Australian Journal of Crop Science AJCS
AJCS 8(5):646-654 (2014) ISSN:1835-2707

Chickpea (*Cicer arietinum* L.) physiological, chemical and growth responses to irrigation with saline water

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Abstract

Chickpea (*Cicer arietinum* L.) is the third most important food legume grown in the world and a favourite food crop in Morocco. Morocco is a semi-arid country with limited fresh water resources. In order to meet the food demand, increasing attention is being given to the use of non-conventional water resources such as saline/brackish water and treated waste water for irrigation. With this in mind, an experiment was conducted in the south of Morocco to investigate the effect of irrigation with saline water on a local variety of chickpea. Irrigation with water of different salinity levels was carried out on pot experiments. Differences in water uptake and plant growth; as well as proline, soluble sugar, and Na^+ and K^+ contents of the plant were quantified. The results showed a negative relationship between increasing water salinity and most of the measured plant growth parameters. Irrigation water salinity has negatively affected growth and biomass accumulation and led to reduced grain yield, water uptake and water productivity. In contrast, proline, soluble sugars, Na^+ and Na^+: K^+ ratio increased with increasing irrigation water salinity. The findings highlighted the role of proline and soluble sugars as osmolytes produced by chickpea to mitigate the effect of salinity stress. The added value of these results is that the crop's responses to salinity are quantified. The obtained values can be used to determine 'threshold values'; should the salinity of the irrigation water go above these threshold values one may expect the crop yield parameters to be affected. The quantified responses also indicate the rate of change of yield parameters in response to the irrigation water salinity level. This could help in avoiding significant yield reduction when deciding on the irrigation water salinity level to be used for the studied chickpea variety.

Keywords: Chickpea (*Cicer arietinum* L.); irrigation; salinity, crop growth; yield; osmolytes; sugar ; sodium and potassium contents in chickpea; stomatal conductance.
Abbreviations: WP_water productivity, HI_harvest index, g_s_stomatal conductance.

Introduction

Semi-arid Mediterranean countries suffer from low and erratic rainfall, high temperature and high evaporation losses. The limited water supply makes it difficult to meet the food demand of the increasing population. Irrigation in the region accounts for 80% of the total fresh water consumption. In order to meet the increasing water demand for food production, attention was given to the use of non-conventional water resources such as saline drainage water, brackish groundwater and treated waste water (Hirich and Choukr-Allah, 2013; Hirich et al., 2011; 2012 a, b; 2013; 2014a). However the use of poor quality water, especially saline water, requires proper management to safeguard the environment and to avoid soil degradation by salinisation (Hamdy et al., 2003). Saline groundwater is often found at shallow depth in irrigated areas of arid and semi-arid regions and is associated with problems of soil salinisation and land degradation (Gowing et al., 2009). Van Weert et al. (2009) reported that the total area where groundwater salinity at shallow or intermediate depth occupies 24 million km^2. This is about 16% of the total land area on earth. According to FAO (2006) the global cost of irrigation-induced salinity is equivalent to an estimated US$11 billion per year. In the Mediterranean region about 1 million ha of irrigated land is affected by secondary salinity induced by poor irrigation management practices (Grove, 1999). In the past, saline water was considered not to be suitable for irrigation. However, but the fresh water shortages, the new gained knowledge about salinity tolerance of crops, and the introduction of new irrigation systems, irrigation strategies and management made the use of saline water for irrigation possible. There are already reports that these new practices have been successfully adopted (Rhoades, 1989; Flowers et al., 2005; Abdel Gawad et al., 2005; Malash et al., 2008 and 2011). In areas where

saline water is the only available water resource, it has already been used for a long time and the local population has learned, most probably by trial and error, the conditions and limitations of its use (Van Hoorn et al., 1997). Chickpea (*Cicer arietinum* L.) is the third most important food legume grown in the world with 12 million ha is cultivated, producing a total grain yield of 11 million ton. Chickpea is grown in over 45 countries (FAOSTAT, 2010). In the Mediterranean countries, chickpea is one of the favourite legumes and an essential part of the diet in some countries such as Morocco, Tunisia, Lebanon and Syria. Chickpea has received the attention of many researchers not only for being one of the primary legume crops but also because of its relatively high protein content (Zia-Ul-Haq et al., 2007; Clemente et al., 1999; Chang et al., 2011). The reluctance towards using saline water for irrigation has possibly led to relatively few publications (Grewal, 2010; Van Hoorn et al., 2001; Katerji et al., 2005a; Katerji et al., 2005b; Samineni et al., 2011; Rasool et al ., 2012 ; Singh, 2004 ; Flowers et al., 2010 ; Vadez et al., 2007). The main objective of this study was to investigate the effect of irrigation water salinity level on the proline, soluble sugar, and sodium and potassium content of chickpea; and to evaluate the effect of irrigation water salinity on chick pea water uptake, plant growth, water productivity, dry matter production and grain yield. The response of different crop parameters to different salinity levels will be quantified and the salinity threshold values, above which significant change is expected, will be identified. These threshold values have practical implications in managing saline water and deciding on the salinity level that would not significantly affect the yield and crop growth. In the Mediterranean region and in Morocco in particular, chickpea is often irrigated with fresh water; this research will improve our knowledge about the possible use of saline water, will illustrate the possibility of using saline water for irrigating chickpea and will quantify the yield in relation to salinity level. Without such quantification, farmers in the Mediterranean region and in Morocco in particular, will not have sufficient knowledge and will be reluctant to use saline water to irrigate chickpea despite the tremendous availability of saline/brackish water in the region.

Results

The following sections will highlight the response of chickpea to different levels of irrigation water salinity in terms of water uptake, dry matter production, yield and water productivity, stomatal conductance, proline and soluble sugar content in leaves and roots, and sodium and potassium accumulation in leaves.

Water uptake

The daily water uptake during the whole growth period for the four water salinity levels is shown in Fig. 1(a), while the cumulative values are shown in Fig. 1(b). The water uptake is expressed in ml pot^{-1} and L pot^{-1} for daily and cumulative values, respectively. Both figures show that the water uptake was affected by irrigation water salinity. Increasing levels of irrigation water salinity significantly decreased the water uptake ($p < 0.05$) and the differences between the treatments became more pronounced during the late growth stages. Compared with the control treatment (T1), the total water uptake was reduced by 31%, 61% and 73% by using saline water with electric conductivity of 4 dS m^{-1} (T2), 7 dS m^{-1} (T3) and 10 dS m^{-1} (T4), respectively.

Dry matter production

The irrigation water salinity level had an impact on the dry matter of chickpea. Figure 2 shows that the dry matter production was significantly affected by irrigation water salinity ($p < 0.01$). The highest dry matter was obtained from the control treatment, irrigated with fresh water. An increase in irrigation water salinity caused a significant decline in dry matter weight. Differences in dry matter weight between the treatments increased and became more pronounced at later stages of crop growth.

Yield and water productivity

The effect of irrigation water salinity on dry matter, root volume, grain yield, harvest index (HI) and water productivity (WP) is reported in Table 1. The results show that the highest dry matter weight has been recorded under the control treatment (T1). In comparison with the control treatment, the biomass production using irrigation water with salinity levels of 4, 7 and 10 dS m^{-1} was reduced by 43, 75 and 84%, respectively. The effect of irrigation water salinity on grain yield was more pronounced than the effect on biomass. In comparison with the control treatment, grain yield was reduced by 47% when irrigating with water of EC of 4 dS m^{-1} (T2). Severe yield reduction was obtained when irrigating with water of salinity level equal to 7 dS m^{-1} (T3) or 10 dS m^{-1} (T4) which resulted in a grain yield amounting to only 10% of that obtained under fresh water treatment (T1). The harvest index (HI) varied greatly with increasing irrigation water salinity. The highest HI was obtained under control treatment (T1), followed by treatments T2 (4 dS m^{-1}), T3 (7 dS m^{-1}) and T4 (10 dS m^{-1}) as the grain yield reduced with increasing irrigation water salinity. Water productivity (WP) was calculated by dividing grain yield by total evapotranspiration. The water productivity was also negatively affected by irrigation water salinity; the highest water productivity of 0.5 kg m^{-3}, was obtained under the control treatment (T1) followed by the T2 treatment with 0.4 kg m^{-3}, and the then by T3 and T4 treatments, both having a productivity of 0.1 kg m^{-3} .

Stomatal conductance (gs)

Exposed to increasing irrigation water salinity, chickpea exhibited a significant decrease in stomatal conductance (g_s) both during growth period (Fig. 3 a) and during the day (Fig. 3 b). During the growth period there was a reduction in the g_s parameter with the increasing irrigation water salinity. Starting from 26 days after sowing, there was a significant difference between treatments and that difference was maintained until the end of the chickpea growth season. The most salinity stressed treatment T4 (10 dS m^{-1}) showed the lowest stomatal conductance and the control treatment T1 (1 dS m^{-1}) showed the highest stomatal conductance. At daily scale, as on day 45th (Fig. 3 b), the difference between treatments was quite obvious during the whole day. For all treatments, the highest stomatal conductance was recorded at 11:00 a.m., it then started to decline towards the end of the day.

Table 1. Dry biomass, root volume, grain yield, harvest index and water productivity (WP) for tested saline treatments.

Treatments	T1	T2	T3	T4
Dry biomass (g pot^{-1})	50.4 a	28.8 b	12.6 c	8.0 c
Root volume (cm^3)	15.9 a	4.2 b	3.5 b	1.3 c
Grain yield (g pot^{-1})	23.0 a	12.3 b	2.4 c	0.7 c
Harvest Index (%)	42.0%	37.1%	11.6%	4.5%
Water Productivity (kg m^{-3})	0.5 a	0.4 a	0.1 b	0.1 b

Letters a, b and c indicate the statistically homogeneous groups. Significant differences have been obtained for all presented parameters.

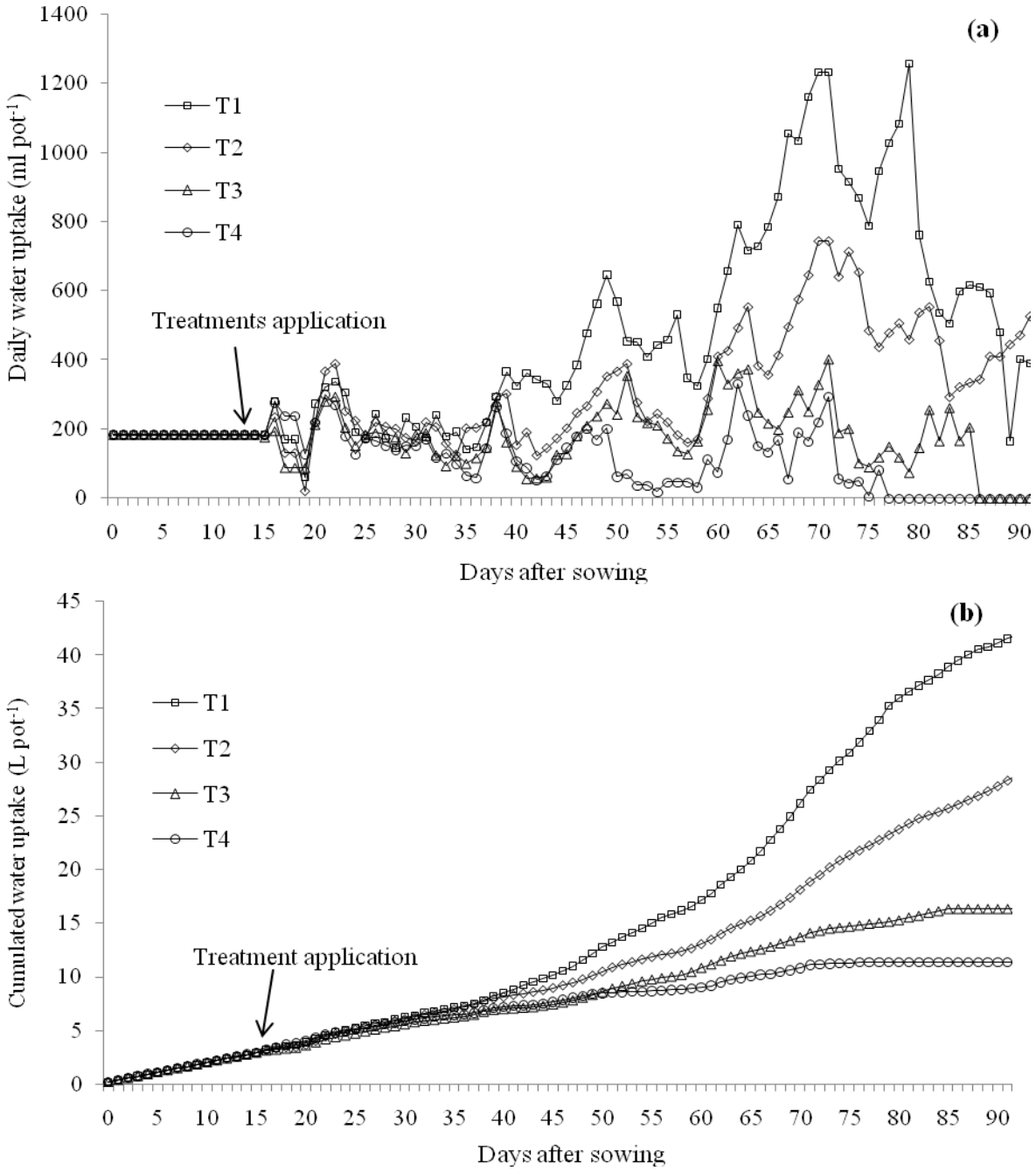

Fig 1. Daily water uptake (a) and cumulated water uptake (b) during the crop growth season.

Proline content in leaves and roots

The concentration of proline in leaves and roots was significantly affected by salinity, as shown in Fig. 4. Proline concentration both in leaves (Fig. 4 a) and roots (Fig. 4 b) has been increased as irrigation water salinity increased. Treatment T4 (EC of irrigation water 10 dS m^{-1}) led to high proline accumulation both in leaves and roots compared to the less saline treatments. Proline concentration in all investigated treatments increased slightly during the crop growth period till 60 days after sowing then declined sharply during the remaining 20 days of the season.

Soluble sugars in leaves and roots and Na^+ and K^+ accumulation in leaves

Data related to soluble sugar concentration in leaves, and roots and Na^+ and K^+ ion contents in leaves are shown in Table 2. For all treatments, the soluble sugars concentration both in leaves and roots increased during crop growth but there were significant differences between treatments as the increase in soluble sugars in leaves and roots was more pronounced with increasing salinity of the irrigation water. The highest soluble sugars concentration was recorded for the treatment with water having an EC of 10 dS m^{-1}. There was a clear relationship between soluble sugars content and irrigation water salinity level. Data indicated that soluble sugars concentration in leaves is higher than in roots. Results regarding the Na^+ and K^+ ion contents in leaves indicated that the Na^+ and K^+ ion concentration, as well as the Na^+:K^+ ratio, were affected by the irrigation water salinity. The higher the water salinity level, the higher were the Na^+ and K^+ levels accumulating in the plant as well as the Na^+: K^+ ratio. The Na^+ content in leaves declined in the period from 40 to 50 days after sowing while the K^+ content increased. Subsequently, there was an increase in the Na^+:K^+ ratio during the same period. At 40 days after sowing, Na^+ and K^+ contents for the investigated treatments were almost equal, with Na^+: K^+ ratio of 0.8 to 1, depending on the salinity of the irrigation water. Ten days later the Na^+: K^+ ratio increased greatly with the increase in salinity level due to the increase in the Na^+ values added through saline water.

Discussion

Salinity is known to have a dual effect on plant growth via the osmotic effect on plant water uptake and the specific ion toxicities (Sheldon et al., 2004). The plant's access to soil water is usually decreased by decreasing the osmotic potential of the soil solution. This is mainly due to the decrease in total soil water potential. Reduced osmotic potential due to increased salinity has been reported to have a negative effect on water and nutrient uptake of chickpea (Grewal, 2010; Sheldon et al., 2004; Hirich et al., 2014b) and other plant species (Razzaghi et al., 2011; Gowing et al., 2009; Katerji et al., 1997). This study clearly showed that irrigation water salinity had a negative effect on plant growth and productivity of chickpea. This finding is in line with some other published work. Flowers et al. (2010) reported that the recent screenings of diverse germplasm suggested significant variation of seed yield under saline conditions. Samineni et al. (2011) demonstrated that chickpea is sensitive to salinity at both the vegetative and reproductive phase, with pod formation being particularly sensitive. Vadez et al. (2007) reported that a screening of 263 accessions of chickpea, including 211 accessions from ICRISAT's mini-core collection showed a 6-fold range of variation for seed yield under salinity (1.9 L of 80 mM NaCl per 7.5 kg Vertisol), with several genotypes yielding 20% more than a previously released salinity tolerant cultivar. Several studies reported that salinity significantly affected chickpea growth and led to early senescence (Katerji et al., 2001; Katerji et al., 2005b). Reduction in yield under elevated salinity may be the result of various factors acting simultaneously like the decline in leaf area and the subsequent reduction in the photosynthesis and stomatal conductance, which would result in a reduction in the accumulated biomass (Samineni et al., 2011; Katerji et al., 2005b; Katerji et al., 2000; Grewal, 2010; Singla and Garg, 2005). Negative effects of salinity on the harvest index and water productivity seemed to be directly correlated with reduced plant biomass production, water uptake and grain yield. A possible explanation for this reduction could be attributed to Cl^- toxicity. Moreover, the reduction in extracting soil water under saline conditions usually results in reduced growth (Sheldon et al., 2004) as plants try to reduce water losses by reducing their canopy size and the Leaf Area Index (LAI). In response to salinity, plants make new amino acids that help them to grow and develop under saline conditions (Goudarzi and Pakniyat, 2009). Among those amino acids, proline is known to build up widely in higher plants and accumulates in large quantities in response to salinity to protect the cell and minimize the salt induced damage (Ábrahám et al., 2003; Shafi et al., 2011). The finding of this research indicated that proline levels, both in the leaves and the roots, have been affected by the increased irrigation water salinity. It was previously reported that salt stress results in extensive proline accumulation in chickpea (Eyidogan and Öz, 2007; Soussi et al., 1998). Rasool et al. (2012) reported that proline and protein contents increased with the increase in salt concentration. Singh (2004) studied the water salinity impact during the germination of chickpea. He reported that at maximum salinity stress there was comparatively more accumulation of protein, proline and phenol in tolerant genotypes. In addition, proline content has been reported to increase under NaCl stress in peas (Ahmad et al., 2008), faba bean (Gadallah, 1999), lentil (Misra and Saxena, 2009), tomato (Ali et al., 2011; Amini and Ehsanpour, 2005), pepper (Chookhampaeng, 2011), sugar beet (Farkhondeh et al., 2012), rice and maize (Turan et al., 2009). The present research evaluated the effect of irrigation water salinity on soluble carbohydrates, such as sugars. They are organic compounds that the plant uses for osmotic adjustment and osmo-protection (Gil et al., 2011; Reina-Sanchez et al., 2005; Hirich et al., 2014 b). The obtained results indicated that there is an increase in soluble sugars concentration with the increased water salinity. Thus, sugar contents can be used as physiological markers of salt tolerance in chickpea. Several studies suggested that the accumulation of soluble carbohydrates was significantly related to salt tolerance (Ahmad et al., 2006; Almodares et al., 2008; Leatherwood et al., 2007; Muscolo et al., 2003; Teimouria et al., 2009; Yin et al., 2010; Zhang et al., 2012; Reina-Sanchez et al., 2005). Singh (2004) studied the water salinity impact during the germination of chickpea.

He reported that at maximum salinity stress, there was comparatively more accumulation of sugar in

Table 2. Soluble sugars concentration (µmol g^{-1} fresh weight) and Na^+ and K^+ content in leaves (mg g^{-1} dry weight).

Treatment	Soluble sugars concentration (µmol g^{-1} fresh weight)					Na^+ and K^+ content in leaves (mg g^{-1} dry weight)					
	Leaves			Roots		Na^+		K^+		Na^+: K^+ ratio	
	30 DAS	40 DAS	50 DAS	40 DAS	50 DAS	40 DAS	50 DAS	40 DAS	50 DAS	40 DAS	50 DAS
T1	0.37	0.40 c	0.49 b	0.21 b	0.36 b	21.33 b	10.57 c	26.00 b	9.50 b	0.82 b	1.11 c
T2	0.40	0.43 b	0.51 ab	0.24 ab	0.37 b	28.27 ab	23.14 b	31.11 a	14.25 ab	0.91 ab	1.62 b
T3	0.43	0.43 b	0.50 b	0.24 ab	0.37 b	31.20 ab	25.86 a	32.89 a	15.50 a	0.95 ab	1.67 b
T4	0.46	0.63 a	0.54 a	0.27 a	0.45 a	32.00 a	26.29 a	31.56 a	11.75 ab	1.01 a	2.24 a

Letters a, b and c indicate the statistically homogeneous groups. Significant differences have been obtained for all presented parameters.

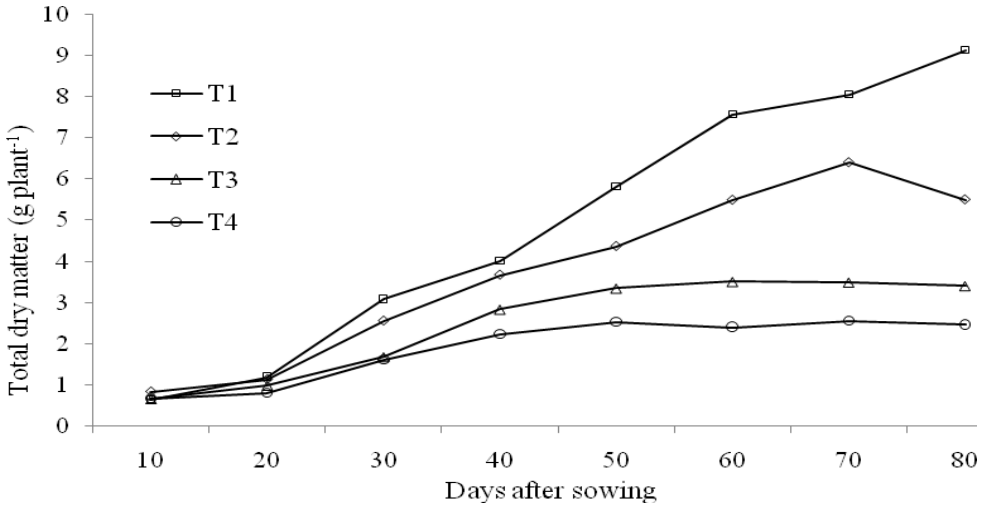

Fig 2. Total dry matter including root, stem and leaves weight following the days after sowing.

tolerant genotypes. Amini and Ehsanpour (2005) found that salinity affected soluble sugars contents of two cultivars of tomato; sugars content increased from 55.5 to 329 mg g^{-1} dry weight for 0 and 160 mM of NaCl, respectively. Total soluble carbohydrates are important solutes that are synthesized and accumulated in the cytosol of rice under salt stress. Total soluble carbohydrates under saline conditions were higher than in the control in both tested rice genotypes (Nemati et al., 2011).

Potassium (K^+) is one of the most important cations for plant growth. It is required as a vacuolar osmoticum and as an enzyme cofactor (Kamel and El-Tayeb, 2004). The similarities between Na^+ and K^+ lead to competition at transport and catalytic sites that normally bind the essential cation K^+ and maintain a high cytosolic K^+:Na^+ ratio which is believed to improve salt tolerance (Mian et al., 2011; Imamul Huq and Larher, 1985b). Our results indicated that the Na^+:K^+ ratio increased with the salinity level of the irrigation water . This means that chickpea accumulates more Na^+ than K^+ under salinity stress which is expected for a salt sensitive crop like chickpea (FAO, 2006). Increasing salt stress ultimately will lead to Na^+ toxicity. The results obtained in this study support the findings obtained by Amini and Ehsanpour (2005) and Saleh (2011) who reported that Na^+: K^+ in shoots of tomato and cotton increased with increasing salinity level. The decrease of K^+ uptake efficiency could be due to direct competition between Na^+ and K^+ for root transporters. Because of the similar physiochemical properties of both ions, Na^+ at high concentration has a strong inhibitory effect on K^+ uptake by the roots (Ghars et al., 2008). Generally in salt resistant crops (halophytes), the Na^+: K^+ ratio remains low, indicating that these crops accumulate K^+ rather than Na^+ to avoid salinity toxicity damage (Samiullah and Asghari Bano, 2011).

Materials and Methods

Experimental setup

The experimental set-up consisted of 192 plastic pots, each of which has a volume of 25 L. At the bottom of the pot a pipe, serving as a drainage outlet was connected to a collecting reservoir. The pots were filled with 5 L of gravel, overlain by 18 L of river sand. To protect the assembly against precipitation the set-up was covered by a sheet of transparent plastic, fixed at 4 m above the pots. Three seeds of a local variety of chickpea were sown on January 20, 2012 in each pot. The plants were harvested on April 16, 2012. The pots were irrigated with water of four different qualities: the control treatment (T1) used fresh water with an electrical conductivity (EC) of 1 dS m^{-1}; and three saline treatments with EC of 4 (T2), 7 (T3) and 10 dS m^{-1} (T4). Saline water of different EC values was obtained by adding the required amount of NaCl to fresh water. The same amounts of fertilizers were added to all treatments: each pot received 6 g of N, 12 g of P_2O_5 and 18 g of K_2O corresponding to 20 kg of N, 40 kg of P_2O_5 and 60 kg of K_2O per hectare. One water tank was available for each treatment. During the first 15 days after sowing, all pots were irrigated with fresh water to obtain homogenous seedling establishment and healthy initial growth, and then salinity treatments started. The salinity was increased gradually, by 1 dS m^{-1} per day, until the designated salinity level for each treatment was reached.

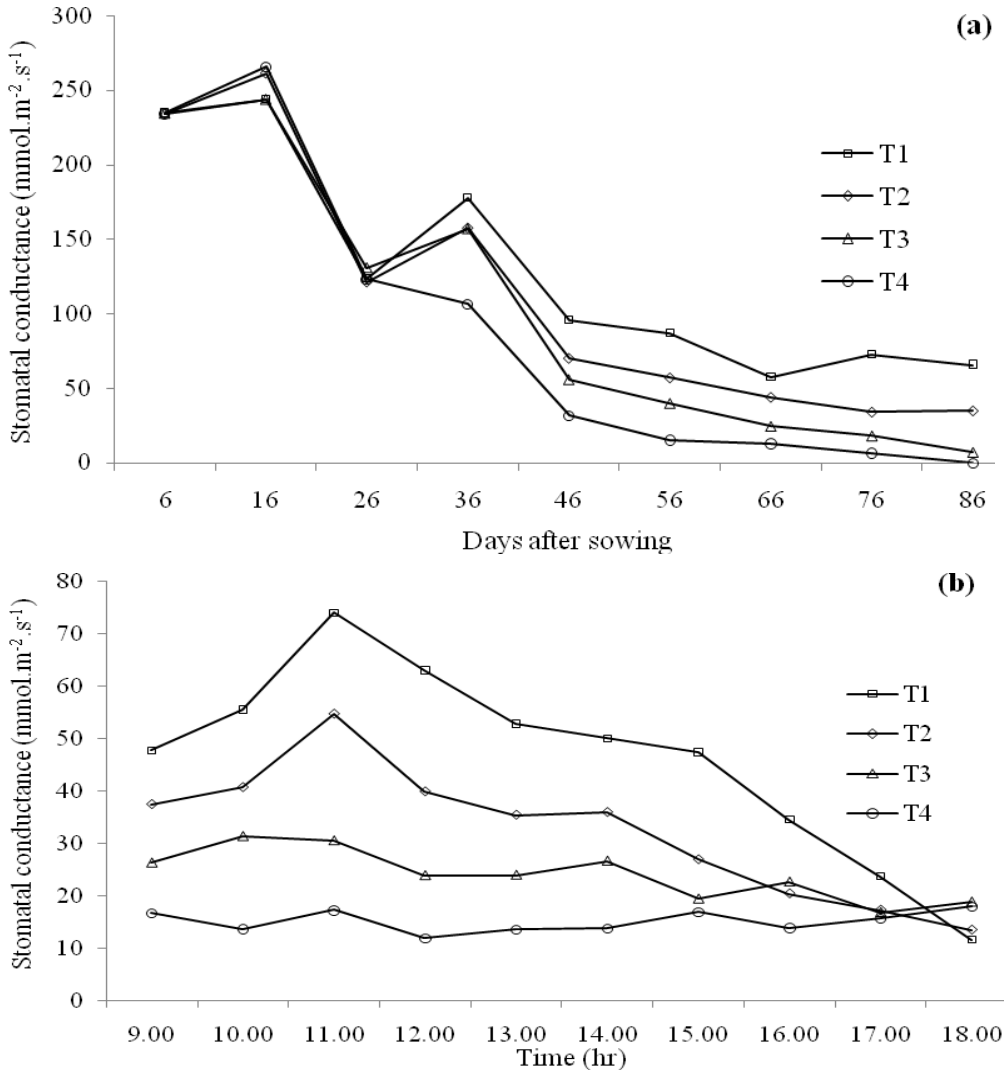

Fig 3. Variation of stomatal conductance during growth period (a) and during the 45th day (b) after sowing.

Irrigation scheduling

The pots were irrigated daily by dripper of a discharge equal to 2 l hr^{-1} and the drainage water volume was measured each day. The recorded data were used to calculate the irrigation requirement for the next day, i. Water uptake or crop evapotranspiration was calculated as the difference between the amount of irrigation water added, I, and drainage water, D, received at the bottom of the pot, multiplied by a leaching fraction of 1.2; as follows:

$$I_i = (I_{i-1} - D_{i-1}) \qquad (1)$$

Growth and yield

Fresh and dry matter of roots, leaves and stems were measured every 10 days for one plant per plot, with a total of 8 plants per treatment. Fresh weight was measured first, followed by the dry matter. At harvest, the yields of grain and straw were measured from all plants present in the pots. The harvest index (HI) was calculated as the ratio between grain yield and total dry biomass (aboveground + roots).

Stomatal conductance measurements and biochemical analysis

Stomatal conductance was measured using the SC-1 Leaf Porometer. Measurements were carried out every 10 days during the growth cycle, in order to assess the variation of the stomatal conductance to irrigation water salinity from seedling emergence till crop harvest. The stomatal conductance was determined at midday on the upper leaf surface of the leaves that were well exposed to sunlight. On the 45th day after sowing, measurements were conducted every hour in order to assess the daily variation in stomatal conductance in response to irrigation water salinity. Proline quantification was carried out by photometry, using the Troll and Lindsley (1955) method modified by Monneveux and Nemmar (1986); soluble sugars were determined by colorimetry (Dubois et al., 1956) and Na^+ and K^+ were determined by flame emission spectrophotometry and their content in leaves was calculated according to Imamul Huq and Larher (1985a).

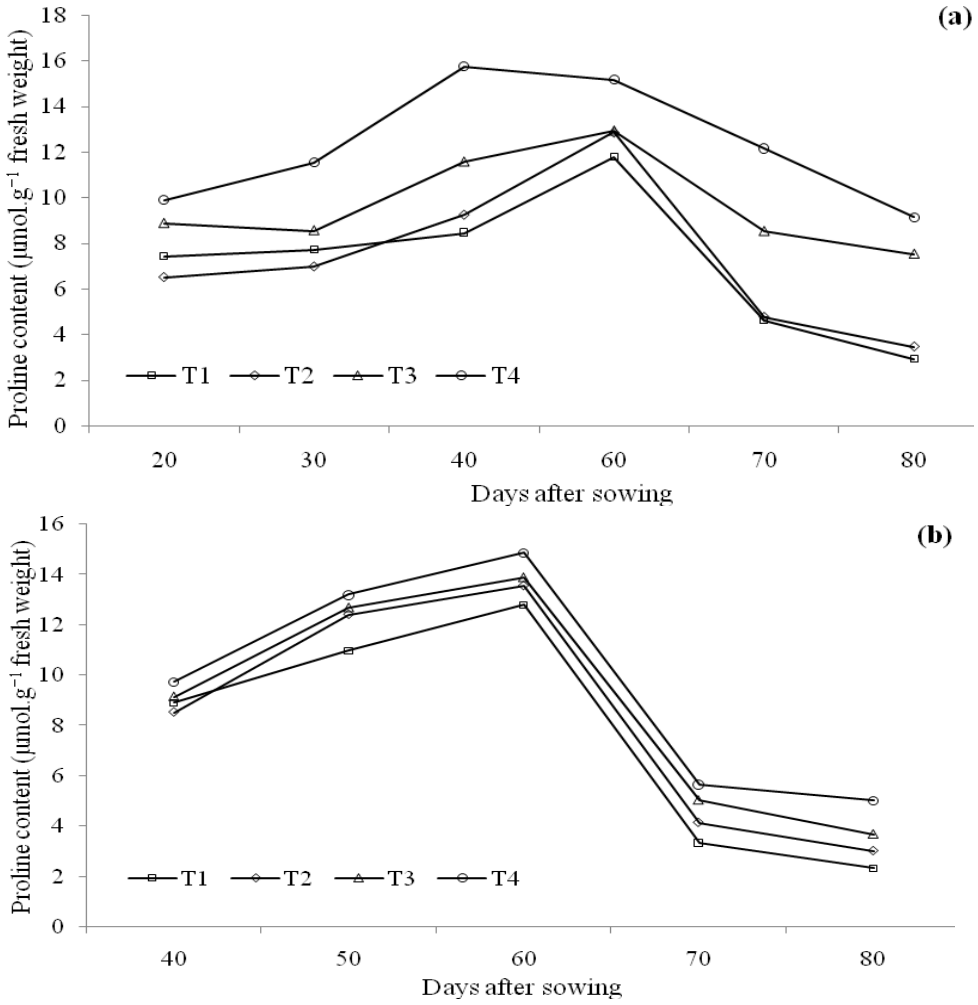

Fig 4. Proline content in leaves (a) and roots (b) during the crop growth season.

Statistical analysis

The adopted experimental design was a crossover with two Latin squares, each containing 16 plots; each of which consisted of 6 pots. All data sets were subjected to a one-way-ANOVA analysis using Statsoft Statistica v7.0.61.0 for Windows statistical data analysis package. Turkey's post-hoc test was employed to determine if significant ($P \leq 0.05$) differences occurred between individual salinity treatments.

Conclusion

The results presented in this paper highlighted and quantified the response of chickpea to irrigation water salinity in terms of water uptake, growth and yield. Under irrigation with saline water, the plant water uptake was reduced due to the increased osmotic effect in the soil solution; this led to a decrease in biomass production and a reduced yield and water productivity. The chickpea response to increased salinity is controlled by several physiological and biochemical mechanisms. In response to salinity, the plant system decreases transpiration losses by reducing the stomatal conductance and by reducing its photosynthesis rate and thus its growth. These mechanisms enabled chickpea to produce a large amount of proline, soluble sugars and cations in the leaves and roots in order to minimize the salt impact. This research quantified the levels of proline and soluble sugars as organic osmolytes to cope with salinity stress under different water salinity levels, while more research is needed to explore the importance of other osmolytes (glycine betaine, other amino acids, alcohol…etc.) and the role of phytohormones as plant growth regulators in response to salinity stress of chickpea. The added value of these results is that the crop's responses to salinity are quantified. The obtained values can be used to determine 'threshold values'; should the salinity of the irrigation water go above these threshold values one may expect the crop yield parameters to be affected. The quantified responses also indicate the rate of change of yield parameters in response to the irrigation water salinity level. This could be help to avoid significant yield reduction when deciding on the irrigation water salinity level to be used for the studied chickpea variety.

Acknowledgements

This research was funded by the EU 7th Framework Programme through the project "Sustainable water use securing food production in dry areas of the Mediterranean region (SWUP-MED)". We are also grateful to the technical staff of the Salinity and Plant Nutrition Laboratory and the Soil-Water-Plant Analysis Laboratory in the IAV-CHA Institute, Agadir, Morocco.

Special thanks go to IAMB Institute and IRSES project for providing funds and supervising this work.

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