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Effects of different tillage on morpho-physiological traits of dryland chickpea (*Cicer arietinum* L.)

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ABSTRACT

The study of different types of tillage can be effective in promoting conservation agriculture for chickpea cultivation. The present experiment was conducted to study the effects of different soil management (reduced tillage (RT), no-tillage (NT), and conventional tillage (CT)) on morpho-physiological characteristics of chickpea in a complete randomized block design field experiment with three replications during the 2017–2019 cropping seasons. In this experiment effects of soil management on mycorrhizal colonization and sporulation, rhizobium nodules, relative leaf water content, leaf area index, chlorophyll and carotenoids, chlorophyll fluorescence, canopy temperature, enzymes, proline, hydrogen peroxide, malondialdehyde, leaf soluble proteins, carbohydrates, and leaf P and N content were investigated in early grain filling were evaluated. The highest activity of antioxidant enzymes including catalase (0.066 U/mg^{-1}Protein min^{-1}), peroxidase (1.22 U/mg^{-1}Protein min^{-1}), and superoxide dismutase (0.69 U/mg^{-1}Protein min^{-1}) were observed in the plants grown under CT practice, while the lowest were observed under NT system. In NT, the lowest amounts of malondialdehyde and carotenoids were retrieved compared to the conventional tillage. CT determined a reduction in chlorophyll in leaves compared to RT (13 %) and NT (36.2 %), respectively. The highest rate of canopy temperature depression occurred in no-tillage system. The highest remobilization from stems to seeds was respectively obtained from conventional tillage, reduced tillage and no-tillage treatments. In no-tillage, leaf area index, quantum efficiency of PSII (chlorophyll fluorescence), relative water content, leaf nitrogen, and leaf phosphorus increased compared to reduced tillage and conventional tillage, respectively. The rate of colonization and sporulation of mycorrhizal fungi and the number of rhizobium nodules significantly increased under NT compared to RT and CT systems. The results in this experiment showed that the NT system improved relative leaf water content, reduced antioxidant enzymes, and positive effects on measured morpho-physiological traits increased the yield and stability of chickpea compared to RT and CT systems under rainfed conditions.

1. Introduction

Chickpea (*Cicer arietinum* L.) is an annual plant of the Fabaceae family that makes biological nitrogen fixation from the atmosphere into the soil through symbiosis with rhizobium bacteria (*Rhizobium leguminosarum*) and has a special place in rotation with cereals (Cherr et al., 2006). Chickpea with high protein content (15–30 %) is applied in human nutrition which helps the sustainable agricultural systems (Hegde et al., 2018). This plant is one of the most important legumes that is cultivated in 35 countries, including Iran, and it is ranked 19th in terms of area under cultivation among crops (FAO, 2016). Due to the fact that chickpeas are cultivated rainfed in many countries of the world, drought stress and its damages decrease the yield of chickpea by up to 50 % (Varshney et al., 2014). Therefore, one of the ways to deal with drought stress can be conservation agriculture. Conservation agriculture offers three management principles on farms: Minimal soil corrosion (including NT), Rotation, and Preservation of plant residue at the soil surface (FAO, 2016). Thus, due to the lack of soil plow under NT system, soil moisture reserving and provides more accessible water to the plant (Sapkota et al., 2014; Safari et al., 2014). Also, Lampurlanés et al. (2016) reported that NT reduces soil evaporation and increases the available water-holding capacity of the soil. Different types of tillage can be

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effective in absorbing elements and soil organic carbon (SOC). Shiwakoti et al. (2019) reported that the highest amount of SOC, phosphorus, and potassium extractable soil was in NT compared to other tillage systems in the semi-arid region of Oregon, USA. Thus, a 0.5 % increase in SOC causes to increase in the yield by 20 % and the highest grain yield occurs in soils with 1–2 % organic matter (Oldfield et al., 2019). Some researchers (Hansen et al., 2012; Rusinamhodzi et al., 2011; Ngwira et al., 2012) have reported sustained or increased crop yields as a result of conservation farming implementation. The NT system resulting to improved soil water storage capacity, increased net photosynthesis (Pn), water transfer efficiency in the plant, and accumulated dry matter, which finally increased yield in wheat (Wang et al., 2015; Hemmat and Eskandari, 2006; Piggin et al., 2015; Chaieb et al., 2020) and chickpea (Piggin et al., 2015; Kaschuk et al., 2010). The reduction of soil water and drought stress causes oxidative stress in plants (Kapoor et al., 2020). Plants have an effective antioxidant defense system (enzymatic and non-enzymatic) to deal with oxidative stress caused by reactive oxygen species (ROS) (Anjum et al., 2011b; c). Enzymatic antioxidants such as peroxidase (POD), superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPX), ascorbate peroxidase (APX) and non-enzymatic antioxidants such as alpha-tocopherol (vitamin E), ascorbic acid (vitamin C), glutathione (GSH), flavonoids and carotenoids, reduce the effects of oxidative stress (Gill and Tuteja, 2010). The benefits of RT and NT systems include improved soil structure and aeration, soil biological activities, and microbial diversity such as nitrogen fixation bacteria and root nodulation (Torabian et al., 2019; Rowland et al., 2015; Dogan et al., 2012). So, the symbiosis of mycorrhiza fungi with the roots of host plants enables them to tolerate drought stress, which can play an essential role in improving crop production, especially in arid regions (Al-Karaki et al., 2004). The extant research shows that mycorrhizal symbiosis probably results in up taking more water in plants by altering the root morphology, elongation the root system of the host plant, increasing the level of uptake by fungal mycelium and improving the water relations of the host plant (Auge et al., 2015). This symbiotic relationship contributes significantly to plant nutrition and growth, as well as increasing relative leaf water content (RWC) and leaf protein (Auge, 2001). According to the above studies, Thierfelder and Wall (2010) reported that NT significantly increased the root colonization compared to CT system.

When the grain filling process begins, the nitrogen stored in all plant vegetative parts is transferred to the grain. Total nitrogen accumulated in chickpea grains include 30 % from leaves, 20 % from pod walls, 11 % from roots and 10 % from stems (Schiltz et al., 2005). Chickpea in the grain filling stage is associated with drought stress and the highest amount of nitrogen transfer to the grain is in the leaves, thus; RT and NT systems can probably help the leaves duration by more reserve soil water. Neumann et al. (2007) reported that conservation tillage (minimum-tillage) and intercropping systems increase the grain nitrogen content in pea. Regarding the effects of different types of tillage on morpho-physiological characteristics of plants, Wasaya et al. (2017) concluded that the highest leaf area index (LAI), leaf area duration (LAD), and grain dry matter were obtained in RT system. Tillage systems show a significant effect on the concentration of chlorophyll in leaves as the highest content of leaf chlorophyll belongs to plants under NT and the lowest content to CT system (Fiorentini et al., 2019). One of the important parameters for identifying tillage-dependent drought stress is canopy temperature depression (CTD). According to Blum (1988) research, comparing canopy temperature changes with air temperature makes it possible to identify different genotypes for genetic improvement of cereals for water deficit constraints. Also, several researchers use chlorophyll fluorescence measurements to study plant stresses (Guidi and Calatayud, 2014) such as drought (Flexas et al., 2002; Zivcak et al., 2013) and heat (Kalaji et al., 2011a; Brestic et al., 2012) stress. Piggin et al. (2015) in a 4-year experiment observed notable improvement in grain yield for NT compared to CT system. As reviewed by Torabian et al. (2019), the NT system has increased the amount of nitrogen in different parts of chickpea compared to CT. In this experiment, the effects of different types of tillage on the yield-related morpho-physiological traits of dryland chickpea was aimed in order to conserve the soil fertility and to select the best tillage system of NT, RT, and CT. We hypothesized that NT and RT maybe improve the morpho-physiological response of chickpea. In other words, this experiment aims to answer this question; Does conservation tillage cause morpho-physiological changes and ultimately improve plant growth and performance through possible changes in plant moisture conditions? Therefore, the effect of tillage on the water status of the plant was investigated.

2. Materials and methods

The experiment was carried out based on randomized complete block design (RCBD) in Saral Rainfed Agricultural Research Station located 55 km north of Sanandaj county, Kurdistan province, located in the west of Iran (35º 40’ 09" N; 47º 07’ 45" E; 2100 m above sea level) for two years (2017–2018 and 2018–2019 cropping seasons, from sowing in the first year to harvesting in the second year). Monthly rainfall and mean temperature during two growing seasons at the experiment station were shown in Table 1. The International Classification of Soils based on USDA Taxonomy is fine, mixed, mesic, calcixerollic Xerochreptes; Type: calcixerollic, Order: inceptisols (Soil Survey Staff, 1999, 2014). The soil properties in this experiment are described in Table 2. In this study, the effects of reduce tillage (RT), no-tillage (NT) and conventional tillage (CT) systems were studied on rainfed chickpeas in rotation with wheat (Data not shown). The wheat was planted the year before the experiment, which has been planted alternately with chickpea in the last three years, in other words, these systems (CT, RT, and NT) in each part continuously were applied in the last three years before this experiment. The tractor used in this study was Massey Ferguson (model ITM 800 4WD, Tabriz Co, Iran) with 82 horsepower and 3480 kg weight. In reduced tillage, a conservation suitable field cultivator machine (model DELTA-5, Sazeh Kesht Kaveh Co, Iran; ASABE S591.1, 2018) was used to prepare the seedbed in summer. In NT system, no operations were performed to prepare the seedbed, though the weeds were controlled chemically with lentagran (pyridate 2 L ha^{-1}) and supergalant (0.7 L ha^{-1}) in spring. In CT, traditional tillage operations were performed with a moldboard plow (25 cm depth) followed by a disc harrow (model Tandem, Sazeh Kesht Kaveh Co, Iran; ASAE S290.2, 2004). In all tillage systems, planting was done with a special multi crop no-tillage planter (fertilizer seeder) machine with 900 kg weight (model ASKE-2200, Kaveh Bukan Co, Iran; ASABE S506, 2010) mounted on the tractor with three-point hitch.

2.1. *crop management*

In this experiment, Saral chickpea cultivar with autumn growth for cold regions was used, which was prepared by Kurdistan Agricultural Research and Training Center. In both experimental years, the amount of planted seed was 80 kg/ha (30 plant/m^2) with a row distance of 35 cm and a plant distance of 10 cm. Sowing occurred in October 18–2017 and October 9–2018. In order to meet the nutritional needs of the plants, 20 kg N ha^{-1} (urea) and 23 kg P_2O_5 ha^{-1} (triple super phosphate) were used at sowing (Table 2).

2.2. *Sampling*

All sampling of mycorrhizal colonization and sporulation, rhizobium nodules, relative leaf water content, leaf area index, chlorophyll and carotenoids, chlorophyll fluorescence, canopy temperature, enzymes, proline, hydrogen peroxide, malondialdehyde, leaf soluble proteins, carbohydrates, and leaf P and N content took place in early grain filling stage (R5 stage) according to Muehlbauer et al. (1982), (June 17–2018 equivalent to 908 GDD (Growing Degree Days) and 243 DAS (Days After

Table 1
Monthly rainfall and temperature in experiment station.

	October	November	December	January	February	March	April	May	June	July	August	September
2017–2018												
Precipitation (mm)	2	21	3.9	19.7	83.4	12.8	117	73.8	0	0.3	0	0
Min-temperature (C°)	-1	-7.2	-10.2	-15.6	-10.2	-3.2	-5.1	2.6	7.9	12	11.6	7.5
Max-temperature (C°)	24.7	16.6	12.4	11	11.4	22.4	18.3	23.6	33.2	33.8	33.6	32.4
Temperature below zero (Day)	3	11	24	30	21	10	3	0	0	0	0	0
2018–2019												
Precipitation (mm)	36.4	86.8	78.5	52.8	35	54.7	98.4	40.2	10.6	0	1.6	-
Min-temperature (C°)	0.7	-3.8	-9.2	-12.6	-11	-8.4	-3.5	3.1	8	10.2	13.5	-
Max-temperature (C°)	25.6	12.4	8.6	8.5	7.6	9.8	17.2	25.3	33	35.7	35.5	-
Temperature below zero (Day)	0	4	25	30	28	21	9	0	0	0	0	-
Average 30-year												
Precipitation (mm)	25.5	45.2	31.1	30.1	32.2	45	59.8	27	3.7	3.2	2	2.3
Min-temperature (C°)	5.5	-0.6	-4.7	-7.5	-6.4	-2.3	2.6	6.9	11	14.6	14.42	10.4
Max-temperature (C°)	18.6	9.09	4.03	0.7	2.5	8	14.1	20.1	26.7	30.5	30.55	26
Temperature below zero (Day)	1.3	10.6	26.3	28.3	27.3	24.3	13	1.6	0	0	0	0

Table 2
Soil properties of experimental site.

Sampling depth (cm)	EC (ds m^{-1})	pH	Available phosphorus (mg kg^{-1})	Available potassium (mg kg^{-1})	Organic matter	Nitrogen	Sand	Silt	Clay	Soil texture
					(%)					
0–30	0.375	8.05	7.3	103	0.62	0.07	43.56	37.28	19.16	Loam

Sowing), and June 17–2019 equivalent to 872 GDD and 252 DAS).

2.3. *Yield, yield components and plant height*

To determine the grain yield, biological yield, and harvest index (HI) at full maturity (July 12–2018 equal to 1362 GDD and 268 DAS, July 12–2019 equal to 1320 GDD and 277 DAS), After removing the margin effects, 3 m^2 was hand-harvested from the middle parts of each experimental unit. To determine the 100-grain weight, four samples of 100 grains were used from each experimental unit. To measure the plant height, 10 plants were selected randomly from each experimental at full maturity (July 12–2018 equal to 1362 GDD and 268 days after sowing, July 12–2019 equal to 1320 GDD and 277 DAS) (Mafakheri et al., 2010).

2.4. *Mycorrhizal colonization and sporulation, rhizobium nodules*

In the early stage of grain filling, 5 plants in each experimental unit were randomly selected and removed with rhizosphere soil and placed in a large water container for 5 h to better clean, then, the roots were washed with distilled water. The roots are washed-out with 10 % KOH and were stained according to Vierheilig et al. (1998). The percentage of root colonization was determined by the gridline intersection method (Giovannetti and Mosse, 1980). At maturity, to determine the number of spores, the soil of rhizosphere belonged to 5 plants, which was randomly sampled to a depth of 30 cm, were isolated (Jiang et al., 2018; Brundrett et al., 1994), then the spores were counted by the microscope (Olympus IX51, Japan). To determine the number of rhizobium nodules, 6 plants were selected randomly from each plot at flowering stages (Man 27, 2018 amounts to 642 GDD and 222 DAS; May 30, 2019–594 GDD and 234 DAS) and the nodules were counted on the roots, then their mean was calculated (Erman et al., 2011).

2.5. *Remobilization*

Based on the Papakosta and Gagianas (1991) and Wang and Shangguan (2015), samples (6 plants) were harvested from 0.2 m^2 area at 50 % flowering stages (Man 27, 2018 amounts to 642 GDD and 222 DAS; May 30, 2019–594 GDD and 234 DAS), and maturity (July 12, 2018 and 2019, amounts to 1362 GDD and 268 DAS, and 1320 GDD and 277 DAS). At each stage, after separating the leaves, pods, seeds, and stems, samples were oven-dried at 75°C for 48 h and weighed. The remobilization and its indices were calculated as below:

1) Remobilization = dry mater of stem at anthesis - dry mater of stem at maturity
2) Stem efficiency in remobilization % = (Remobilization/dry mater of stem at anthesis) × 100
3) Contribution of remobilization in grain % = (Remobilization/ Grain weight at maturity) × 100
4) Contribution of photosynthesis in grain % = (100 - Contribution of remobilization in grain)

2.6. *Canopy temperature depression (CTD), Relative water content (RWC)*

To determine the temperature difference between the canopy and the environment, a handheld infrared thermometer (Model Kiray100, KIMO Co, France) was used. Measurements were taken at noon and in order to minimize the effect of the angle of the sun, the southward direction was selected. At the same time, an ordinary thermometer was used to measure the ambient temperature. The difference between ambient and canopy temperature was considered as CTD (Reynolds et al., 2007). To measure the relative water content based on the method of Sairam et al. (2002), the 20 same size and fully developed leaves were collected and weighted to determine Fresh weight (FW) and turgid weight (TW), then oven-dried at 75°C for 48 h and weighed again to determine dry weight (DW) and then was calculated according to the following formula:

$$RWC\ (\%) = (FW - DW)/\ (TW - DW) \times 100$$

2.7. *Leaf area index (LAI), specific leaf area (SLA)*

To measure the leaf area index, 6 plants (0.2 m^2) were collected. Then the leaf area was measured with a leaf area meter (Delta-T, UK). The dry weight of the leaves was measured after oven drying at 70°C for 48 h and specific leaf area (SLA) was calculated $m^2\ g^{-1}$ (Ramamoorthy et al., 2016).

2.8. Chlorophyll fluorescence

For measurement of All fluorescence chlorophyll parameters randomly selected 10 leaves samples from each experimental unit on chickpea. then by miniPPM-300 pocket device (EARS Co, Netherlands) according to the.

method of Zivcak et al. (2013) with a slight change including twice in the early night due to the adaptation to darkness and daylight at 10 am (day) were measured.

5) (Fv/Fm) = (Fm-Fo)/Fm = Maximum photochemical quantum yield of PSII in the dark-adapted state (Night)
6) (Fv'/Fm') = (Fm'-Fo')/Fm' = Maximum photochemical quantum yield of PSII in the light-adapted (Day)

Fm, Fm' = maximal level of fluorescence in the dark and light-adapted state.

F0, F0' = minimal level of fluorescence in the dark and light-adapted state.

Fv, Fv' = variable fluorescence.

2.9. Chlorophyll and carotenoids

Randomly selected leaf samples from each experimental unit were placed in liquid nitrogen, leaf extract by acetone 80 % was extracted. then the amount of chlorophyll a, b, total chlorophyll, and carotenoids according to Wellburn (1994) and Xu et al. (2019) were measured at 646, 663, and 470 nm wavelengths by spectrophotometer (model Spekol 2000, Analytic Jena Co, Germany).

2.10. Antioxidant enzymes

After grinding the frozen leaves in liquid nitrogen, they were homogenized in 50 mM sodium phosphate buffer (pH 7.0) containing 2 mM EDTA, 5 mM mercaptoethanol and 4 % (w/v) polyvinylpyrrolidone_40 (PVP-40). The material was centrifuged for 20 min at 20,000 rpm at 4 °C (Model 3K30 manufactured by Sigma, Germany). Supernatant was used to evaluate antioxidant enzymes (CAT, SOD, POD).

2.10.1. Catalase (CAT), Peroxidase (POD), Superoxide dismutase (SOD)

To measure the catalase enzyme, the method of Aebi (1984) was used by sodium phosphate buffer and reaction to changes in hydrogen peroxide at a wavelength of 240 nm by spectrophotometer machine (Model Spekol 2000, Analytic Jena Co, Germany). According to MacAdam et al. (1992), to measure peroxidase enzyme by) using sodium phosphate buffer and guaiacol due to enzymatic changes, the presence of hydrogen peroxide as an electron acceptor was measured by spectrophotometer at 470 nm. Activity of superoxide dismutase enzyme was evaluated by the method of Dhindsa et al. (1981) based on the inhibition of light reduction of nitroblutetrazolium (NBT) at 560 nm.

2.11. Hydrogen peroxide (H_2O_2)

Hydrogen peroxide (H_2O_2) was measured According to method of Loreto and Velikova (2001). For this purpose, 0.5 g of leaf sample was pounded with liquid nitrogen in a container and mixed with 5 ml of 1 % trichloroacetic acid (TCA), then, the samples were centrifuged at 4 °C for 15 min at 12,000 rpm. After that 0.75 ml of supernatant was mixed with 0.75 ml of 10 mM potassium phosphate buffer (pH 7) and 1 ml of 1 M potassium iodide solution (KI). The concentration of hydrogen peroxide in the samples was calculated by a spectrophotometer at 390 nm with a standard curve.

2.12. Malondialdehyde (MDA)

Malondialdehyde concentration was measured by the method of Zang and Qiu (2004). Crush 0.5 g of the frozen samples in liquid nitrogen and add 5 ml of 1 % trichloroacetic acid (TCA) and mixed, then was centrifuged (Model 3K30 made by Sigma, Germany) for 5 min at 14, 000 rpm. Next, 2 ml of supernatant was mixed with 2 ml of 0.5 % thiobarbituric acid (TBA) and the mixture was heated for 30 min (Bain--marie) at 95 °C, after that, it was quickly cooled and centrifuged for 10 min at 10,000 rpm. Finally, the samples were measured by spectrophotometer at 450, 532 and 600 nm and the concentration of malondialdehyde was calculated based on the following formula as nmol g^{-1} FW:

$$\text{MDA} = 6{\cdot}45 \times (A532 - A600) - 0{\cdot}56 \times A450$$

2.13. Proline

According to Bates et al. (1973), 0.5 g of fresh leaf sample was homogenized with 3 % (w/v) liquid sulfosalicylic acid. Then, with ninhydrin reagent, and glacial acetic acid was incubated at 100 °C for 1 h. The reaction was arrested in an ice bath the concentration of soluble proline in liquid toluene was measured by spectrophotometer at 520 nm.

2.14. Leaf Soluble protein and carbohydrates

Bradford (1976) method was used to measure soluble proteins. Gently mix 10 μl of the test sample (extract) with 990 μl of Bradford solution and then the absorbance was read with a spectrophotometer at 595 nm. After sampling the leaves in the early stage of grain filling, the amount of water-soluble carbohydrates was measured according to the method of Dubois et al. (1956).

2.15. Leaf phosphorus, Leaf, and grain nitrogen

To determine the leaf P by yellow Vanadate/molybdate method (Kitson and Mellon, 1944) after dried leaves were heated at 550 °C and using 1 M hydrochloric acid, and then the extract was prepared and after filtering, it was measured at a wavelength of 430 nm. Leaf nitrogen (Early stage of grain filling) and grain (Maturity stage, July 12, 2018 and 2019) was measured by Kjeldahl method (1883). The grain protein was calculated by multiplying the nitrogen by 5.7 (Owusu-Apenten, 2002).

2.16. Statistical analysis

To determine the effect of the treatments over the experiment, Two-year data in a randomized complete block design field experiment with three replications were analyzed (general linear model, GLM) using SAS software. The mean values were compared by Duncan's range test at $P \leq 0.05$. The Pearson correlation between related traits and significant values of the correlation coefficient were reported.

3. Results

According to the combined ANOVA of 2-year data (Tables 3–6), the effect of tillage system on the yield, harvest index (HI), plant height, remobilization, leaf relative water content (RWC), canopy temperature depression (CTD), leaf area index (LAI), specific leaf area (SLA), chlorophyll a, b, total chlorophyll, carotenoids, chlorophyll fluorescence, Enzymes: Catalase (CAT), peroxidase (POD) superoxide dismutase (SOD), malondialdehyde (MDA), hydrogen peroxide (H_2O_2), proline, leaf soluble proteins and carbohydrates, leaf phosphorus and nitrogen concentration, grain nitrogen, grain crude protein, root colonization and fungal sporulation, rhizobium nodules were significant ($P \leq 0.01$). The

Table 3
Combined (2-year data) analysis of variance and means comparisons of some traits (remobilization and yield of chickpea) affected by different tillage treatments.

Source of variation	Remobilization (kg ha^{-1})	Stem efficiency in Remobilization (%)	Contribution of Remobilization in grain yield (%)	Contribution of Photosynthesis in grain yield (%)	Plant height (cm)	Grain yield (kg ha^{-1})	Biologic yield (kg ha^{-1})	HI
Year (Y)	* *	*	*	*	* *	* *	* *	* *
2018	150.7a	33.29b	14.79a	85.2a	36.9a	1053.8a	2380.2a	44a
2019	121.7b	35.82a	15.88a	84.11b	28.3b	797.5b	1852b	42.7b
Tillage (T)	* *	* *	* *	* *	* *	* *	* *	* *
(NT)	123.68b	25.12c	11.05c	88.94a	35.1a	1120a	2328.67a	48a
(RT)	131.08b	33.68b	14.84b	85.15b	32b	887.67b	2095b	42.31b
(CT)	153.95a	44.86a	20.12a	79.87c	30.6c	769.5c	1924.67c	39.88c
Y × T	ns	ns	ns	ns	ns	ns	ns	ns
CV (%)	7.3	6.35	5.02	0.911	3.3	1.44	2.3	1.46

No-tillage (NT); Reduce tillage (RT); Conventional tillage (CT)
Mean values followed by different letters in each column are statistically different based on Duncan's range test at P = 0.05
* and * *, respectively, 5 % and 1 % level of significance, ns is not significant

Table 4
Combined (2-year data) analysis of variance and means comparisons of some physiological traits of chickpea affected by different tillage treatments.

Source of variation	CTD (C°)	LAI (m^2)	SLA (m^2 g^{-1})	Chlorophyll a (mg g^{-1} FW)	Chlorophyll b (mg g^{-1} FW)	Total Chlorophyll (mg g^{-1} FW)	Carotenoids (mg g^{-1} FW)	Fv/Fm	Fv'/Fm'
Year (Y)	*	* *	*	ns	ns	ns	*	* *	* *
2018	2.02a	2.41a	0.0110a	1.72a	0.30a	1.99a	0.234a	78.02a	45.43a
2019	1.91b	1.74b	0.0103b	1.68a	0.28a	1.92a	0.222a	76.61b	42.08b
Tillage (T)	* *	* *	* *	* *	* *	* *	* *	* *	* *
(NT)	3a	2.95a	0.0118a	2.1a	0.39a	2.48a	0.296a	79.96a	49.93a
(RT)	1.76b	1.72b	0.0101b	1.58b	0.27b	1.81b	0.211b	77.13b	39.46c
(CT)	1.13c	1.56b	0.0100b	1.42c	0.21c	1.58c	0.178c	74.85c	41.88b
Y × T	ns	ns	ns	ns	ns	ns	ns	ns	ns
CV (%)	6.11	5.95	5.41	4.16	11.13	5.77	5.26	2.31	0.58

Canopy temperature depression (CTD); leaf area index (LAI); Specific leaf area (SLA) photosynthetic efficiency Night- Day (Fv/Fm; Fv'/Fm'); No-tillage (NT); Reduce tillage (RT); Conventional tillage (CT)
Mean values followed by different letters in each column are statistically different based on Duncan's range test at P = 0.05
* and * *, respectively, 5 % and 1 % level of significance, ns is not significant

Table 5
Combined (2-year data) analysis of variance and means comparisons of relative water content, enzymatic and non-enzymatic activity in chickpea affected by different tillage treatments.

Source of variation	RWC (%)	CAT (Units mg^{-1} Protein min^{-1})	SOD (Units mg^{-1} Protein min^{-1})	POD (Units mg^{-1} Protein min^{-1})	MDA (nmol g^{-1} FW)	H_2O_2 (mmol g^{-1} FW)	Proline (μmol g^{-1} FW)	Leaf Soluble protein (mg g^{-1} FW)	Leaf Soluble carbohydrates (mg g^{-1} DW)
Year (Y)	*	* *	*	* *	* *	* *	* *	* *	* *
2018	66.18a	0.056b	0.52b	0.99b	189.39b	1.00b	0.70b	0.84a	0.40a
2019	65.14b	0.061a	0.57a	1.15a	217.88a	1.15a	0.80a	0.74b	0.31b
Tillage (T)	* *	* *	* *	* *	* *	* *	* *	* *	* *
(NT)	73.78a	0.05c	0.39c	0.88c	175.69c	0.89c	0.49c	0.91a	0.49a
(RT)	63.72b	0.06b	0.55b	1.11b	201.49b	1.02b	0.79b	0.80b	0.32b
(CT)	59.48c	0.066a	0.69a	1.22a	233.72a	1.32a	0.98a	0.66c	0.24c
Y × T	ns	ns	ns	ns	ns	ns	ns	ns	* *
CV (%)	1.51	3.25	7.36	6.66	2.67	3.46	3.79	5.64	2.37

Relative water content (RWC); Catalase (CAT); superoxide dismutase (SOD); peroxidase (POD); Malondialdehyde (MDA); Hydrogen peroxide (H2O2); No-tillage (NT); Reduce tillage (RT); Conventional tillage (CT)
Mean values followed by different letters in each column are statistically different based on Duncan's range test at P = 0.05
* and * *, respectively, 5 % and 1 % level of significance, ns is not significant

effects of "year * tillage" on the leaf water soluble carbohydrates were significant (Table 5) ($P \leq 0.01$). Chlorophyll a, chlorophyll b, total chlorophyll, root colonization, sporulation of mycorrhizal fungi and the number of rhizobium nodules were not significantly different in both years (Tables 4 and 6) ($P > 0.05$).

3.1. Yield-related traits

The highest amounts of grain yield (1120 kg/ha), biological yield (2328.67 kg/ha) and harvest index (48 %) were obtained from NT system. The lowest grain yield (769.5 kg ha^{-1}), biological yield (1924.67 kg ha^{-1}) and harvest index (39.88 %) was obtained from CT. In this study, the grain yield in NT and RT were respectively higher by 31 % and 13 % compared to CT. These increases were 17 % and 8 % for biological yield, and 17 % and 6 % for harvest index, respectively. Grain yield, biological yield and harvest index in the first year were significantly higher than the second year (Table 3). The maximum plant height (35.1 cm) was observed under NT system, which showed about of 3.1 and 4.5 cm increasing compared to RT and CT, respectively (Table 3).

Table 6
Combined (2-year data) analysis of variance and means comparisons of mycorrhizal fungi, rhizobia nodules, leaf elements and grain protein of chickpea affected by different tillage treatments.

Source of variation	Leaf N (%)	Leaf P (%)	Grain N (%)	Grain crude protein (%)	Colonization (%)	Number of Spores (No, per 10 g Soil)	Number of rhizobium nodules (No, per Plant)
Year (Y)	* *	* *	* *	* *	ns	ns	ns
2018	3.22a	0.52a	4.18a	23.87a	23.11a	121.55a	24a
2019	2.84b	0.45b	3.68b	20.82b	23.44a	127.11a	22.3a
Tillage (T)	* *	* *	* *	* *	* *	* *	* *
(NT)	3.43a	0.54a	4.14a	23.4a	35.16a	211.66a	41.66a
(RT)	2.92b	0.47b	3.88b	22.03b	21.5b	109.5b	22b
(CT)	2.73c	0.43c	3.77c	21.6c	13.16c	51.83c	5.83c
Y × T	ns	ns	ns	ns	ns	ns	ns
CV (%)	2.76	2.36	1.27	0.61	8.79	8.34	10.64

No-tillage (NT); Reduce tillage (RT); Conventional tillage (CT)
Mean values followed by different letters in each column are statistically different based on Duncan's range test at P = 0.05
Nitrogen (N); phosphorus (P); * and * *, respectively, 5 % and 1 % level of significance, ns is not significant

3.2. *Mycorrhizal and rhizobial symbiosis*

In this study, the highest colonization, sporulation, and the number of rhizobium nodules on chickpea root were under NT system, which is significantly more than RT and CT systems (Table 6). The number of rhizobium nodules under NT and RT systems increased by 85 % and 47 %, respectively, compared to CT. These increases for sporulation were 75 % and 48 %, respectively. The percentage of root colonization in NT was 13.66 % and 22 % were higher than RT and CT, respectively. Based on the results, a significant correlation ($r = 0.96^{**}$) was observed between leaf phosphorus and mycorrhizal fungus colonization, as well as leaf nitrogen and the number of rhizobium nodules ($r = 0.88^{**}$) (Fig. 1-C, D).

3.3. *Remobilization*

According to the results (Table 3), the highest remobilization was observed under CT (153.95 kg ha^{-1}), followed by RT (131.08 kg ha^{-1}) and NT (123.68 kg ha^{-1}). Remobilizations in RT and NT, with no significant differences, were respectively by 14.8 % and 19.6 % lower than CT. In NT, the contribution of current photosynthesis in yield (88.94 %) was significantly higher than the other two treatments (RT, CT), so that CT (79.87 %) had the lowest one. On the contrary, the increasing order of contribution of remobilization in grain yield was for NT (11.05 %), RT (14.84 %) and CT (20.12 %). Likewise, the remobilization, CT (44.86 %), RT (33.68 %) and NT (25.12 %) showed the decreasing trend in the percentage of stem efficiency in remobilization. Due to climatic conditions (Table 1), the rate of remobilization was higher in the first year of the experiment, but the efficiency of stem in remobilization was higher in the second year (Table 3).

3.4. *Physiological traits*

3.4.1. *Relative water content (RWC), Canopy temperature depression (CTD), Leaf area index (LAI), specific leaf area (SLA)*

Based on the results (Table 4), the highest leaf relative water content (RWC) of chickpea was observed in NT (73.78 %) followed by RT (63.72 %) and CT (59.48 %), respectively. Also, the highest CTD was in NT (3ºC) and the lowest was in CT (1.3ºC), which showed a positive and significant correlation ($r = 0.98^{**}$) with RWC (Fig. 1-M). According to the results, LAI obtained from NT (2.95) was significantly higher compared to RT and CT. Also, the SLA of the plants under CT and RT systems had no statistical difference, but SLA in both tillage was significantly lower than NT (SLA = 0.0118) (Table 4).

3.4.2. *Chlorophyll fluorescence (Quantum efficiency of photosystem II)*

The highest quantum efficiency in day and night (light and darkness) was related to NT followed by quantum efficiency in RT and CT at night, identically. However, during the day the efficiency of CT was higher than RT (Table 4). In this study, a significant correlation ($r = 0.95^{**}$) was observed between the maximum quantum efficiency of photosystem 2 and total chlorophyll (Fig. 1-E).

3.5. *Biochemical responses*

3.5.1. *Chlorophyll and carotenoids*

The concentration of chlorophyll a, b and total chlorophyll, were identically occurred at RT and NT compared to conventional tillage. The highest amount of leaf carotenoids was in NT (0.296 mg g^{-1} FW) with decreasing trends for RT (0.211 mg g^{-1} FW) and CT (0.178 mg g^{-1} FW), respectively (Table 4). According to the results (Fig. 1-L), a significant correlation ($r = 0.89^{**}$) was observed between the total chlorophyll and grain nitrogen percentage.

3.5.2. *Antioxidant enzymes*

Means comparison of enzymes data (Table 5) showed that the highest amount of catalase (CAT), superoxide dismutase (SOD) and peroxidase was observed in the plants under in CT system, other tillage systems including: RT and NT were in the lowest category. In conventional tillage (CT), CAT (24.2 %), SOD (43.4 %) and POD (27.8 %) increased compared to NT, and however CAT (9 %), SOD (20.2 %) and POD (9 %) were higher than RT. In this study, a significant correlation was observed between the RWC and antioxidant enzymes (Fig. 1-G-I-J).

3.5.3. *Malondialdehyde (MDA), Hydrogen peroxide (H_2O_2) and proline*

According to the results, plants under CT system had the highest levels of H_2O_2, MDA and proline, and the lowest ones were similarity obtained from RT and NT. The amount of H_2O_2 in RT and NT were respectively 22.7 % and 32.5 % lower than CT. The reductions were 13.7 % and 24.8 % for MDA, 19.3 % and 50 % for proline, respectively (Table 5). A significant correlation ($r = 0.97^{**}$; $r = 0.87^{**}$) was observed between the RWC with H_2O_2 and proline (Fig. 1-F-H).

3.5.4. *Leaf carbohydrates and soluble proteins*

The highest concentration of leaf soluble protein (0.91 mg g^{-1} FW) and carbohydrates (0.49 mg g^{-1} DW) were obtained from plants growing under NT system and their values were statistically lower in RT and CT systems, respectively (Table 5).

3.5.5. *Leaf phosphorus and nitrogen, Nitrogen and crude grain protein*

The highest levels of leaf nitrogen (3.43 %), leaf phosphorus (0.54 %) and grain nitrogen (4.14 %) were obtained under NT and their values, which were lower in RT and CT systems, respectively. In this study, leaf nitrogen and phosphorus in NT and RT increased significantly compared to CT. The lowest crude protein content of chickpea grains showed an increasing trend for CT (21.6 %), RT (22.03 %) and NT (23.4 %)

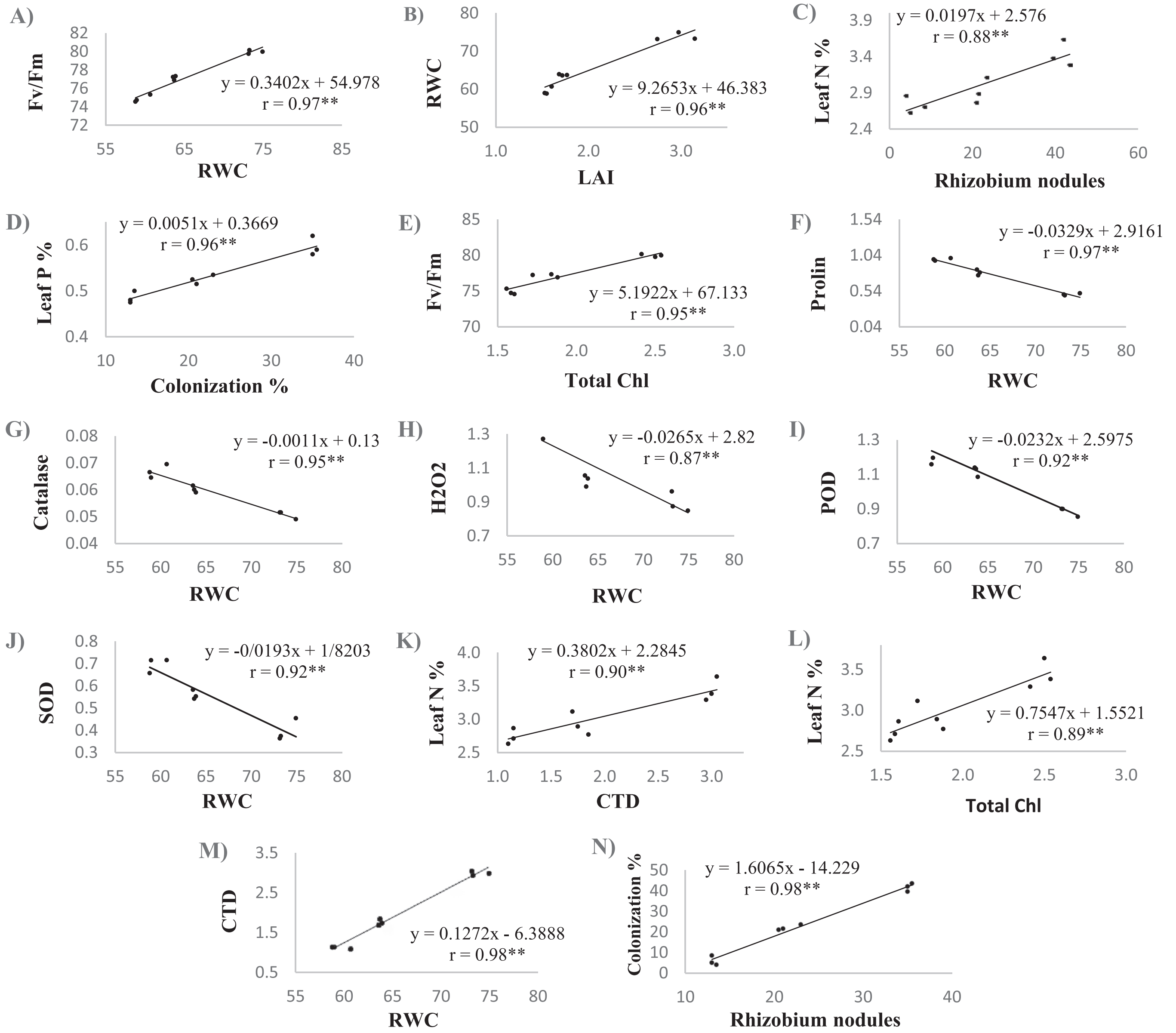

Fig. 1. Correlation of studied traits A: Quantum efficiency of photosystem II (Fv/Fm) and relative water content (RWC); B: relative water content (RWC) and leaf area index (LAI); C: Number of Rhizobium nodules and leaf nitrogen; D: colonization of mycorrhiza fungus and leaf phosphorus; E: Quantum efficiency of photosystem II and total chlorophyll; F: and relative water content (RWC) and proline; G: RWC and Catalase (CAT); H: RWC and hydrogen peroxide (H_2O_2); I: RWC and Peroxidase (POD); J: RWC and Superoxide dismutase (SOD); K: Canopy temperature depression (CTD) and leaf nitrogen; L: Total chlorophyll and leaf nitrogen; M: RWC and CTD; N: Number of Rhizobium nodules and mycorrhizal fungus colonization * and * *, respectively, 5 % and 1 % level of significance, based on Table of Critical Values for Pearson's r.

(Table 6). In this experiment, a positive and significant correlation ($r = 0.90^{**}$) was observed between leaf nitrogen content and CTD (Fig. 1-K).

4. Discussion

4.1. Yield -related traits

In this experiment, the yield increased with decreasing tillage, which probably due to the NT and the presence of plant residue, resulted in more available moisture to the plant and less exposed to water deficiency. In the same study Piggin et al. (2015) declared that among legume crops, chickpea has the highest adaptation under NT and observed increase grain yield in NT compared to CT Which is consistent with the results of this study. Other researchers (Zhang et al., 2012) have suggested that the use of conservation tillage (including NT and RT) instead of CT can increase crop yield (8–35 %) and water use efficiency, which is more significant under drought and rainfed conditions. In this regard as well Hemmat and Eskandari (2004, 2006), in their studies in Iran, reported a 24–57 % increase in chickpea yield in NT compared to RT and CT because of higher storage and greater access of plants to water. In this study, we have also obtained a 13 % and 31 % increase yield of chickpea in RT and NT, respectively, compared to CT. Moreover, Kaschuk et al. (2010) reported increase chickpea yield in NT, but Chaieb et al. (2020) reported increase wheat yield under RT compared to CT systems. In the present study, the reasons for lower height in CT can be related to deficiency of leaf water content, chlorophyll reduction, and efficiency of photosystem (Tables 4,5). Furthermore, the presence of mycorrhizal fungus (Table 6) can be resulted from increasing phosphorus and nutrients uptake. Moreover, Auge et al. (2001, 2015) also pointed to the role of mycorrhizal fungi in better nutrition and plant growth. In particular, with regard to this study the height of chickpea under RT and NT systems, 2 and 5 cm was higher than CT respectively, Borstlap and Entz (1994) also showed that the height of the chickpea (7–12 cm) will be higher under NT than CT. Lafond and Loeppky (1988) observed a 4 cm increase in plant height in NT compared to CT. Mafakheri et al. (2010) also reported a decrease in chickpea height due to water deficit. Changes in plant height in two years can be due to the greater number of days with low temperatures in spring (Table 1).

4.2. Mycorrhizal and rhizobial symbiosis

One of the most important factors to help the growth of plants of the legume family is their symbiosis with a variety of fungi and soil bacteria. Mycorrhizal fungi, by spreading their mycelium to increase the uptake of water and minerals for the plant, improve the growth condition and also increase the uptake of more nutrients from the rhizosphere (Auge et al., 2015; Auge, 2001). Also, the symbiosis of rhizobium bacteria has a very important role in nitrogen fixation and helps the growth of chickpea (Torabian et al., 2019). In this study, based on the above results by other researchers it is possible that in NT due to no turning the soil, the protection of mycorrhiza mycelium, and proper growth with better fixation by rhizobium bacteria, the symbiosis percentage was higher, which helped to better absorb water and nutrients in chickpeas. However, in CT, due to soil plowing and exposure of microorganisms to sunlight, the plant showed poor symbiosis (Table 6). In this regard, Rosner et al. (2020) also reported an increase in the colonization of mycorrhizal fungi with chickpea root in NT and RT compared to CT. The positive effects of NT have been reported on the population of soil microorganisms, especially mycorrhizal fungi and rhizobium bacteria (Torabian, 2019). Accordingly, in this experiment, due to better growth conditions for mycorrhizal fungi in NT and RT, fungal reproduction and spore production were in more favorable conditions than CT. In this study, a positive relationship was significantly observed between the number of rhizobium nodules and mycorrhizal colonization (Fig. 1-N). It means NT system can support soil micro-organisms due to a lack of tillage preservation. The significant increase in the number of rhizobium nodules under NT and RT (Table 6) obtained in this experiment which was also proved by Dogan et al. (2012) in soybean and Lopez-Bellido et al. (2011a, 2011b) in chickpea and faba bean plant roots. According to the reports of Torabian et al. (2019), conservation tillage specifically improves node production and nitrogen fixation due to the increase in soil moisture. Reiter et al. (2002) stated that NT and RT systems are likely to increase biological nitrogen fixation in chickpea roots compared to CT. Mycorrhizal symbiosis of chickpea root was improved in both two years (Table 6), regardless of climatic conditions (Table 1) and according to its needs. However, it seems that changes in the number of rhizobium nodules were very low in both years (7 %) despite climate change (Table 6).

4.3. Remobilization

Due to the fact that chickpea plants grown under CT system had a higher canopy temperature and less water in their leaves; therefore, they were probably more exposed to stress and had more remobilization from the stem to compensate for the lack of current photosynthesis during grain filling. Also, due to plant residues in the soil surface and higher plant moisture in RT and NT systems, the rate of current photosynthesis was higher and remobilization was less (Table 3); thus, the efficiency of the stem due to the stress caused by material transfer and grain filling was significantly higher in CT, especially under stress condition. Decreased chlorophyll a and b, as well as lower photosynthetic efficiency (Tables 3,4), proved this matter. In this study, weather conditions (Table 1) can be one of the reasons for the difference in remobilization between two years (Table 3). Hemmat and Eskandari (2006) also reported better growth of chickpea plants due to higher soil moisture in NT. Schiltz et al. (2005) stated the importance of chickpea replanting during grain filling, which coincides with water stress. Other researchers have evaluated the increase in remobilization in drought stress compared to favorable conditions in some crops. (Papakosta and Gagianas, 1991; Schiltz et al., 2005; Masoni et al., 2007).

4.4. Physiological traits

4.4.1. Leaf relative water content (RWC), Canopy temperature depression (CTD), Leaf area index (LAI), specific leaf area (SLA)

leaf relative water content (RWC) is an important indicator of soil water absorption by roots (Schonfeld et al., 1988), and it is one of the most important traits for showing the proper activity of plants. (Kaushal and Wani, 2016). It is possible that chickpea with high RWC due to higher soil moisture to uptake more water. Likewise, in the present experiment, plants grown under NT system had a higher RWC than the other two tillage systems (Table 5). Other researchers have reported increased soil water in NT (Zhang et al., 2012; Hemmat and Eskandari, 2006). Due to the no turning the soil in NT and more root penetration plus plant residue, likely the plant had more availability to soil moisture and had been more leaf water. Therefore, higher RWC in chickpeas under NT and RT systems may be obtained due to more soil water availability. Increased soil moisture and more water in plants owing to conservation tillage and NT have also been reported by other researchers (Sapkota et al., 2014; Safari et al., 2014; Lampurlanés et al., 2016). Also, in this study according to our focus on plant moisture, more water uptake and consequently higher RWC could be due to the high symbiosis of mycorrhizal fungi in RT and NT compared to CT (Tables 5, 6). It was found that the canopy temperature is directly related to tillage, so the lower tillage showed the greater difference between canopy temperature and leaf temperature (Table 4). In this regard, researchers have reported that the higher RWC in NT through more root penetration, plant residues, water uptake as a result of better gas exchange causing the CTD (Fang and Xiong, 2015; Roohi et al., 2015). Blum (1988) and Hatfield et al. (1987) also reported lower canopy temperatures due to higher soil moisture in their experiments. Roohi et al. (2015) in their studies reported a significant difference in canopy temperature due to drought stress and its relationship with soil moisture at the time of filling wheat, barley and aqueous triticale. Balota et al. (1993) found a positive and significant relationship between yield and CTD. The relationship between leaf relative water content and stomatal exchanges and plant roots has been investigated in other studies (Gupta et al., 2001; Fang and Xiong, 2015).

Leaf area index (LAI), is an important indicator to determine the proper growth status of plants in different conditions. In this study, NT, and RT due to plant residue, soil organic matter and mycorrhizal fungi have caused more water storage in the plant structure (Table 4); therefore, the chickpea plant has been able to expand further and increase its leaf area. A positive and significant correlation ($r = 0.96^{**}$) was also observed in the tillage process between LAI and RWC (Fig. 1-B). In this regard, Wasaya et al. (2017) observed an increase in LAI in RT. Due to the lower RWC in RT and CT, probably in the stage of grain filling, stress has risen and the chickpea plant reduced its leaf area by counteracting it and maintaining moisture, and also increased SLA. In this experiment, chickpeas under NT system had less SLA (Table 4). Emam et al. (2010) also reported a decrease in leaf area due to moisture stress in the bean plant.

4.4.2. Chlorophyll fluorescence (Quantum efficiency of photosystem II)

One of the most important indicators for measuring the health of the photosynthetic cycle is the quantum efficiency of photosystem II (chlorophyll fluorescence), which can be measured without any damage to the plants. In the present experiment, due to dryland conditions and reduction of chlorophyll concentration, leaf nitrogen and water in plants under CT and RT compared to NT (Tables 4,5), quantum efficiency also decreased, which had a straight and significant relationship with these factors (Fig. 1- E-A), Therefore, the activity of fluorescence chlorophyll in dark and light conditions has been significantly affected by different types of tillage (Table 4). In this regard, Kaushal and Wani (2016) reported that a decrease in chlorophyll fluorescence (quantum yield, Fv/Fm) indicates an increase in leaf damage. Khan et al. (2019) also reported that the chlorophyll fluorescence ratio decreases by 50–63 % under the influence of moisture stress, which is consistent with the findings of this study. Roohi et al. (2015) reported a significant relationship between drought stress and chlorophyll fluorescence at the grain filling stage in wheat, barley and irrigated triticale.

4.5. Biochemical responses

4.5.1. Chlorophyll and carotenoids

Pigment concentration is an important indicator of plant growth status and photosynthetic conditions that is strongly related to photosynthetic capacity and therefore chlorophyll content can indicate damage due to different stress and can be considered as a good indicator to determine photosynthesis (Cenzano et al., 2013; Nageswara et al., 2001). In the present study, chickpeas under NT system probably had higher chlorophyll a and b levels due to more moisture, higher nitrogen and phosphorus in leaves (Tables 5,6). The positive and direct relationship ($r = 0.89^{**}$) between leaf nitrogen increase and chlorophyll can also be one of the reasons for the higher amount of chlorophyll in NT than RT and CT systems (Fig. 1-L). Fiorentini et al. (2019) in their experiments observed a significant difference in the concentration of chlorophyll in leaves and tillage systems, as the highest concentration of leaf chlorophyll was in plants under NT and the lowest was in RT and CT systems, respectively. Also, Munyao et al. (2019) in their experiments on bean showed that in the flowering stages and after, the concentration of chlorophyll in conventional tillage decreases compared to zero tillage. The above findings are consistent with the results of this study. Due to the rain-fed condition chickpea plants under RT and CT systems probably were under more drought stress compared to NT (Table 5), which is consistent with the research of Mafakheri et al., (2010, 2011) on reducing the chlorophyll content of chickpea due to moisture stress. Carotenoids are key photosynthetic pigments that act as the major components of light-absorbing antennas in photosynthetic reactions (Zakar et al., 2016). Hence, in our study, chickpeas cultivated under CT and RT systems had less carotenoids because of under stress due to lack of water (Table 5), fewer leaf elements (Table 6) and low efficiency of photosystem II (Table 4) compared to NT. Also, with the increase of reactive oxygen species (Table 5), the photosynthetic activities of the plant have been affected and the chlorophyll concentration and carotenoids decreased. Wang et al. (2010) reported that the carotenoids decreased under stress and could not play their protective role, and that the carotenoids content decreased due to the presence of active oxygen and the destruction of their structure.

4.5.2. Antioxidant enzymes and H_2O_2

Plants have special mechanisms to deal with various biological and non-biological stresses. The activity of reactive oxygen species (ROS) increases during oxidative stress (Reddy et al., 2004). In the present experiment, due to the dryland conditions, the tillage treatment had significant effects on the activity of reactive oxygen species (ROS), which was directly related to the amount of plant moisture and antioxidant enzymes (Fig. 1-G-I-J). Due to the more canopy temperature in chickpeas grown by CT (Table 4), the amount of hydrogen peroxide (H_2O_2) has probably increased due to drought stress, lack of nitrogen, phosphorus and chlorophyll in the leaves, so the plant also neutralizes the enzyme and increased its antioxidants including SOD, POD and CAT (Tables 5,6). Other researchers have reported increased activity of hydrogen peroxide and antioxidant enzymes in environmental stresses (Dat et al., 2000). Probably due to less plowing of the soil and more residue plant, quantum efficiency of photosystem II and high concentration of chlorophyll in RT and NT systems, resulting in the chickpea plant with higher absorption of water than CT system; therefore, less exposed to drought stress and ROS, Eventually, it has lowered the production of antioxidant enzymes. Khan et al. (2019) reported that the levels of catalase, superoxide dismutase and peroxidase enzymes have increased under water deficit in chickpea. Mafakheri et al. (2011) in their study reported the increase of antioxidant enzymes in chickpea plants due to moisture stress. The results of Wang et al. (2018) on the significant increase of antioxidant enzymes due to drought stress are consistent with the findings of this study.

4.5.3. MDA

In this experiment, chickpea plants in CT were exposed to dehydration and temperature stress due to lower RWC (Table 5) and CTD (Table 4), which caused damage to cell membranes and increased MDA but less of it was observed in RT and NT systems. Man et al. (2017) reported that the content of MDA increases in the grain filling stage in terms of maturity due to moisture stress, which is consistent with the results of this experiment. In line with the results of this study, other researchers have also reported low levels of MDA in NT compared to CT systems (Huang et al., 2012).

4.5.4. Proline

Proline is one of the most common compatible osmolytes in plants, whose metabolism is mainly studied in response to drought stress (Verbruggen and Hermans, 2008). In this experiment, proline was directly related to different tillage and RWC (Fig. 1-F). Due to the fact that reducing tillage improves the water status of the plant (Table 5), less proline amino acid was observed under NT system. Chickpea plants under CT due to high canopy temperature also had less activity of mycorrhizal fungi and more proline (Tables 5,6), as the plant reacted to water deficit stress and temperature to regulate osmotic pressure. Other researchers have reported similar results regarding water deficit stress and canopy temperature (Wilkinson and Davies, 2002) and proline concentration (Reddy et al., 2004). Shinde and Singh (2017) also reported an increase in proline due to water deficit stress compared with mycorrhizal plants in sweet corn.

4.5.5. Leaf carbohydrates and soluble proteins

The concentrations of soluble carbohydrates in the leaves can be the result of starch decomposition due to stress or better photosynthetic performance of the plant. Under NT system, due to the high chlorophyll level, higher water content of the plant, and higher quantum yield as well as higher symbiosis with mycorrhizal fungi (Tables 4, 5, 6), the plant has been able to produce more carbohydrates due to more photosynthesis, While the mentioned conditions are less under RT and CT systems, it has reduced the concentration of carbohydrates. These results are consistent with the findings of other researchers (Auge et al., 1987; Auge, 2001; Subramanian and Charest, 1995) in the chickpea plant. It seems that the significant difference between leaf soluble carbohydrates in the two years of study of this experiment is due to differences in temperature and different climatic conditions (Table 1). In CT system, due to the low content of leaf nitrogen, chlorophyll and chlorophyll fluorescence (Tables 4, 6), it seems that the plant had less photosynthesis. Therefore, the concentration of soluble protein in leaves was less than RT and NT systems. Huang et al. (2012) also observed an increase in the concentration of soluble leaf proteins under NT rice fields compared to CT in their experiments, which is consistent with the results of this study. Other researchers have reported the reduction of soluble protein in leaves due to water deficit and photosynthesis (Mafakheri et al., 2011; Shinde and Singh, 2017).

4.5.6. Leaf phosphorus and nitrogen, Nitrogen and crude grain protein

Considering that in this experiment, mycorrhizal fungi colonization and RWC of plants under NT system was better than other tillage systems (Tables 5,6), it is possible that the uptake of elements, especially phosphorus, from the soil was due to higher symbiosis of roots with mycorrhiza fungi (Fig. 1-D). More uptake of phosphorus from the soil by mycorrhizal fungi due to NT system and its significant increase in chickpea compared to CT system, which has also been reported by Rosner et al. (2020). Rahimzadeh and Pirzad (2017) reported that the increase in phosphorus uptake rate by the host plant is due to the presence of numerous branches of mycorrhiza hyphae inside the root cells of the plant, which provides a large area for the transfer of nutrients, especially phosphorus, to the host plant and grain. In this study, the concentration of phosphorus in chickpea leaves cultivated under RT and CT systems is lower than NT. The results correspond with studies by other researchers (Eke et al., 2016; Liu et al., 2007, 2000a) that suggest that mycorrhiza has an important role in absorption and access to nutrients, especially in immobile soil elements such as phosphorus, is consistent. Huang et al. (2012) also reported an increase in phosphorus concentration in rice plants under NT, which is consistent with the results of this experiment. In this study, chickpea under NT system were compared to RT and CT systems due to using mycorrhizal fungi and better nitrogen fixation due to more rhizobium nodules on the root as well as higher RWC (Tables 5, 6). They also provide more nitrogen for leaves and then transfer to the grains; therefore, they also cause an increase in grains protein. In this regard, Torabian et al. (2019) have suggested increase in nitrogen concentration in different parts of the chickpea plant grown under NT system compared to CT system. Subramanian and Charest (1999) also reported that nitrogen uptake was higher in plants whose roots host mycorrhizal fungi and its concentration in plant foliage was 32 % higher than in non-mycorrhizal plants. The results of this experiment are consistent with the report of Dogan et al. (2012) on the reduction of nitrogen in the vegetative organs of the soybean plant under CT compared to RT and NT systems. A significant increase in atmospheric nitrogen fixation under NT system compared to CT has been reported in other studies (Ruisi et al., 2012; Mohammad et al., 2010). Due to the higher concentration of chlorophyll in NT than RT and CT systems in this experiment (Table 4) and its relationship ($r = 0.89^{**}$) with leaf nitrogen (Fig. 1-L) probably influenced increasing leaf nitrogen. Evans (1989) also reported a close relationship between chlorophyll concentration and leaf nitrogen level.

5. Conclusion

In this study, chickpea plants under NT and RT systems showed greater yields and the highest height. Relative water content (as leaf water status) and canopy temperature depression were in order of NT, RT and CT systems. Moreover, leaf area index, as light receiver in plants, were greater in reduced tillage (NT and RT). Under the CT system, the maximum quantum efficiency of photosystem II, the concentration of carotenoids and chlorophyll in the chickpea were the lowest, and they were significantly more under NT and RT systems. For this reason, the stem reserve for remobilization and efficiency of this reserve to the grains is lower. Also, the activity of H2O2 and MDA was more under CT system, which were increased the activity of antioxidant enzymes and proline due to the protection of biochemical processes. The percentage of colonization, sporulation of mycorrhizal fungi, and rhizobium nodules were higher under NT and RT, respectively. In general, the results of this experiment showed that the RT and NT systems in rainfed conditions due to positive morpho-physiological changes in the plant, it seems to be effective in improving growth conditions and increasing yield.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Data Availability

No data was used for the research described in the article.

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