Food Chemistry 303 (2020) 125402

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Supplementary irrigations at different physiological growth stages of chickpea (*Cicer arietinum* L.) change grain nutritional composition

Ihsan Serkan Varol[a,*], Yusuf Murat Kardes[b], Hasan Ali Irik[a], Halil Kirnak[c], Mahmut Kaplan[d]

[a] *Department of Biosystems Engineering, Faculty of Agriculture, University of Erciyes, Kayseri, Turkey*
[b] *Department of Field Crops, Faculty of Agriculture, University of Bilecik Seyh Edebali, Bilecik, Turkey*
[c] *Department of Civil Engineering, Faculty of Engineering, University of Adnan Menderes, Aydin, Turkey*
[d] *Department of Field Crops, Faculty of Agriculture, University of Erciyes, Kayseri, Turkey*

ARTICLE INFO

Keywords:
Amylose
Crude oil
Amino acids
Mineral content
Resistant starch
Starch properties
Protein

ABSTRACT

Chickpea is one the most important legumes consumed all around the world because of high protein content. The current study was conducted to investigate the effects of irrigation treatments at different physiological growth stages (before flowering, beginning of flowering, pod-set and pod-fill periods) on nutritional attributes of chickpea. The findings showed that one or two irrigations at different physiological growth stages instead of full irrigation treatment might be sufficient to improve the nutritional attributes of chickpea. Flowering and pod-fill periods were identified as the critical periods for irrigations and affected the nutritional component levels. The greatest protein level (29.52%) was obtained from the samples irrigated before flowering while the greatest total starch (36.30%) was obtained from the samples irrigated at the beginning of flowering. It was concluded based on present findings that physiological growth stages should be taken into consideration in irrigation practices of chickpea farming.

1. Introduction

Chickpea (*Cicer arietinum* L.) is among the oldest and largely consumed legumes, especially in tropical and sub-tropical regions. Chickpea is mostly produced and consumed in the Mediterranean, Near East, Central Asia and America (Mohammed, Tana, Singh, Korecha, & Molla, 2017). Seeds contain 59% carbohydrate, 29% protein, 5% oil, 3% fiber and 4% ash (Iqbal, Ateeq, Khalil, Perveen, & Saleemullah, 2006). Chickpea is also a rich source of vitamins, amino acids, calcium, phosphorus, iron, magnesium, and potassium (Akibode & Maredia, 2011). Besides, chickpea is known to have protective effects against cardiovascular diseases, cancer and diabetes (de Camargo et al., 2019). As with other edible legumes, chickpea also enhances the protein content and quality of cereal-based diets. Easy digestion and essential amino acids are important indicators of protein quality (Mokrane et al., 2010). Chickpea with a good balance of amino acids and high protein bio-availability is thus considered as a good source of diet protein (Xu et al., 2016). Amino acids are the building blocks of protein required for body development, repair and maintenance, as well as regeneration of tissues (Yigit, 2015). Protein deficiency may result in serious disorders. Legumes contain sufficient lysine levels, but they are insufficient in S-containing amino acids (methionine and cystine) (Jukanti, Gaur, Gowda, & Chibbar, 2012).

Starch is an important component of chickpea seed and it is a long-chain carbohydrate. Obesity and diabetes-like diseases are closely related to carbohydrate consumption (Bray & Popkin, 2014). Besides the quantity, digestibility is also an important attribute of starch. Resistant starch is defined as the fraction of diet starch indigestible in intestines of healthy individuals and it is considered as a functional source of fiber and probiotics playing a significant role in digestion physiology (Sajilata, Singhal, & Kulkarni, 2006). Resistant starch has positive correlations with amylose content (Raigond, Ezekiel, & Raigond, 2015).

Plant-originated foodstuffs constitute the primary source of minerals especially for low-income families (Ortiz-Monasterio et al., 2007). Minerals are essential parts of the human diet, mostly required for the functioning of important enzyme systems (Ozlu, Aydemir Atasever, Urcar, & Atasever, 2012). Chickpea is a rich source of iron, zinc, calcium, magnesium, potassium, copper and selenium (Thavarajah & Thavarajah, 2012).

Chickpea is mostly grown under rain-fed conditions; thus, drought is the most significant abiotic stressor limiting production activities (Mehta, Verma, & Ravi, 2015). There is a strong relationship between soil moisture content and the availability of plant nutrients. Adequate soil moisture increases the protein, starch and fat content of grain (Kale,

---

* Corresponding author.
E-mail address: svarol@erciyes.edu.tr (I.S. Varol).

https://doi.org/10.1016/j.foodchem.2019.125402
Received 2 May 2019; Received in revised form 19 August 2019; Accepted 20 August 2019

Kaplan, Ulger, Unlukara, & Akar, 2018; Kaplan, Karaman, Kardes, & Kale, 2019). It was observed that a single irrigation (Muniratham & Sangita, 2009), two irrigations (Abraham, Sharma, Thenua, & Shivakumar, 2010; PAU, 2011) or three irrigations (Mansur, Palled, Halikatti, Chetti, & Salimath, 2010) throughout the growing season significantly improved the yield and yield parameters of chickpea (Singh, Sekhon, & Sharma, 2011). Previous studies have mostly focused on water-yield relationships, but the number of studies on biochemical attributes is quite limited.

This study was conducted to investigate the effects of supplementary irrigations performed in seven different physiological stages on i) proximate composition and starch characteristics, ii) protein and amino acid composition and iii) mineral content of chickpea.

Table 2
Weather parameters of the study area for both years of the study.

Months	Temperature (°C)			Precipitation (mm)			Relative Humidity (%)		
	2016	2017	Long-Term*	2016	2017	Long-Term*	2016	2017	Long-Term*
April	14.0	14.2	10.6	0	25.9	52.1	45.4	53.9	62.7
May	14.8	14.9	14.8	151.8	57.2	51.8	57.2	59.1	61.5
Jun	20.4	19.6	19.0	25.6	50.6	39.5	54.5	56.6	55.6
July	23.3	23.7	22.4	2.0	0	10.5	43.6	42.7	50.7
August	25.4	25.3	22.0	0	3.3	8.8	41.2	47.6	49.5
Mean	19.6	19.5	17.8	–	–	–	48.4	52.0	56.0
Total	–	–	–	179.4	137	162.7	–	–	–

*Covers the years 1985–2017.

2. Material and methods

2.1. Field experiments and samples

Experiments were conducted for two years (2016 and 2017) in the Kayseri province of Turkey. The research site with an altitude of 1094 m is located between 340 56′–360 59′ east longitudes and 370 45′–38 18′ north latitudes. Aksu chickpea cultivar, which was registered in 2009, was used as the plant material of the experiments. Experimental soils were clay-loam in texture. Soil moisture at field capacity was 30.3%, soil moisture at permanent wilting point was 10.5% and soil infiltration rate was 23.3 mm/h (Table 1). Mean temperature was 19.6 °C in the first year and 21.5 °C in the second year. Mean relative humidity was 48.4% in the first year and 51.9% in the second year (Table 2). The rainfall in the April-August period was 179.4 mm and 137 mm in 2016 and 2017, respectively. The average annual rainfall in this period is 162.7 mm.

Experiments were conducted in a randomized blocks design with three replications. Seeds were sown as to have 35 cm row spacing and 5 cm on-row plant spacing. Each plot had 6 rows. Side rows and 0.5 m sections from the top and the bottom were considered as the side effects and observations and harvests were performed from mid-rows. Plots had 5 × 1.75 m dimensions, 1.2 m spacing was provided between the plots and 2.5 m spacing was provided between the blocks to prevent interactions. Based on soil analysis results, 15 kg/da DAP (18-46-0) was applied at sowing and fertilizer was incorporated into the soil with a rototiller. Sowing was performed on 13.04.2016 in the first year and on 13.04.2017 in the second year. Harvest was made separately from each block at physiological maturity period.

Irrigation systems were installed over the experimental fields on 28 April 2016 in the first year and on 30 April 2017 in the second year. Polythene (PE) lateral lines (16 mm) with inline emitters of 2 L/h spaced 25 cm apart were placed by each row. In irrigation treatments, deficit moisture within the effective root zone of the plants was brought to field capacity. There were seven different irrigation treatments in this study: I_1 – rainfed without irrigation, I_2 – single irrigation before the flowering period, I_3 – single irrigation at the beginning of flowering, I_4 – single irrigation at 50% pod set, I_5 – two irrigations (one at 50% flowering and another one at 50% pod fill periods), I_6 – two irrigations (one before flowering and another one at 50% pod set), I_7 – full irrigation from sowing to full grain fill period as to bring the deficit moisture to field capacity when 40% (± 5) of available moisture at 60 cm soil profile was depleted. Soil moisture was measured with the aid of a TDR device placed within a 10 cm vicinity of each row. Amount of irrigation water to be applied in each irrigation was determined with the equation given below:

$$d = \frac{(P_{vfc} - P_{vp})}{10} \times D \times P$$

where d: Amount of water applied, mm; Pvfc: volumetric moisture content at the field capacity %; Pvp: volumetric moisture content before irrigation %; D: depth of soil to be irrigated, cm, and P: plant cover ratio.

2.2. Biochemical assays

Immediately after harvest, the samples were dried to a constant weight at 65 °C and ground in a mill and kept in vacuum bags at +4 °C until the analyses. Harvested seeds were ground in a mill (IKA MF 10.1, Staufen, Germany) for biochemical analyses and preserved at +4 °C. All analyses were performed in 3 replicates.

Crude protein: About 0.2 g ground samples were used get nitrogen ratios with the aid of the Kjeldahl method. Resultant nitrogen values were multiplied by 6.25 to get the crude protein ratio of the samples (AOAC, 1990).

Crude oil: About 3 g sample were placed into the cartridge of Soxhelet device, dissolved in ether, oil was removed, kept in drying cabin at 95 °C for 1 h, cooled down in a desiccator, weighed to get crude oil content with the aid of relevant equations (AOAC, 1990).

Total, resistant and non-resistant starch content: Megazyme Resistant Starch Assay (K-RSTAR, Megazyme International Ireland Ltd, Co. Wicklow, Ireland) kit developed in accordance with AOAC 2002.02 Method and AACC 32-40 Method was used to determine resistant starch content. Samples were incubated in α-amylase and amyloglucosidase at 37 °C for 16 h. Non-resistant starch was dissolved and disintegrated into glucose. Resistant starch was obtained from the precipitate of the centrifuge process. Resultant precipitate was washed through ethanol, dissolved in potassium hydroxide and hydrolyzed into glucose with the aid of an amyloglucosidase enzyme. Resistant and total starch contents were separately determined through spectrophotometric glucose readings.

Amylose/Amylopectin content: Megazyme Amylose/Amylopectin Analysis Kit (K-AMYL) was used to determine amylose and amylopectin

Table 1
Soil characteristics of the research site.

Depth	Texture	EC	pH	FC	PWP	BD	OM	Lime	N	P_2O_5	K_2SO_4
0–30	Loamy	0.220	8.13	29.5	10.73	1.27	1.25	2.54	2.15	2.05	27.16
30–60	Loamy	0.173	8.17	31.5	11.38	1.24	1.05	5.83	1.05	1.15	37.64
60–90	Clay-Loam	0.258	8.14	30.0	9.30	1.22	0.69	3.15	0.40	0.60	31.01

EC: Electrical conductivity, ***FC:*** Field capacity, ***PWP:*** Permanent wilting point, ***BD:*** Bulk density, ***OM:*** Organic matter.

fractions of the starch. Starch samples were totally dispersed in hot-water bath supplemented with dimethyl sulphoxide. Starch was precipitated with ethanol supplementation and oils were removed through recycle of precipitate. Starch precipitate was dissolved in acetate, supplemented with conA and centrifuged to remove amylopectin. Then, amylose and total starch were hydrolyzed to D-glucose and amylose quantity was determined spectrophotometrically with glucose oxidase peroxidase supplementation.

Amino acid composition: Samples (1 g) were prepared for amino acid analysis through wet digestion with 10 ml 6 N HCl at 150 °C for 2 h. Resultant mixtures were filtered through 45 µm Syringe filter (Millipore Millex-HV). The HPLC method specified in Aristoy and Toldra (1991) and Antoine, Wei, Littell, and Marshall (1999) was used for free amino acid composition. Na_2HPO_4, $Na_2B_4O_7$ and NaN_3 (pH 8.2) were used as Elution Buffer A and Acetonitrile: methyl alcohol: water (45:45:10) was used as Elution Buffer B. An HPLC (Shimadzu LC-20 AT Prominence Liquid Chromatograph) system equipped with prominence fluorescence detector and Zorbax Eclipse-AAA (4.6 × 150 mm, 3.5 µm) was used for free amino acid composition of the samples. OPA (orthophthalaldehyde) and FMOC (9-fluorenylmethyl chloroformate) were used as derivatization reagents and 0.4 N Borate (pH of 10.2) was used as the buffer solution. Flow rate was 1.5 ml/min and readings were performed at 2% Buffer B concentration for 40 min. An RF20A detector was used to determine cystine, valine, hydroxyproline, aspartic acid, glutamic acid, asparagine, serine, glutamine, histidine, glycine, thionine, arginine, alanine, tyrosine, methionine, tryptophan, phenylalanine, isoleucine, leucine, lysine and proline (analytical standards Sigma Aldrich) amino acids.

Mineral content: For minerals, chickpea samples were washed and dried for two days at 65 °C and ground to pass through a 60 mesh screen. Sample preparation was performed using the Mars 5 Microwave Digestion System (CEM, Kamp-Lintfort, Germany). After adding 500 mg 250–350 mg of sample and 10 ml nitric acid (65%), the samples were predigested for a period of 20 min. The basic microwave digestion in this procedure was as follows: temperature time ramp for 20 min with a final temperature of 180 °C (356 °F), then 20–30 min hold time at 1200 W for more than six vessels. Following this, the samples were cooled to room temperature and the vessels were uncapped. The clear sample solutions were transferred to a volumetric flask (50 ml) and filled with ultrapure water. After get digestion, P, K, Ca, Mg, Na, Fe, Mn, Zn, Cu and B contents of the seed samples were determined using an ICP OES spectrometer (Inductively Couple Plasma spectrophotometer) (PerkinElmer, Optima 4300 DV, ICP/OES, Shelton, CT 06484-4794, USA) (Mertens, 2005).

2.3. Data analysis

Experimental data were subjected to variance analysis with Statistical Analysis System (SAS) Software 9.0. Least significant difference (LSD) multiple comparison procedure was used to compare treatment means at 1% significance level.

3. Results and discussion

This study was conducted to investigate the effects of irrigations at different physiological stages on chickpea nutritional attributes. Starch, crude protein, crude ash and oil contents are provided in Table 3. Effects of irrigation treatments on crude ash ratios were found to be significant at a 5% level and the effects of irrigation treatments on the other parameters were found to be significant at a 1% level. Years had significant effects only on resistant starch contents ($P \leq 0.01$). Year × Treatment interactions had significant effects on resistant starch and total starch at a 1% level and significant effects on non-resistant starch at a 5% level. Seed chemical composition can be altered under water stress through changes in metabolic and enzyme activities induced by changes in transportation of assimilates (Carvalho, Ricardo, & Chaves, 2004). Water stress also negatively influences photosynthetic activity of the plants (Ali, Ashraf, & Anwar, 2010). Temperatures throughout the growth stages varied significantly in experimental years and such variations in temperature may influence chemical composition. Variations in temperature yield narrower leaves and result in various changes in cells such as stomal closure, cell wall thickening, recessed cell growth and development (Mahajan & Tuteja, 2005). Such a case then resulted in differences in investigated parameters of the years. Amylose contents of the samples varied between 15.39 and 39.11%, amylopectin contents between 60.89 and 84.61% and total starch contents between 24.88 and 36.30%. In different studies, water deficits were studied for both cereals and legumes. Li et al. (2015) informed that water deficits significantly influenced starch quality and quantity and also Yu et al. (2016) reported that water deficits reduced starch, amylose and amylopectin content of barley. As a different finding from Yu et al. (2016), amylose content increased with water deficit treatments of the present study (Table 3). Present findings comply with the results of Kaplan et al. (2019) reporting decreasing amylopectin content and increasing amylose content of maize with increasing irrigation water quantities. Crude protein contents varied between 25.22 and 29.52% with the lowest protein content in rain-fed treatment. Similar to present findings, Xie, Jiang, Cao, Dai, and Jing (2003) indicated that drought stress significantly reduced starch synthesis, thus altered protein and total starch contents. Under stress conditions, carbohydrate quantity decreases through stomal closure and proline and glycine-like carbohydrates and protein metabolics accumulated in leaves (Pelleschi, Rocher, & Prioul, 1997). Since these nutrients accumulated in leaves and are not transported to seeds, seed protein and starch contents decrease under water deficit conditions.

The greatest resistant starch, non-resistant starch and total starch contents were obtained from I_3 treatment (beginning of flowering) while the greatest crude protein and crude ash contents were obtained from I_2 treatment (before flowering). Resistant starch is a component of total starch non-hydrolyzed by the enzymes of digestive tract but fermented by bacteria of the large intestines, and plays an important role in prevention of several diseases (Jiang, Lio, Blanco, Campbell, & Jane, 2010). Therefore, it is quite significant to know the effects of cultural practices on resistant starch. Kaplan et al. (2019) reported increasing resistant starch contents with increasing irrigation water quantity. Wang, Hatcher, Tyler, Toews, and Gawalko (2010) reported RS content of different chickpea varieties as between 8.7 and 21.9 g/kg DM. In present study, the greatest value was obtained as 2.01 g/100 g of DM.

Crude ash contents varied between 2.42 and 3.05% and crude oil contents varied between 3.93 and 5.32% and the greatest crude oil contents were obtained from I_4 treatment (50% pod-set). Queiroz et al. (2015) informed that water stress reduced carbohydrate, protein and ash content and increased fiber content, but generally did not influence lipids. But in present study, water deficits reduced crude oil content and the lowest value was obtained from the rain-fed treatment. Irrigation generally prolongs grain-fill period, then healthier seeds with greater oil contents are achieved (Bewley & Black, 1994). Similarly, the lowest ash content was obtained from the rain-fed treatment, but no statistical differences were observed in ash contents of I3, I5 and I6 treatments.

Amino acid contents of the samples are provided in Table 4. Irrigation treatments had significant effects on the amino acids ($P \leq 0.01$) except lysine. Years did not have significant effects on threonine, glycine, lysine, proline and total essential amino acids, but had significant effects on asparagine, alanine and phenylalanine at a 5% level and on the other amino acids at a 1% level. While year × treatment interactions did not have significant effects on threonine, phenylalanine, lysine and proline, interactions had significant effects on methionine and total essential amino acids at 5% level and on the other amino acids at 1% level.

The greatest total essential amino acids were obtained from I_2 and I_6 treatments, the greatest total sulphurous amino acids were obtained from I_5 treatment and the greatest total amino acids were obtained from

Table 3
Effects of irrigation treatments at different growth stages on starch, crude protein, ash and oil content of chickpea.

Irrigation treatments	Amylose	Amylopectin	RS	NRS	TS	CP	CA	CO
	(g/100 g of DM)							
I_1	39.11^{a}	60.89^{e}	0.66^{d}	24.23^{d}	24.88^{d}	25.22^{c}	2.42^{b}	3.93^{d}
I_2	26.51^{c}	73.49^{c}	1.38^{bc}	28.21^{c}	29.59^{c}	29.52^{a}	3.05^{a}	4.80^{bc}
I_3	31.92^{b}	68.08^{d}	2.01^{a}	34.29^{a}	36.30^{a}	27.23^{b}	2.59^{b}	4.42^{c}
I_4	21.78^{d}	78.22^{b}	1.51^{b}	31.51^{b}	33.02^{b}	27.12^{b}	3.05^{a}	5.32^{a}
I_5	15.39^{e}	84.61^{a}	1.41^{bc}	30.77^{b}	32.18^{b}	27.95^{b}	2.62^{b}	4.98^{ab}
I_6	20.82^{d}	79.18^{b}	1.30^{c}	30.88^{b}	32.17^{b}	27.00^{b}	2.50^{b}	4.76^{bc}
I_7	27.05^{c}	72.95^{c}	1.54^{b}	30.72^{b}	32.26^{b}	27.56^{b}	2.82^{ab}	4.55^{bc}
Treatment	**	**	**	**	**	**	*	**
Year	NS	NS	**	NS	NS	NS	NS	NS
Y × T	**	**	**	*	**	NS	NS	NS
LSD	2.05	2.05	0.18	1.25	1.23	1.04	0.40	0.45

I_1: rain-fed; **I_2:** before flowering; **I_3:** beginning of flowering; **I_4:** 50% pod-set; **I_5:** 50% flowering + 50% pod-fill; **I_6:** before flowering + 50% pod-set; **I_7:** full irrigation; ***RS:** resistant starch; **NRS:** non-resistant starch; **TS:** total starch; **CP:** crude protein; **CA:** crude ash; **CO:** crude oil; **:* $P \leq 0.01$; ***:** $P \leq 0.05$; ***NS:** non-significant; **LSD:** least significant difference; **DM:** dry matter.*

I_3 and I_4 treatments. Amino acid composition of foodstuffs is a significant attribute of human diets. Chickpea is rich in the essential amino acids of arginine, leucine and lysine and the non-essential amino acids of glutamic acid and aspartic acid (Ghribi et al., 2015; Iqbal et al., 2006). Chickpea is also quite rich in sulphurous amino acids (methionine and cystine) (Zia-Ul-Haq et al., 2007). Hammad and Ali (2014) stated that decreasing water levels reduced total amino acids and increased proline contents and such findings comply with the present ones. Proline contributes to stabilization of sub-cellular structures (membranes and proteins), scavenging of free radicals and buffering of cellular redox potential under stress conditions (Iqbal, 2009). Ahmed et al. (2013) reported different responses of barley genotypes to water stress and stated that significant increases were detected in all amino acids (except for methionine) under drought stress as compared to the control treatments. Besides, while sulphurous amino acids were not influenced in some barley genotypes similarly to present findings, they were influenced in some others (Ahmed et al., 2013).

Mineral contents of chickpea irrigated at different physiological growth stages are provided in Table 5. Irrigation treatments had highly significant effects on all minerals ($P \leq 0.01$). Besides, years also had highly significant effects on S and Na contents ($P \leq 0.01$) and significant effects on Mg, P, Cu and Mn contents ($P \leq 0.05$). Year × Treatment interactions had significant effects on Ca, Mg, K, P, Fe, B, Mn and Ni contents ($P \leq 0.01$).

For macro elements, the greatest Ca content was obtained from I_2 treatment, the greatest Mg content was obtained from I_5 treatment, the greatest K and S contents were obtained from I_6 treatment, the greatest P content was obtained from I_4 treatment and the greatest Na content was obtained from I_7 treatment. For micro elements, the greatest Cu content was obtained from I_2 treatment, the greatest Fe, B, Mn and Ni contents were obtained from I_3 treatment and the greatest Zn content was obtained from I_6 treatment (Table 5). In general, chickpea is rich in minerals. It is rich in iron and zinc, while mostly deficient micro elements and calcium, magnesium, potassium and phosphorus-like macro

Table 4
Effects of irrigation treatments at different growth stages on amino acid composition of chickpea.

Amino Acids	Irrigation Treatment							Treat.	Year	Year × Treat.	LSD
g/100 g DM of protein	I_1	I_2	I_3	I_4	I_5	I_6	I_7				
Aspartic acid	14.44^{f}	18.79^{d}	24.03^{a}	23.23^{b}	22.55^{c}	23.87^{ab}	15.47^{e}	**	**	**	0.65
Glutamic acid	14.75^{b}	11.13^{d}	14.45^{b}	14.36^{b}	14.65^{b}	15.85^{a}	12.86^{c}	**	**	**	0.95
Serine	4.16^{c}	4.01^{c}	4.64^{b}	5.29^{a}	3.15^{d}	3.96^{c}	3.02^{d}	**	**	**	0.37
Asparagine	2.89^{d}	3.85^{c}	5.75^{a}	5.47^{a}	3.99^{c}	3.71^{c}	4.69^{b}	**	*	**	0.66
Glutamine	2.91^{d}	3.93^{bc}	4.62^{ab}	4.24^{b}	4.48^{b}	5.33^{a}	3.26^{cd}	**	**	**	0.74
Threonine	1.36^{c}	4.27^{ab}	4.26^{ab}	4.60^{a}	4.14^{ab}	3.92^{b}	3.78^{b}	**	NS	NS	0.69
Glycine	1.51^{d}	2.61^{cb}	3.05^{ab}	3.22^{a}	2.35^{c}	2.72^{cb}	1.64^{d}	**	NS	**	0.47
Histidine	2.49^{a}	2.61^{a}	2.17^{bc}	2.16^{bc}	2.38^{ab}	2.38^{ab}	1.95^{c}	**	**	**	0.30
Alanine	6.35^{b}	7.14^{a}	7.51^{a}	6.34^{b}	6.26^{b}	7.16^{a}	5.84^{b}	**	*	**	0.58
Arginine	5.60^{c}	4.22^{d}	6.75^{ab}	6.38^{b}	6.96^{a}	5.83^{c}	4.21^{d}	**	**	**	0.53
Tyrosine	1.57^{bc}	2.03^{a}	1.37^{cd}	1.76^{b}	1.22^{d}	1.16^{d}	1.47^{c}	**	**	**	0.23
Cysteine	1.11^{d}	1.11^{d}	1.97^{ab}	1.28^{d}	1.76^{bc}	2.04^{a}	1.65^{c}	**	**	**	0.26
Valine	4.20^{e}	7.24^{b}	5.63^{d}	7.72^{b}	6.55^{c}	9.10^{a}	9.54^{a}	**	**	**	0.65
Methionine	2.68^{c}	2.01^{d}	3.18^{ab}	1.73^{d}	3.48^{a}	2.86^{bc}	1.61^{d}	**	**	*	0.50
Phenylalanine	6.38^{e}	8.43^{d}	8.63^{c}	9.43^{ab}	9.13^{cb}	9.68^{a}	8.76^{cd}	**	*	NS	0.54
Isoleucine	3.43^{d}	5.31^{a}	4.52^{bc}	4.08^{c}	4.51^{bc}	4.09^{c}	4.68^{b}	**	**	**	0.45
Leucine	7.08^{c}	7.24^{c}	8.30^{b}	9.00^{a}	8.71^{ab}	6.41^{d}	7.40^{c}	**	**	**	0.53
Lysine	7.50^{ab}	7.66^{a}	7.15^{b}	7.58^{ab}	7.26^{ab}	7.19^{b}	7.32^{ab}	NS	NS	NS	0.43
Proline	4.77^{a}	3.36^{c}	3.48^{bc}	3.74^{b}	3.43^{c}	2.66^{d}	3.44^{bc}	**	NS	NS	0.31
TEAA	36.67^{c}	46.80^{ab}	45.21^{b}	48.05^{a}	47.39^{a}	46.79^{ab}	46.52^{ab}	**	NS	*	1.70
TSAA	3.79^{b}	3.12^{c}	5.15^{a}	3.01^{c}	5.24^{a}	4.90^{a}	3.26^{c}	**	**	**	0.49
TAA	95.17e	106.95^{c}	121.47^{a}	121.61^{a}	116.95^{b}	119.93^{ab}	102.59^{d}	**	**	**	3.16

I_1: rain-fed; **I_2:** before flowering; **I_3:** beginning of flowering; **I_4:** 50% pod-set; **I_5:** 50% flowering + 50% pod-fill; **I_6:** before flowering + 50% pod-set; **I_7:** full irrigation; ***TEAA:** total essential amino acids; **TSAA:** total sulphurous amino acids; **TAA:** total amino acids; **:* $P \leq 0.01$; ***:** $P \leq 0.05$; ***NS:** non-significant; **LSD:** least significant difference; **DM:** dry matter.*

Table 5
Effects of irrigation treatments at different growth stages on mineral content of chickpea.

Irrigation Treatments	Macro Elements (ppm)					
	Ca	Mg	K	P	S	Na
I_1	823^{cd}	779^{b}	7920^{d}	1692^{c}	1097^{c}	142^{c}
I_2	1046^{a}	837^{ab}	8622^{bc}	1893^{ab}	1243^{ab}	183^{ab}
I_3	1020^{a}	892^{ab}	9126^{a}	1960^{a}	1321^{a}	193^{a}
I_4	988^{ab}	888^{ab}	8765^{b}	1961^{a}	1222^{b}	156^{bc}
I_5	762^{d}	931^{a}	9146^{a}	1877^{ab}	1060^{c}	181^{ab}
I_6	912^{bc}	859^{ab}	9252^{a}	1854^{b}	1330^{a}	199^{a}
I_7	1012^{ab}	889^{ab}	8396^{c}	1807^{b}	1199^{b}	210^{a}
Treatment	**	**	**	**	**	**
Year	NS	*	NS	*	**	**
Year × Treatment	**	**	**	**	NS	NS
LSD	100.96	115.04	243.09	87.64	97.51	32.81

Irrigation Treatments	Micro Elements (ppm)					
	Cu	Fe	B	Mn	Zn	Ni
I_1	7.77^{c}	65.33^{c}	7.56^{d}	12.82^{c}	16.26^{c}	1.04^{bc}
I_2	8.67^{a}	80.69^{b}	7.64^{cd}	13.37^{bc}	19.53^{ab}	1.15^{ab}
I_3	8.28^{abc}	90.56^{a}	8.56^{a}	14.01^{a}	18.26^{b}	1.21^{a}
I_4	7.93^{bc}	81.94^{ab}	7.59^{d}	13.43^{abc}	18.33^{b}	1.10^{ab}
I_5	7.90^{c}	76.31^{b}	7.68^{cd}	12.01^{d}	18.25^{b}	1.02^{bc}
I_6	8.64^{ab}	80.88^{b}	8.44^{ab}	13.28^{bc}	20.20^{a}	1.04^{bc}
I_7	7.96^{ab}	84.16^{ab}	8.05^{bc}	13.55^{ab}	19.12^{ab}	0.97^{c}
Treatment	NS	**	**	**	**	**
Year	*	NS	NS	*	NS	NS
Year × Treatment	NS	**	**	**	NS	**
LSD	0.74	8.84	0.43	0.62	1.64	0.13

I_1: rain-fed; **I_2:** before flowering; **I_3:** beginning of flowering; **I_4:** 50% pod-set; **I_5:** 50% flowering + 50% pod-fill; **I_6:** before flowering + 50% pod-set; **I_7:** full irrigation; ***: $P \le 0.01$; *: $P \le 0.05$;* ***NS:*** *non-significant;* ***LSD:*** *least significant difference.*

elements. There is a positive correlation between soil available moisture and root nutrient uptake (Albrizio, Todorovic, Matic, & Stellacci, 2010). Root nutrient uptake and nutrient transport from roots to shoots decrease under drought stress (Nagasuga, Murai-Hatano, & Kuwagata, 2011). It was reported that water stress significantly limited root development (Kaplan, Kamalak, Kasra, & Güven, 2014). A weak root system then reduces uptake of various nutrients from the soils. Water stress negatively influences plant nutrient uptakes (Ferreira, Magalhaes, Duraes, Vasconcellos, & de Araujo Neto, 2008). Paiva et al. (2017) informed that Fe and Zn were greatly influenced by irrigation practices. Kayodé, Linnemann, Hounhouigan, Nout, and van Boekel (2006) indicated that Fe and Zn contents were greatly influenced by environmental factors rather than genetic characteristics. These findings support the present ones, and the rain-fed treatment caused decreases in Fe and Zn contents.

4. Conclusion

The results of this study revealed that there was no need for full irrigation to improve nutritional attributes of chickpea. One or two irrigations to be performed at different physiological growth stages may yield quite good outcomes for starch, protein, oil, amino acid and mineral content. Among the physiological stages, flowering and pod-fill stages were identified as the most sensitive stages for irrigation. Nutritional attributes varied with the years. Therefore, further studies are recommended under different climate and soil conditions for repetitive years.

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.

References

Abraham, T., Sharma, U. C., Thenua, O. V. S., & Shivakumar, B. G. (2010). Effect of levels of irrigation and fertility on yield and economics of chickpea (*Cicer arietinum*) and Indian mustard (*Brassica juncea*) under sole and intercropping systems. *Indian Journal of Agricultural Sciences, 80*, 372–376.

Ahmed, I. M., Cao, F., Han, Y., Nadira, U. A., Zhang, G., & Wu, F. (2013). Differential changes in grain ultrastructure, amylase, protein and amino acid profiles between Tibetan wild and cultivated barleys under drought and salinity alone and combined stress. *Food Chemistry, 141*(3), 2743–2750.

Akibode, Sitou, & Maredia, Mywish (2011). *Global and regional trends in production, trade and consumption of food legume crops.* East Lansing, Michigan: Michigan State University.

Albrizio, R., Todorovic, M., Matic, T., & Stellacci, A. M. (2010). Comparing the interactive effects of water and nitrogen on durum wheat and barley grown in a Mediterranean environment. *Field Crops Research, 115*(2), 179–190.

Ali, Q., Ashraf, M., & Anwar, F. (2010). Seed composition and seed oil antioxidant activity of maize under water stress. *Journal of American Oil Chemists Society, 87*, 1179–1187.

Antoine, F. R., Wei, C. I., Littell, R. C., & Marshall, M. R. (1999). HPLC method for analysis of free amino acids in fish using o-phthaldialdehyde precolumn derivatization. *Journal of Agriculture and Food Chemistry, 47*, 5100–5107.

AOAC (1990). *Official method of analysis* (15th ed.). Washington: Association of Official Analytical Chemists66–88.

Aristoy, M. C., & Toldra, Õ. F. (1991). Deproteinization techniques for HPLC amino acid analysis in fresh pork muscle and dry-cured ham. *Journal of Agricultural and Food Chemistry, 39*, 1792–1795.

Bewley, J. D., & Black, M. (1994). *Seeds: Physiology of development and germination.* New York: Plenum Press.

Bray, G. A., & Popkin, B. M. (2014). Dietary sugar and body weight: Have we reached a crisis in the epidemic of obesity and diabetes? Health be damned! Pour on the Sugar. *Diabetes Care, 37*(4), 950–956.

Carvalho, I. S., Ricardo, C. P., & Chaves, M. (2004). Quality and distribution of assimilates within the whole plant of lupins (*L. albus* and *L. mutabilis*) influenced by water stress. *Journal of Agronomy and Crop Science, 190*, 205–210.

de Camargo, A. C., Favero, B. T., Morzelle, M. C., Franchin, M., Alvarez-Parrilla, E., de la Rosa, L. A., et al. (2019). Is chickpea a potential substitute for soybean? Phenolic bioactives and potential health benefits. *International Journal of Molecular Sciences, 20*(11), 2644.

Ferreira, V. M., Magalhaes, P. C., Duraes, F. O., Vasconcellos, C. A., & de Araujo Neto, J. C. (2008). Concentration and partitioning of macro nutrients in two maize genotypes as related to soil water availability. *Revista Brasileira de Milho e Sorgo, 7*(1), 1–17.

Ghribi, A. M., Gafsi, I. M., Blecker, C., Danthine, S., Attia, H., & Besbes, S. (2015). Effect of drying methods on physico-chemical and functional properties of chickpea protein concentrates. *Journal of Food Engineering, 165*, 179–188.

Hammad, S. A., & Ali, O. A. (2014). Physiological and biochemical studies on drought tolerance of wheat plants by application of amino acids and yeast extract. *Annals of Agricultural Sciences, 59*(1), 133–145.

Iqbal, S. (2009). Physiology of wheat (Triticum aestivum L.) accessions and the role of phytohormones under water stress. Ph.D. Thesis, Fac. of Biological Sci., Quaid-i-azam Univ., Islamabad, pp. 83-154.

Iqbal, A., Ateeq, N., Khalil, I. A., Perveen, S., & Saleemullah, S. (2006). Physicochemical characteristics and amino acid profile of chickpea cultivars grown in Pakistan. *Journal of Foodservice, 17*(2), 94–101.

Jiang, H., Lio, J., Blanco, M., Campbell, M., & Jane, J. L. (2010). Resistant-starch formation in high-amylose maize starch during kernel development. *Journal of Agricultural and Food Chemistry, 58*, 8043–8047.

Jukanti, A. K., Gaur, P. M., Gowda, C. L. L., & Chibbar, R. N. (2012). Nutritional quality and health benefits of chickpea (*Cicer arietinum* L.): A review. *British Journal of Nutrition, 108*(S1), S11–S26.

Kale, H., Kaplan, M., Ulger, I., Unlukara, A., & Akar, T. (2018). Feed value of maize (*Zea mays var. indentata* (Sturtev.) LH bailey) grain under different irrigation levels and nitrogen doses. *Turkish Journal of Field Crops, 23*(1), 56–61.

Kaplan, M., Kamalak, A., Kasra, A. A., & Güven, I. (2014). Effect of maturity stages on potential nutritive value, methane production and condensed tannin content of *Sanguisorba minor* Hay. *Journal of the Faculty of Veterinary Medicine, Kafkas University, 20*, 445–449.

Kaplan, M., Karaman, K., Kardes, Y. M., & Kale, H. (2019). Phytic acid content and starch properties of maize (*Zea mays* L.): Effects of irrigation process and nitrogen fertilizer. *Food Chemistry, 283*, 375–380.

Kayodé, A. P. P., Linnemann, A. R., Hounhouigan, J. D., Nout, M. J. R., & van Boekel, M. A. J. S. (2006). Genetic and environmental impact on iron, zinc, and phytate in food sorghum grown in Benin. *Journal of Agricultural and Food Chemistry, 54*, 256–262.

Li, C., Li, C. Y., Zhang, R. Q., Liang, W., Kang, X. L., Jia, Y., & Liao, Y. C. (2015). Effects of drought on the morphological and physicochemical characteristics of starch granules in different elite wheat varieties. *Journal of Cereal Science, 66*, 66–73.

Mahajan, S., & Tuteja, N. (2005). Cold, salinity and drought stresses: An overview. *Archives of Biochemistry and Biophysics, 444*, 139–158.

Mansur, C. P., Palled, Y. B., Halikatti, S. I., Chetti, M. B., & Salimath, P. M. (2010). Effect of dates of sowing and irrigation levels on biometric growth parameters of kabuli chickpea. *Karnataka Journal of Agricultural Sciences, 23*, 566–569.

Mehta, G., Verma, P. K., & Ravi, M. (2015). Correlation studies in chickpea grown under rainfed and irrigated conditions in Northern Plains of India. *Journal of Agroecology and Natural Resource Management, 2*(5), 388–390.

Mertens, D. (2005). AOAC Official Method 975.03. Metal in Plants and Pet Foods. Official Methods of Analysis, 18th ed. Horwitz, W., Latimer, G. W., (Eds). Chapter 3, pp. 3-4, AOAC-International Suite 500, 481. North Frederick Avenue, Gaitherburg, Maryland 20877-2417, USA.

Mohammed, A., Tana, T., Singh, P., Korecha, D., & Molla, A. (2017). Management options for rainfed chickpea (*Cicer arietinum* L.) in northeast Ethiopia under climate change condition. *Climate Risk Management, 16*, 222–233.

Mokrane, H., Amoura, H., Belhaneche-Bensemra, N., Courtin, C. M., Delcour, J. A., & Nadjemi, B. (2010). Assessment of Algerian sorghum protein quality [*Sorghum bicolor* (L.) Moench] using amino acid analysis and in vitro pepsin digestibility. *Food Chemistry, 121*(3), 719–723.

Muniratham, P., & Sangita, M. S. (2009). Influence of sowing dates and irrigations on growth and yield of chickpea. *Legume Research, 32*(3).

Nagasuga, K., Murai-Hatano, M., & Kuwagata, T. (2011). Effects of low root temperature on dry matter production and root water uptake in rice plants. *Plant Production Science, 14*(1), 22–29.

Ortiz-Monasterio, J. I., Palacios-Rojas, N., Meng, E., Pixley, K., Trethowan, R., & Pena, R. J. (2007). Enhancing the mineral and vitamin content of wheat and maize through plant breeding. *Journal of Cereal Science, 46*(3), 293–307.

Ozlu, H., Aydemir Atasever, M., Urcar, S., & Atasever, M. (2012). Mineral Contents and Heavy Metal Contamination in Kashar Cheeses Consumed in Erzurum Province, Turkey. *Journal of the Faculty of Veterinary Medicine, Kafkas University, 18*(2), 205–208.

Paiva, C. L., Queiroz, V. A. V., Simeone, M. L. F., Schaffert, R. E., de Oliveira, A. C., & da Silva, C. S. (2017). Mineral content of sorghum genotypes and the influence of water stress. *Food Chemistry, 214*, 400–405.

PAU (2011). Package of Practices for Crops of Punjab, Rabi 2011–12. Ludhiana, India: Punjab Agricultural University.

Pelleschi, S., Rocher, J. P., & Prioul, J. L. (1997). Effect of water restriction on carbohydrate metabolism and photosynthesis in mature maize leaves. *Plant, Cell & Environment, 20*, 493–503.

Queiroz, V. A. V., da Silva, C. S., de Menezes, C. B., Schaffert, R. E., Guimarães, F. F. M., Guimarães, L. J. M., et al. (2015). Nutritional composition of sorghum [*Sorghum bicolor* (L.) Moench] genotypes cultivated without and with water stress. *Journal of Cereal Science, 65*, 103–111.

Raigond, P., Ezekiel, R., & Raigond, B. (2015). Resistant starch in food: A review. *Journal of the Science of Food and Agriculture, 95*(10), 1968–1978.

Sajilata, M. G., Singhal, R. S., & Kulkarni, P. R. (2006). Resistant starch: A review. *Comprehensive Reviews in Food Science and Food Safety, 5*, 1–17.

Singh, G., Sekhon, H. S., & Sharma, P. (2011). Effect of irrigation and biofertilizer on water use, nodulation, growth and yield of chickpea (*Cicer arietinum* L.). *Archives of Agronomy and Soil Science, 57*, 715–726.

Thavarajah, D., & Thavarajah, P. (2012). Evaluation of chickpea (*Cicer arietinum* L.) micronutrient composition: Biofortification opportunities to combat global micronutrient malnutrition. *Food Research International, 49*(1), 99–104.

Wang, N., Hatcher, D. W., Tyler, R. T., Toews, R., & Gawalko, E. J. (2010). Effect of cooking on the composition of beans (*Phaseolus vulgaris* L.) and chickpeas (*Cicer arietinum* L.). *Food Research International, 43*(2), 589–594.

Xie, Z., Jiang, D., Cao, W., Dai, T., & Jing, Q. (2003). Effects of post-anthesis soil water status on the activities of key regulatory enzymes of starch and protein accumulation in wheat grains. *Acta Photophysiologica Sinica, 29*, 309–316.

Xu, Y., Cartier, A., Obielodan, M., Jordan, K., Hairston, T., Shannon, A., & Sismour, E. (2016). Nutritional and anti-nutritional composition, and in vitro protein digestibility of Kabuli chickpea (*Cicer arietinum* L.) as affected by differential processing methods. *Journal of Food Measurement and Characterization, 10*(3), 625–633.

Yigit, A. (2015). *Determination of protein, amino acid distribution and antioxidant activity of widely grown bread wheat (Triticum aestivum L.) varieties in Turkey*. Adnan Menderes University Phd Thesis.

Yu, X., Li, B., Wang, L., Chen, X., Wang, W., Gu, Y., et al. (2016). Effect of drought stress on the development of endosperm starch granules and the composition and physicochemical properties of starches from soft and hard wheat. *Journal of the Science of Food and Agriculture, 96*(8), 2746–2754.

Zia-Ul-Haq, M., Iqbal, S., Ahmad, S., Imran, M., Niaz, A., & Bhanger, M. I. (2007). Nutritional and compositional study of desi chickpea (*Cicer arietinum* L.) cultivars grown in Punjab, Pakistan. *Food Chemistry, 105*, 1357–1363.