4* were widely upregulated. Cluster 7 had also only 1 gene (*HvNRT2.3*, 4.2%), which was upregulated in different treatments compared to T1 with the highest expression in T3. Cluster 8 contains *HvAMT1* gene (4.2%), which was mainly upregulated only in T3 and T4 and revealed no change in transcript accumulation in T2 compared to T1. Cluster 9 mainly consists of 3 genes (*HvNRT1.1, HvPHT1.1* and *HvNRT2.1*, 12.5%) with significant upregulation in T4 compared to T1. Moreover, the expression of *HvPHT1.1* and *HvNRT2.1* was upregulated in T3; however, *HvNRT1.1* was practically unchanged.

Table 7
Effect of different treatments application on barley plant mineral concentration (N, P and K) and organic matter (OM) at heading stage. All values are means of 3 replicates (±SE). Different letters indicate significant difference at $P < 0.05$ (Duncan's test) probability level.

Treatment	N (%)	P (%)	K (%)	OM (%)
T1	1.73 ± 0.03^{b}	0.40 ± 0.02^{b}	2.31 ± 0.12^{b}	81.12 ± 0.94^{a}
T2	1.72 ± 0.02^{b}	0.43 ± 0.01^{b}	2.29 ± 0.01^{b}	81.61 ± 0.70^{a}
T3	2.33 ± 0.05^{a}	0.51 ± 0.01^{a}	2.55 ± 0.02^{a}	83.09 ± 0.36^{a}
T4	2.09 ± 0.12^{a}	0.51 ± 0.02^{a}	2.61 ± 0.03^{a}	82.69 ± 0.84^{a}

4. Discussion

Using date palm leaves compost and its extract as organic fertilizer revealed a significant effect on barley plant height, shoot and root dry weight and growth performance compared to control plants (T1). These results concur with those presented by Rekaby et al. (2020) in barley who reported that compost application increased plant height and dry biomass. Similar results were reported by Hountin et al. (1995) and Jan et al. (2014) in barley and wheat, respectively.

Interestingly, the combined application of compost and its extract (T4) gave the best results, suggesting the capability of date palm leaves compost as organic fertilizer in supplying plant needs which may be associated with balanced availability of the micro- and macro-nutrients in the soil (Rahimi et al., 2019). Wong et al. (2016) reported that this effect may be due to the presence of plant growth regulators such as phytohormones in organic fertilizers that promote plant growth and development. Moreover, with a C/N ratio of 15.36 (Table 2) at the suggested optimal range, compost used in this study could promote N mobilization and therefore, have an impact on plant

Table 8
Effect of different treatments on nutrient content of grain of barley plants.

Parameters	T1	T2	T3	T4
Total N (g kg^{-1})	19.60	20	20.20	20.80
Total P (g kg^{-1})	4.53	4.91	4.95	5.01
k (g kg^{-1})	6.35	6.42	6.51	6.94
Mg (g kg^{-1})	1.28	1.35	1.39	1.41
Ca (mg kg^{-1})	643	680	710	736
Na (mg kg^{-1})	345	346	466	495
Fe (mg kg^{-1})	31.10	32.70	33.60	34.90
Zn (mg kg^{-1})	38.20	40.80	40.90	42.40
Mn (mg kg^{-1})	5.00	6.31	7.05	9.16
Cu (mg kg^{-1})	3.80	4.04	3.97	4.15
Cd (mg kg^{-1})	<0.07	<0.07	<0.07	<0.07
Cr (mg kg^{-1})	<1.07	<1.07	<1.07	<1.07
Ni (mg kg^{-1})	<0.07	<0.07	<0.07	<0.07
Pb (mg kg^{-1})	<0.20	<0.20	<0.20	<0.20

growth and production (Wolkowski, 2003). It is well reported by Siavoshi and Laware (2013) that nutrients provided by added organic fertilizers in soil could be assimilated by plants and used in different metabolic pathways for synthesis of photosynthetic pigments necessary for their development. In this study, untreated plants (T1) exhibited the lowest Chla, Chlb, Chlt and total carotenoids content suggesting the positive effect of the application of organic fertilizers on the amount of photosynthesizing pigments in organic barley. Ali et al. (2015) reported that the application of organic fertilizers significantly increased chlorophyll concentration in wheat plants. Other similar results were also found in pear jujube (*Ziziphus jujuba* Mill.) plants (Ye at al., 2020). In this study, it was observed that the application of compost increased photosynthetic pigments content in leaves of barley plants which are required and play an important role in plant photosynthesis, thereby resulting in increased plant growth. Thus, date palm leaves compost used in this study may be adequate for the supply of nutrients responsible for photosynthetic pigments biosynthesis. Moreover, the present investigation indicated that combination treatment of compost and its extract respond better with respect to chlorophyll content compared to other treatments. Table 5 shows that Chla/b in T1 and T2 is the highest and the lowest in T3 and T4. This could be due to the effect of compost on Chlb synthesis that is greater than that of Chla, which significantly increases the absorption of blue-green light and enhances the photosynthetic activity of leaves (Ye at al., 2020).

Several studies reported that cereal grain crops such as wheat and barley gave significant response to organic fertilizers including compost application resulting in improved crop growth (Mohamed et al., 2019). The present investigation showed the improvement of barley growth and yield grown under organic agriculture with the use of compost (T3 and T4) when they were compared with T1 and T2. The data obtained confirms the results of previous studies with wheat (Mohamed et al., 2019), soybean (Yagoub et al., 2012) and teff (Assefa et al., 2016) which are crops with similar or different growing patterns than barley. Growth-promoting effects of compost may be associated to its effect on enhancing availability and content of macro (N, P, K, Ca, Mg and Na) and micro (Fe, Mn, Zn, and Cu) nutrients (Table 2) and their use efficiency that lead to promoting barley growth and yield productivity (Nadjet et al., 2014). These results were in agreement with those reported by Abera et al. (2018) and Tadesse et al. (2018) which observed maximum grain yield of barley with application of compost. The significant difference in grain yield among different treatments may be associated with the difference in nutrient absorption and support previous studies (Ibrahim et al., 2008) who reported that compost application improves plant growth parameters, thus enhancing grain yield. Consequently, the highest biological yield in T3 and T4 might be due to the fact that compost

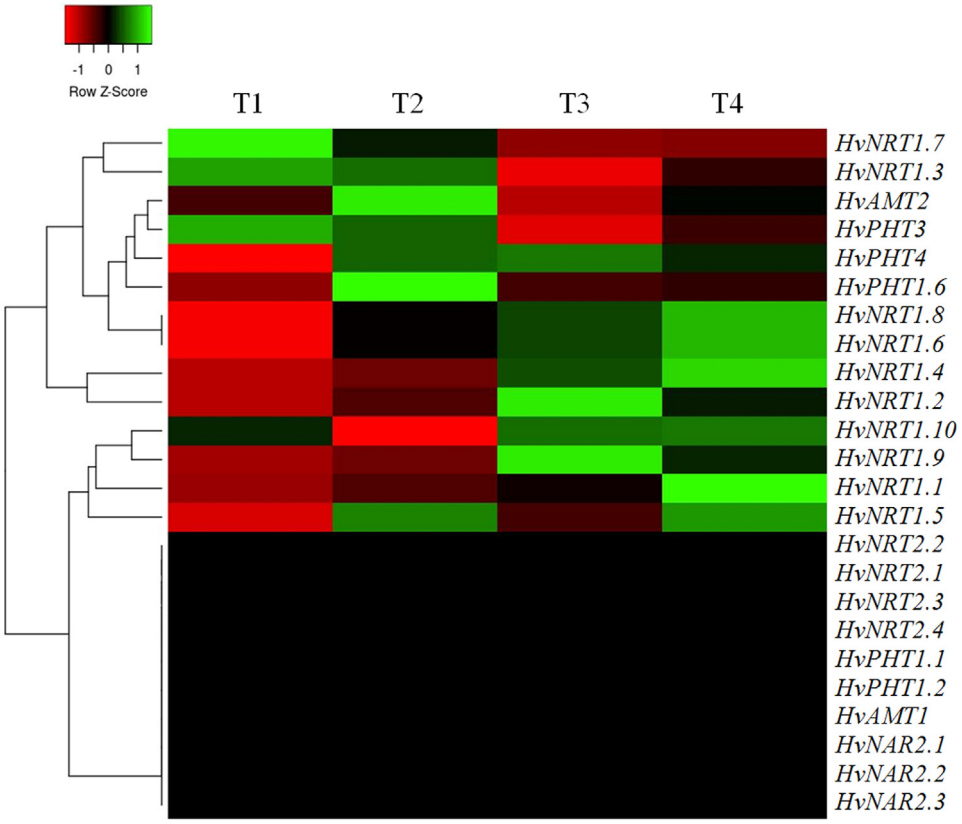

Fig. 1. Heat map representation of the effects of different treatments on the genes expression in the leaves of barley cultivar Sahli. Green and red indicate higher and lower expression values, respectively. Intensity of the colors is proportional to the absolute value of log2 of the fold difference in expression.

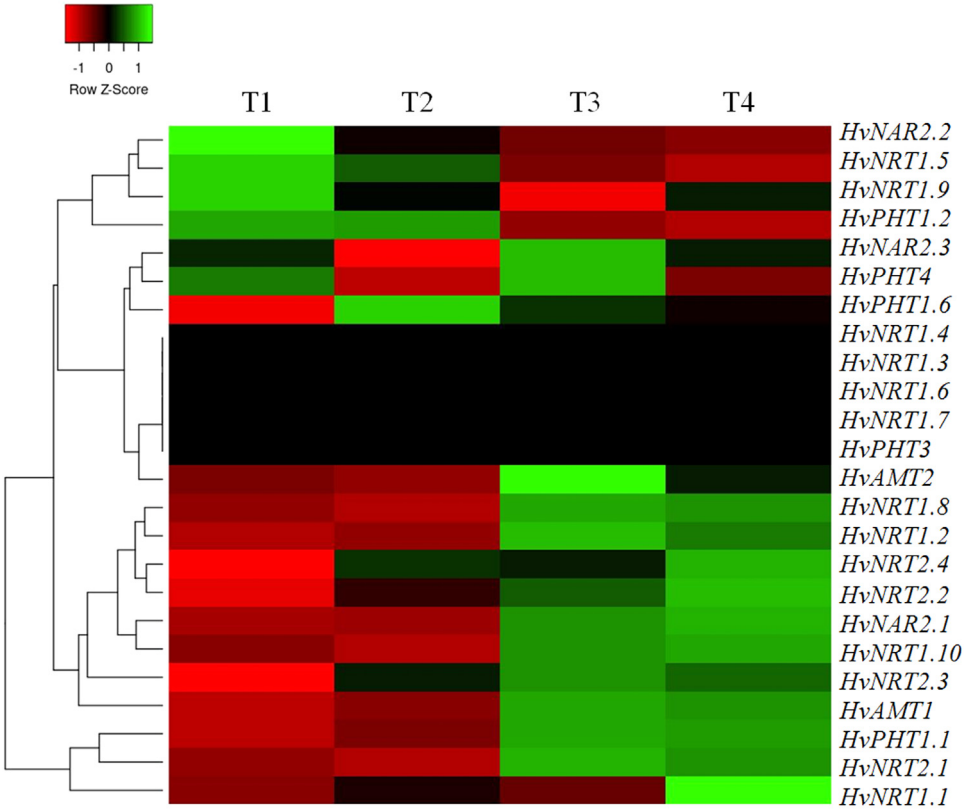

Fig. 2. Heat map representation of the effects of different treatments on the genes expression in the roots of barley cultivar Sahli. Green and red indicate higher and lower expression values, respectively. Intensity of the colors is proportional to the absolute value of log2 of the fold difference in expression.

directly supplies the essential nutrients to the plant. Similarly the dominant effect of compost on barley, wheat and soybean biological yield was also reported by Abera et al. (2018), Mohamed et al. (2019) and Yagoub et al. (2012), respectively. Data regarding the harvest index exhibited no significant difference between treatments and were found statistically similar to each other. This result was in disagreement with Abera et al. (2018) who found significant increase in the harvest index with application of compost. These non-significant results for harvest index might be due to a short term experiment (only for one growing season). Combined application of compost with its extract (T4) increased straw and biological yield more than a single application (Table 6). Such a promoting effect of T4 might be associated to hormonal activity of compost extract like cytokinins, indoleacetic acid, and gibberellins that stimulate plant growth and development resulting in increased yield and yield components. This data was in line with various investigations which reported an increase in wheat straw and biological yield with dual application of compost and its extract (Youssef et al., 2013).

Several studies have shown that the application of organic fertilizers such as compost to soil can increase plant nutrient concentration and uptake, thereby increasing the nutrient content of grain (Gopinath et al., 2008). Similarly Mohamed et al. (2019) also reported that either sludge or compost increased the nutrient content of wheat grain. All treatments registered significantly higher Ca, Na and Mn contents in barley grain than with T1. No significant differences among T1 and T2 were observed in grain nutrient concentration for N, P, K, Mg, Fe, Zn, and Cu. On the other hand, compost application (T3 and T4) registered higher N, P, K, Mg, Fe, Zn, and Cu contents in barley grain than with T1 and T2. Indeed, T4 was found different from other treatments in barley grain nutrient and showed the highest values, whereas only minor variations occurred in grain N, P, K, Mg, Fe, Zn, and Cu concentrations between T1 and T2. Similarly the significant effect of organic composts on rice grain nutrient was also reported by Saha et al. (2007). Such effect may be associated with the higher content of all nutrients needed by plants in compost and their availability (Gopinath et al., 2008).

It is well reported that nutrient uptake and allocation into root cells was regulated by various transporter proteins (Nath and Tuteja, 2016). Both, nitrogen (N) and phosphorus (P) are essential nutrients determining plant growth and productivity. Nitrate and ammonium are the major source of N absorbed by roots from soil through various major families of transporters (Lamichhane et al., 2021) including, low affinity nitrate transporters (NRT1), high affinity nitrate transporters (NRT2), NRT3 (also named NAR2) proteins, which interact with NRT2 members and contribute to high-affinity nitrate uptake and ammonium transporters (AMTs). On the other hand, high affinity phosphate transporters (PHT1) and low affinity phosphate transporters (PHT2/4, PHT3 and PHT4) are responsible for phosphate uptake from soil and for internal transport (Młodzinska and Zboinska, 2016).

In order to gain more insight in to the potential role of a number of specific genes involved in nitrogen and phosphorus metabolism on organic barley plant growth and development, the expression profiles of 24 barley genes in response to different treatments (T1, T2, T3 and T4) were analyzed by quantitative real-time PCR in leaves (Fig. 1) and roots (Fig. 2) tissue. These genes expressed diversely in different organs tested under T1, T2, T3 and T4 suggesting their potential role in regulating barley plant growth and development.

Previous studies in *Arabidopsis thaliana* have shown that *AtNRT1.1*/NRT1-PEPTIDE TRANSPORTER FAMILY 6.3 (*AtNPF6.3*) gene plays an important role in nitrate uptake, translocation from root to shoot, root system architecture and as a sensor of external nitrate availability (Bouguyon et al., 2015). In rice (*Oryza sativa* L.), *OsNRT1.1*/*OsNPF8.9* was downregulated by N deficiency in roots, and overexpressed under high N supply, suggesting a potential for improving N use efficiency (NUE) which leads to increased rice growth and yield

(Hu et al., 2015). The two maize (*Zea mays* L.) orthologues of *NRT1* (*ZmNPF6.6* and *ZmNPF6.6*) showed differentially expressed to N supply and improved NUE of maize (Wen et al., 2017). On the other hand, 4 *NRT1.1* orthologues (*TaNPF6.1, TaNPF6.2, TaNPF6.3* and *TaNPF6.4*) showed differentially expressed in different organs of wheat (*Triticum aestivum* L.) plant.

In the current study *HvNRT1.1* was differentially expressed in barley leaf and root tissues and highly expressed in root than leaf. Moreover, T2, T3 and T4 increased the expression of *HvNRT1.1* compared to T1 suggesting that the expression patterns of *HvNRT1.1* were responsive to compost as organic fertilizer supply. These data indicate a possible important role of *HvNRT1.1* in barley N allocation and utilization. *AtNRT1.2* another member of the Arabidopsis *NRT1*, showed expression mainly in the plasma membrane of roots and was involved in nitrate uptake (Liu et al., 1999), abscisic acid (ABA) transporter, seed germination, transpiration and seedling development (Li et al., 2020). In this study, *HvNRT1.2* was induced by T2, T3 and T4 and expressed in both leaves and root tissue, suggesting that this gene implicated in root nitrate uptake in barley and possibly nitrate translocation and distribution to aerial parts. *HvNRT1.3* showed expression only in leaf tissues under all treatments suggesting that this gene may play a role in the step of nitrate supply to photosynthesizing cells. This result was in line with Tong et al. (2016) in Arabidopsis, which found that *AtNRT1.3* expression is induced by nitrate in shoots and showed expression in flowers, stems and leaves, suggesting that expression of *AtNRT1.3* was restricted to parenchymal tissues but not in epidermal tissues. Similarly to *NRT1.3, NRT1.4* showed expression in the shoot but predominantly in the petiole (Chiu et al., 2004), suggesting a crucial role in leaves nitrate homeostasis. *HvNRT1.4* showed expression differentially in different treatments suggesting its role in regulation of nitrate accumulation and nitrate homeostasis in leaves of barley.

NRT1.5 is another member of low-affinity transporters which expressed in root pericycle cells and is involved in loading nitrate into xylem. *HvNRT1.5* was expressed in both root and leaf tissues and was more induced under different treatments in root than leaf tissues suggesting that is involved in nitrate transport and accumulation from root to shoot in barley. Similarly, Chen et al. (2012) reported that the *AtNRT1.5* gene is expressed mainly in root pericycle cells close to the xylem in order to load nitrate into xylem and its transport from root to shoot. Moreover, it has been reported that *AtNRT1.6* is involved in nitrate storage in seeds and thereby seed development through delivering nitrate from maternal tissue to developing embryo (Almagro et al., 2008). According to these authors, *AtNRT1.6* is only expressed in reproductive tissue. In barley, *HvNRT1.6* showed expression in leaf tissues suggesting that nitrate and organic nitrogen stored in source leaves can be remobilized via phloem to feed reproductive young tissues. Similarly, RT-PCR analysis revealed that expression of *HvNRT1.7* is only detectable in leaf tissue and is downregulated under different treatments compared to T1. *AtNRT1.7* expressed in the phloem of the Arabidopsis leaf vein, suggesting its responsibility in nitrate remobilization from source tissues like older tissues to nitrogen-demanding tissues such as younger tissues (Fan et al., 2009).

All *AtNRT1.8, AtNRT1.9* and *AtNRT1.10* predominantly expressed in roots and play a role in the uptake of nitrate and fine-tune root-to-shoot nitrate long distance transport (Nour-Eldin et al., 2012). Expression patterns of *HvNRT1.8, HvNRT1.9* and *HvNRT1.10* indicate that barley uses these nitrate transporters to regulate xylem-to-phloem transfer at both leaf and root organs.

In order to contribute to high-affinity nitrate uptake, most NRT2 members encode to high-affinity nitrate transporters which function at low external nitrate concentrations to interact with NRT3 (also named NAR2) proteins. A total of 10 putative *HvNRT2* and 3 putative *HvNAR2* genes were found in the barley genome (Guo et al., 2020). *HvNRT2*.1, *HvNRT2.2, HvNRT2.3, HvNRT2.4, HvNAR2.1, HvNAR2.2* and *HvNAR2.3* were isolated from barley root tissues and were induced differentially in the roots under low and high N levels. In the present study we found that *HvNRT2*.1, *HvNRT2.2, HvNRT2.3* and *HvNRT2.4* were differentially expressed mainly in the root but not or very weakly in leaf tissues under different treatments, suggesting that these genes play a crucial role in barley root nitrate absorption. Similarly in Arabidopsis the expression levels of NRT2 genes are generally higher in roots than in shoots (Orsel et al., 2002). Interestingly, *HvNAR2* genes showed similar expression patterns to *HvNRT2* genes in leaf and root tissues, suggesting that *HvNRT2*.1, *HvNRT2.2, HvNRT2.3* and *HvNRT2.4*, require *HvNAR2.1, HvNAR2.2* and *HvNAR2.3* or either of *HvNAR2* genes for root acquisition and transport of nitrate. Six Arabidopsis *NRT2* family members (*AtNRT2.1, AtNRT2.2, AtNRT2.3, AtNRT2.4, AtNRT2.5* and *AtNRT2.6*) showed interaction with *AtNAR2.1*, while only 3 *OsNRT2* members (*OsNRT2.1, OsNRT2.2* and *OsNRT2.3*) interact with *OsNAR2.1* during nitrate transport activity (Kotur et al., 2012; Yan et al., 2011). In order to confirm the proposed complementary roles of NRT2 and NAR2 proteins in chrysanthemum (*Chrysanthemum morifolium*) an important ornamental specie, *CmNRT2* showed interaction with *CmNAR2* in the plasma membrane of transgenic *Arabidopsis thaliana* lines, suggesting that both proteins serve as partners to nitrate uptake (Gu et al., 2014).

Quantitative PCR results showed that *HvAMT1* and *HvAMT2* were expressed in leaf and root tissues, but they displayed different expression patterns under different treatments, suggesting that these genes may execute special functions depending on differential tissue expression. Interestingly, compost treatment (T3 and T4) induced the expression of *HvAMT1* and *HvAMT2* in root tissues compared to control (T1) and T2, indicating that compost may affect the genes expression pattern. According to Loqué et al. (2006), the expression of *AMT* genes in Arabidopsis is not only dependant on ammonium concentration, but also on N deprivation. In this study, compost treatments (T3 and T4) may increase nitrogen uptake compared to T1 and T2 which is caused by increased *HvAMT1* and *HvAMT2* mRNA expression.

The phosphate transporter (PHT) genes have been reported in plant species including barley as key regulators of phosphate (P) uptake from soil and translocation between plant tissues and organs. qRT-PCR data showed that *HvPHT1.1* and *HvPHT1.2* were expressed mainly in roots tissue but not or very weakly in leaves. Moreover, under compost treatments (T3 and T4) *HvPHT1.1* showed upregulated in the roots, while *HvPHT1.2* was downregulated. Taken together, these findings are in line with the results of Preuss et al. (2011) who found that *HvPHT1.1* and its paralogs *HvPHT1.2* function as a high-affinity Pi transporter and mediate phosphate uptake when phosphate is limited in soils. *HvPHT1.6* is expressed in both roots and leaves. These results were in agreement with Huang et al. (2008). Moreover, *HvPHT1.6* showed upregulated in T2, but downregulated in T3 and T4 compared to T1, suggesting that this member function as a low-affinity Pi transporter responsible for Pi remobilization in the whole plant (Huang et al., 2008). *HvPHT3* was expressed only in roots and was upregulated under T1 and T2 treatment suggesting that this Mitochondrial Phosphate Transporter (MPT) member encodes a high-affinity PHT and was upregulated by low P treatment in the field experiment. The PHT4 gene family is involved in the phosphorus transport in plastids and the Golgi apparatus (Karlsson et al., 2015). Recently, Ruili et al. (2020) reported that OsPHT4 proteins are involved in phosphorus transport and distribution between the cytoplasm and chloroplast or Golgi apparatus in rice leaves and roots and are also involved in abiotic stress responses such as salt stress tolerance. In the present study, the transcriptional patterns of the *HvPHT4* gene in response to different treatments have demonstrated that *HvPHT4* is differentially expressed in leaves and roots tissue under T2, T3 and T4 compared to T1. Moreover, *HvPHT4* displays greater expression in leaves than in roots, suggesting that it may function primarily in the chloroplast and is also targeted in the heterotrophic plastids (Guo et al., 2008).

5. Conclusion

The results of the present study demonstrate that the application of compost, alone or combined with their water extract, positively affects plant growth, plant development, yield and yield components as well as macronutrient uptake of barley grains. Indeed, all the yield parameters such as number of grains per spike, 1000-grain weight and grain yield were significantly increased in compost treatments (T3 and T4) compared to all other treatments. Hence, the results indicate that compost as a biofertilizer could be helpful in increasing the grain yield of barley in organic crop production. These increases in plant growth parameters by compost application were associated with the expression of several genes involved in nitrate, ammonium and phosphorus uptake and transport in roots and leaves of barley, suggesting their potential role in improving N and P uptake, assimilation and translocation capacity of plant under organic fertilizers. Overall, these findings provide novel information about the effects of compost on plant development and nutrient uptake regulations. As a future study, it would be interesting and useful to apply multi-omics approaches for the discovery of novel genes, proteins and metabolites for better understanding the underlying mechanisms and mode of action of compost on plant growth and development.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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