

Effects of seeding date and seeding rate on yield, proximate composition and total tannins content of two Kabuli chickpea cultivars

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Abstract

Experiments were conducted in open field to assess the effect of seeding season and density on the yield, the chemical composition and the accumulation of total tannins in grains of two chickpea (*Cicer arietinum* L.) cultivars (*Pascià* and *Sultano*). Environmental conditions and genetic factors considerably affected grain yield, nutrient and total tannins content of chickpea seeds, giving a considerable range in its qualitative characteristics. Results confirmed cultivar selection as a central factor when a late autumn-early winter sowing is performed. In effect, a more marked resistance to *Ascochyta* blight (AB) of *Sultano*, allowed better agronomic performances when favourable-to-AB climatic conditions occur. Winter sowing appeared to be the best choice in the Mediterranean environment when cultivating to maximise the grain yield (+19%). Spring sowing improved crude protein (+10%) and crude fibre (+8%) content, whereas it did not significantly affect the accumulation of anti-nutrients compounds such as total tannins. The most appropriate seeding rate was 70 seeds m^{-2} , considering that plant density had relatively little effect on the parameters studied.

Introduction

Chickpea (*Cicer arietinum* L.) is an annual grain legume traditionally cultivated in semi-arid tropics (Asia and India), Australia and Mediterranean regions and has recently extended its

acreage and cultivation area to higher latitudes (Knights *et al.*, 2007).

Chickpeas, as other pulses, play a significant role in human and animal diets, especially as protein and energy source.

The major constraint to chickpea cultivation is represented by *Ascochyta* blight (AB), a necrotrophic disease caused by the fungus *Ascochyta rabiei* (Pass.) Labrousse. Several epidemics of AB causing complete yield loss have been reported in Pakistan, India, European countries and Mediterranean regions (Jettner *et al.*, 1999). Also, the occurrence of bioactive compounds with anti-nutritional effects, such as phenolic compounds (tannins), may represent a limiting factor for chickpeas consumption. In fact, especially in monogastric animals, nutrient absorption from the gastrointestinal tract can be impaired, with the onset of detrimental effects on health and growth (Muzquiz and Wood, 2007; Verma *et al.*, 2013). Conversely, these secondary compounds appear to be largely inactivated by rumen fermentation (Bampidis and Christodoulou, 2011).

The content of anti-nutrients and the proximate composition of chickpeas under different treatments have been widely investigated (Singh *et al.*, 1991; Attia *et al.*, 1994; Rincón *et al.*, 1998; El-Adawy, 2002; Nikolopoulou *et al.*, 2006). There has also been different studies on the effect of genotype, growing season and agronomic technique on chickpea growth and grain yield under rainfed conditions (Brown *et al.*, 1989; Horn *et al.*, 1996; Koutroubas *et al.*, 2009). However, limited knowledge exists on the combined influence of environmental and agronomic factors on the proximate composition and anti-nutrients content of chickpeas. Saxena (1984) and López-Bellido *et al.* (2008) reported on the dramatic increases in yield obtainable by winter planting of AB tolerant and low-temperature tolerant kabuli chickpea, but they did not comment on quality parameters.

Bampidis and Christodoulou (2011) reported on factors influencing chickpea grain protein utilisation and some processing techniques to improve the nutritional value of chickpea. Anyway, in this review no data were presented about the effect of agronomic techniques on grain yield, proximate composition and content of secondary compounds.

Singh *et al.* (1990) studied the combined effect of growing season, location and planting time on hundred seed weight (HSW), protein content (PC) and cooking time (CT) of different chickpea genotypes. The authors related these quality parameters with environmental conditions and found that winter planting decreased PC by 8 $g\ kg^{-1}$, whereas HSW increased by 1.2 g per 100 seeds as compared with spring planting. However, in this research no data were presented about other nutritional parameters (*e.g.* crude fat, starch, crude fibre, aminoacidic profile, *etc.*) and anti-nutrients content.

Oluwatosin (1999) and Nikolopoulou *et al.* (2006) found that the variability in the levels of some antinutritional factors (*i.e.* tannins and phytic acid) in chickpea and cowpea seeds depends largely on the environment where they are grown. Moreover, both

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authors indicated a high degree of heritability for all studied parameters. One way to change the environmental variables where a plant grows is to change the sowing date. Sowing practices have often been studied to determine the best time to plant for optimum yields. Although yield is still a crucial factor, studying how sowing date affects chemical and nutritional composition of seeds, may be helpful in achieving the desired quality of the product. Sowing date and densities have already been reported to influence the chemical composition of different legume crops such as chickling vetch (De Falco and Pardo, 1999; Rao and Northup, 2008), bean (Greven *et al.*, 2004; Getachew *et al.*, 2015), green field pea (Gubbels, 1977), faba bean (Hegab *et al.*, 2014), soybean (Li, 2014) but, according to our knowledge, no data are available for chickpea. Hence, the aim of this study was to verify the hypothesis that different seeding practices (time and rate) can affect not only grain yield, but also nutritional composition, total tannins content and the susceptibility to *Ascochyta* blight of two chickpea cultivars (*Sultano* and *Pascià*) currently cultivated in the Mediterranean basin.

Materials and methods

Experimental set up and plant growing conditions

Two chickpea Italian varieties were used in the experiments: *Sultano* and *Pascià*. They were chosen to verify their adaptability to winter sowing, assessed mainly as resistance to *Ascochyta rabiei* and to represent a range of genetic variation in morphological traits (Paolini *et al.*, 2006). Information on these varieties are reported in Table 1. To test the effects of sowing date and seeding density on grain yield and chemical composition of the two cultivars, a randomised complete block design experiment with three replicates was performed in 2006-2007 (Trial 1) and 2007-2008 (Trial 2) growing seasons. Both the trials were carried out under the same Mediterranean area (Tarquinia, Central Italy, 42°11'N, 11°45'E, 22 m a.s.l.). Soil tillage consisted of one pass of mouldboard plough at 0.3 m depth followed by disk harrow and spring harrow.

The early seeding (hereinafter referred to as winter) was carried out on 28 December 2006 and 18 December 2007 for Trial 1

and Trial 2, respectively. The late seeding (hereinafter referred to as spring) occurred on 2 March 2007 and 14 March 2008 for Trial 1 and Trial 2, respectively. Two seeding rates, 70 and 110 seeds m⁻², were compared only in Trial 2. They were chosen depending on normal and high-yielding situations in Mediterranean-type environments (Pande *et al.*, 2006). In Trial 1, 70 seeds m⁻² was applied.

Individual plots (8 x 1.5 m each) consisted of six rows with a row spacing of 0.3 m and a seeding depth of approximately 30 mm. Diammonium phosphate (18-46-0) was applied before sowing at the rate of 200 kg ha⁻¹, and weed control was achieved by using a pre-emergence herbicide at the rate of 2 l ha⁻¹ (Pendimethalin 322 g L⁻¹+ Imazethapyr 22 g L⁻¹). In both Trial 1 and Trial 2, neither irrigation nor pesticides were applied.

The chemical and physical characteristics of soil were: 33% clay, 19% silt and 48% sand, pH 6.8, 0.96% organic matter and 0.054% total N. Preceding crop for both trials was durum wheat.

Meteorological data are shown in Table 2. Both for Trial 1 and Trial 2, the mean air temperature during crop growing season was about 16°C for winter sowing and about 18 °C for spring sowing. Considering winter sowing, total rainfall registered during the chickpea growing season was 227 mm and 307 mm for Trial 1 and Trial 2, respectively; whereas, with regard to spring sowing, it was 134 mm and 162 mm for Trial 1 and Trial 2, respectively.

Ascochyta blight scoring

The reaction to disease was recorded each month starting from 40 days after emergence on 6 randomly selected plants per plot, using a 1-9 rating scale (Kimurto *et al.*, 2013), in which the disease score (DS) was graded from no visible symptoms to aerial part (1) to 100% of plants killed (9). Based on the DS, cultivars were categorised for their resistance to *A. rabiei* infection according to the Pande *et al.* (2006) scale, where 1, asymptomatic; 1.1-3.0, resistant; 3.1-5.0, moderately resistant; 5.1-7.0, susceptible; and 7.1-9.0, highly susceptible. The whole plant disease ratings were averaged across plants and date, to generate mean values of the disease rating for the two varieties before analysis.

Harvesting and sample preparation

Harvesting was performed using a plot harvester after physiological maturity, when about 90% of plants were completely dry

Table 1. Details of the two chickpea varieties tested.

Variety	Seed weight (mg)	Seed type	Plant habit	Resistance to <i>Ascochyta rabiei</i>
<i>Sultano</i>	320	Smooth	Erect	Yes
<i>Pascià</i>	550	Rough	Semi-erect	Yes

Table 2. Minimum, average and maximum temperature, relative humidity and rainfall of chickpea growing season for the years 2007 and 2008. Weather data were collected from Latium Region - Agricultural Department Agro-Meteorological Station, which is located 100 m away from the experimental fields.

	2007					2008				
	Tmin (°C)	Tavg (°C)	Tmax (°C)	RH (%)	Rainfall (mm)	Tmin (°C)	Tavg (°C)	Tmax (°C)	RH (%)	Rainfall (mm)
January	5.5	10.6	16.5	85	26.7	5.1	9.8	15.3	86	49.9
February	5.4	10.7	16.9	82	66.1	3.8	9.1	15.7	80	38.3
March	6.7	11.8	18.0	79	54.5	5.8	11.2	17.2	90	71.7
April	8.6	15.5	22.5	80	17.5	8.5	14.3	20.5	86	36.4
May	12.0	18.8	25.2	79	50.4	12.6	18.6	24.7	82	74.1
June	16.0	22.2	28.1	80	10.8	15.3	21.9	27.6	87	14.4
July	15.8	24.1	30.8	71	0.6	17.4	24.7	30.6	82	2.4

Tmin, minimum temperature; Tavg, average temperature; Tmax, maximum temperature; RH, relative humidity.

(on 30 July 2007 for Trial 1 and 1 August 2008 for Trial 2). After thorough cleaning and removal of foreign material, the grains were stored in paper envelopes at room temperature ($22 \pm 2^\circ\text{C}$) until drying. Seeds were analysed for dry matter (DM) drying at 65°C for 48 h in a forced air oven before grinding through a mill (Retsch, Haan, Germany) to pass 1 mm screen. After thoroughly mixing, milled samples were stored in sealed polyethylene containers until analysis.

Analytical procedures

Crude protein (CP), ether extract (EE), crude fibre (CF) and ash were determined according to AOAC Official Methods 984.13 (A-D), 920.39, 978.10 and 942.05 (AOAC, 2006) respectively. Total starch (TS) concentration was determined by amyloglucosidase- α -amylase method (AOAC Official Method 996.11) (AOAC 2006) using a commercial kit (Total Starch, AA/AMG, Megazyme International Ireland, Wicklow, Ireland). The amino-acidic profile was obtained by reverse phase liquid chromatography (RP-HPLC) on sample hydrolysates following the method developed by Cohen and De Antonis (1994), as modified by Liu *et al.* (1995). Separative column, derivatising agent and chromatographic eluents were available as AccQ•Tag™ kit for HPLC (Waters Co., Milford, MA, USA). Quantification of amino acids was carried out with the external standard calibration technique using high purity L-aminoacids (Sigma-Aldrich Co., St. Louis, MO, USA). Total tannins (TT) were determined by Folin-Ciocalteu method, according to Zielinski and Kozłowska (2000). Analysis was performed in triplicate, using technical grade methanol (MetOH) and Folin-Ciocalteu's reagent (Sigma-Aldrich Co). The final solution was read at 725 nm against blank using an UV-1601 double beam spectrophotometer (Shimadzu Corp., Kyoto, Japan). A standard curve was constructed dissolving purified (+)-catechin hydrate ($\geq 96.0\%$) (Sigma-Aldrich Co.) in MetOH to obtain four calibration standards within the range 0.03-0.30 mg mL⁻¹. Total tannin content of samples was then expressed as g of Catechin-Equivalents (CE) per kg of the sample (g CE kg⁻¹).

Statistical analysis

Response variables measured in both the experiments were subjected to ANOVA, using a year-combined randomised complete block design (McIntosh, 1983). Since different seeding densities were compared only in 2008 (Trial 2) a separate three-way ANOVA was performed just for data collected in this year with cultivar, sowing date and density as factors. Means were separated by the Fisher's least significance difference (LSD) test at the 95% probability level. Data analyses were performed using R 2.4.0 software (RCORE, 2006).

Results

Grain yield and Ascochyta blight resistance

The effect of each treatment on grain yield and AB score is shown in Table 3. The cultivar x year interaction affected both grain yield and DS (Figure 1). Particularly, in 2007 both cultivars yielded more than 2 t ha⁻¹ (2.4 for *Pascià* and 2.3 for *Sultano*), while in 2008, when climatic conditions were favourable to AB spreading, grain production significantly dropped by 57% for *Pascià* and 35% for *Sultano*.

As expected, winter sown chickpeas produced considerably more grain than did spring sown ones both in Trial 1 and Trial 2 ($P < 0.01$ and $P < 0.05$, respectively). Particularly, in Trial 1, winter

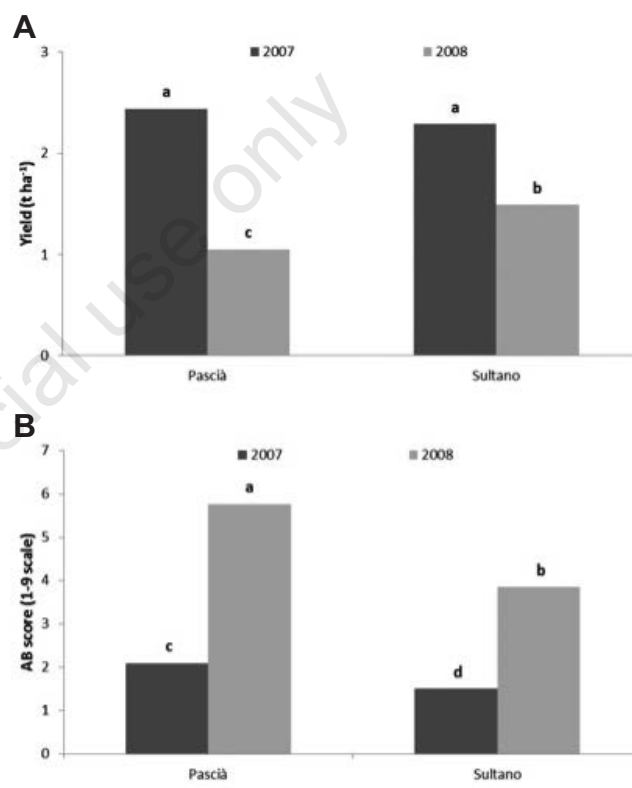


Figure 1. Grain yield (A) and Ascochyta blight score (B) as affected by cultivar x year interaction. a-d: different letters indicate that the samples are statistically different, according to the Fisher's least significant difference test.

Table 3. Grain yield and Ascochyta blight score means assessed in the two trials: effect of cultivar, year and time of sowing, seeding density and their respective interactions.

Trial		C			Y			T			S			Interaction*	
		<i>Pascià</i>	<i>Sultano</i>	P	2007	2008	P	Winter	Spring	P	70 (seeds m ⁻²)	110 (seeds m ⁻²)	P	CxY	SxT
1	Grain yield	1.75	1.89	ns	2.36	1.27	<0.001	1.98	1.66	<0.01	-	-	-	<0.01	-
	AB score	3.95	2.72	<0.001	1.80	4.87	<0.001	3.41	3.26	ns	-	-	-	<0.01	-
2	Grain yield	1.11	1.51	<0.001	-	-	-	1.39	1.23	<0.05	1.27	1.35	ns	-	-
	AB score	5.80	3.94	<0.001	-	-	-	4.92	4.82	ns	4.81	4.93	<0.05	-	<0.01

C, cultivar; Y, year of sowing; T, time of sowing; S, seeding rate; ns, not significant. *Other interactions are not reported as they are not significant. Mean grain yields are expressed as t ha⁻¹; means of Ascochyta blight (AB) score are expressed in the scale of 1-9, where 1, no disease and 9, dead plants. Probability (P) within a single variable and row indicates that the samples are statistically different, according to ANOVA procedure.

sowing yielded 320 kg ha⁻¹ more than spring one.

With regard to Trial 2, sowing rate did not affect grain yield whereas it significantly influenced AB incidence also in interaction with time of sowing (Figure 2).

In detail, significantly higher score was detected in plots sown earlier and with higher seeding density (5.1, susceptible) in comparison with other treatments that were ranked as moderately resistant (score from 4.8 to 4.9).

Proximate composition and total tannins content

Proximate analysis and total tannins content of chickpea seeds are reported in Table 4. Over the two years, CP content was significantly higher in 2008 than in 2007 ($P < 0.001$) and for spring sowing date compared to winter date. The cultivar x time of sowing interaction significantly affected the CP accumulation (Figure 3), reaching a 17% increase in *Sultano* for spring sowing ($P < 0.01$).

Separately, time of sowing and year affected the fat content of grain, with more fat for winter sowing (+4.5% compared with spring date) and 2008 (+4.4% compared with 2007). Cultivar x year interaction also influenced the fat content, with *Pascià* showing a significantly higher concentration in 2008 (49.9 g kg⁻¹) compared with 2007 (45.8 g kg⁻¹).

Differences in CF and ash content were observed between cultivars (*Sultano* > *Pascià*), years (2008 > 2007) and sowing date (spring > winter for CF while winter > spring for ash content). As for the CF content, cultivar x time of sowing interaction was significant (Figure 3). *Sultano* showed a higher CF content in delayed sowing (+13%) and as compared with *Pascià* both in winter (+12.6%) and spring sowing (+24.8%).

Furthermore, TS content in seeds from 2008 trial was 2.7% greater ($P < 0.01$) than 2007, and *Sultano* contained more TS than *Pascià* ($P < 0.01$). Cultivar x sowing date interaction significantly affected TS content (Figure 3). Particularly, *Pascià* showed 2.5% more TS ($P < 0.05$) in spring sowing than winter one (474.0 vs 462.3 g kg⁻¹). Regarding the total tannins (TT) content, *Sultano* contained 25% more TT ($P < 0.01$) than *Pascià*, while the other treatments were not significant.

Amino acids content

Amino acid composition of chickpea seeds produced during Trial 2, grouped in essential, aromatic and sulfur amino acids, is reported in Table 5. The content of essential amino acids was affected by the sowing date ($P < 0.05$) and cultivar ($P < 0.001$). It was 6% greater for winter sowing than spring one and 7.4% higher for *Sultano* than *Pascià*. A sowing date x cultivar interaction ($P < 0.01$) was observed as far as the aromatic amino acids content is concerned. Particularly, a higher aromatic amino acids content was detected for *Sultano* when sown in winter (+38.5%). Furthermore, sowing date affected sulfur amino acids content ($P < 0.05$) showing a 33% increase for winter sowing compared with spring one.

Discussion

In this study, growing chickpea in rainfed cropping systems of the Mediterranean environment resulted in a greater yield for winter than spring sowing.

The year-to-year variability observed in grain yield could be explained by taking into account differences in weather parameters and AB pressure. It was demonstrated that under similar climatic conditions, the improvement in grain yield was positively affected by total rainfall and its distribution over growing season (López-

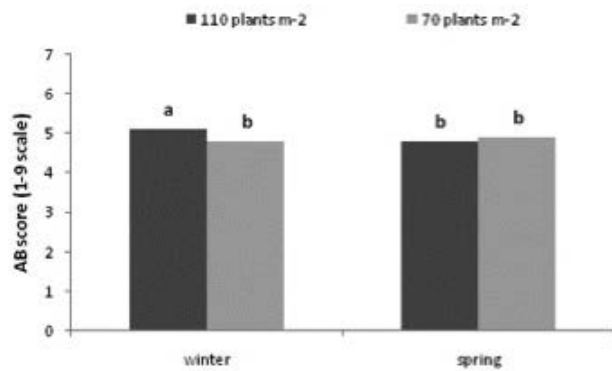


Figure 2. Ascochyta blight score as affected by sowing rate x time interaction. a-b: different letters indicate that the samples are statistically different, according to the Fisher's least significant difference test.

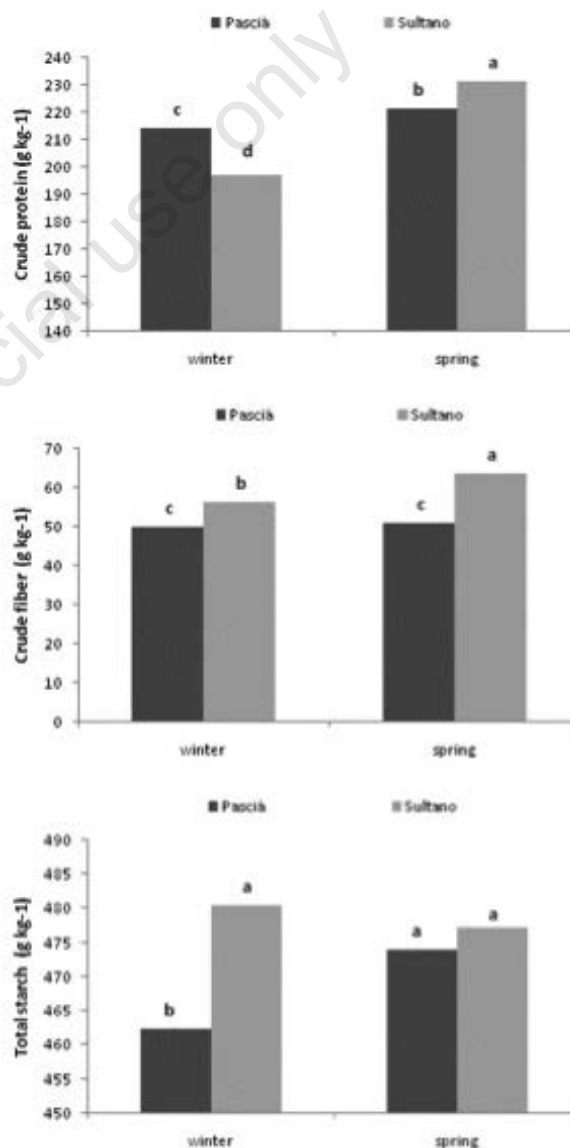


Figure 3. Crude protein, crude fibre and total starch content as affected by sowing date x cultivar interaction. a-d: different letters indicate that the samples are statistically different, according to the Fisher's least significant difference test.

Bellido *et al.*, 2008). However, in the present study the wetter growing season (2007/2008) was characterised by a lower grain yield than the drier one (2006/2007). The lower yields should be attributed to a greater biotic stress occurred during the 2007/2008 growing season. Moreover, it has to be taken into account that 2007 rainfall amount was sufficient to cover the chickpea needs in the useful stages. Trapero-Casas and Kaiser (1992) noted that disease severity increases with the increase in relative humidity, cloudiness and prolonged wet weather, which were the climatic conditions observed from March to early June 2008 in the present study (Figure 1). Considering that AB resistance rapidly diminishes with plant age, in particular at the beginning of anthesis (Chongo and Gossen, 2001), in 2008 the disease pressure was substantially greater than in 2007, due to almost 50% more rainfall during reproductive development (April and May).

The yield reduction observed in 2008 was much higher (57%) in *Pascià* compared to *Sultano* (35%). These results could be explained by the disease score observed in 2008 for *Pascià* (5.8), which can be categorised as susceptible versus the more resistant *Sultano* (score 3.9). This fact evidences that yield loss in chickpea could be strongly affected by AB outbreaks even though moderately resistant cultivars are used (Pande *et al.*, 2006).

About sowing date, winter sowing increased yields (19%) in comparison to spring sowing ($P < 0.01$). This result confirms findings from previous studies conducted under rainfed Mediterranean conditions (Saxena, 1984; Zaiter and Barakat, 1995; López-Bellido *et al.*, 2008). In that environment, early sowing allows the crop to take more advantage of stored soil water from late winter and early spring rain events. This opportunity, coupled with the absence of frost or disease damages, suggests that grain yield

increased in long season chickpeas because leaf area duration was longer (López-Bellido *et al.*, 2008) and water use efficiency was greater than that for late winter or spring sowings (Yau, 2005).

However, the advantages associated with early sowing can be lost when climatic conditions are unfavorable. In our study, this was particularly important during the critical growth stages, such as flowering and pod-filling, when plants had the maximum susceptibility to AB infection. As for differences between cultivars, *Sultano* (more resistant to AB) shall be regarded as more advisable from an economic and environmental point of view, because it does not need fungicide applications during the growing season. Moreover, for the same reason *Sultano* could be more advisable for organic farming than *Pascià*.

In general, the proximate composition of the chickpea cultivars under study agrees with the values found in the literature. References report protein varying from 13.7 to 34.0% and fat from 3.4 to 4.6%; CF, TS and ash were also in line with previously published data (Nikolopoulou *et al.*, 2006; Bambidis and Christodoulou, 2011). Also aminoacidic profiles, observed in the present study, are in line with findings by Bampidis and Christodoulou (2011).

The effect of cultivation year on the composition of chickpeas was also reported by Nikolopoulou *et al.* (2006), though we observed a higher fat content in the rainy season (2008). The higher fat content could be attributed to the lower grain yield as recently found by Li *et al.* (2014) in soybean.

Higher CP content in delayed sowings was also reported for chickpea by Singh *et al.* (1990), Kaya *et al.* (2010) and Dehal *et al.* (2016). The effect of spring or autumn planting on protein content is well known in wheat, in which a delay in sowing date was asso-

Table 4. Proximate composition and total tannins content assessed in the two trials: effect of cultivar, year, time of sowing, seeding density and their respective interactions.

Trial		C		Y			T			S			Interactions*						
		<i>Pascià</i>	<i>Sultano</i>	P	2007	2008	P	Winter	Spring	P	70 (seeds m ⁻²)	110 (seeds m ⁻²)	P	CxY	CxT	YxT	CxS	TxS	
1	CP	217.8	214.3	ns	204.2	228.0	<0.001	205.6	226.5	<0.001	-	-	-	<0.001	-	-	-	-	-
	Fat	47.8	47.1	ns	46.5	48.5	<0.05	48.5	46.4	<0.05	-	-	-	<0.05	-	-	-	-	-
	CF	50.4	59.9	<0.01	30.2	80.1	<0.05	53.0	57.2	<0.05	-	-	-	-	<0.05	-	-	-	-
	Ash	36.8	38.2	<0.01	36.3	38.7	<0.01	37.9	37.1	<0.05	-	-	-	-	-	<0.001	-	-	-
	TS	469.0	477.9	<0.01	467.1	478.2	<0.05	472.2	474.8	ns	-	-	-	-	<0.05	-	-	-	-
	TT	1.6	2.0	<0.01	1.6	1.9	ns	1.8	1.7	ns	-	-	-	-	-	-	-	-	-
2	CP	230.2	226.8	ns	-	-	-	216.8	240.2	<0.05	229.0	228.0	ns	-	-	-	-	-	-
	Fat	49.6	49.8	ns	-	-	-	52.7	46.7	<0.05	48.5	50.9	<0.05	-	-	-	<0.001	<0.01	-
	CF	77.5	83.4	<0.05	-	-	-	78.9	81.9	ns	80.1	80.8	ns	-	-	-	<0.01	-	-
	Ash	38.0	38.9	ns	-	-	-	37.3	39.6	<0.05	38.7	38.12	ns	-	-	-	-	-	-
	TS	474.8	484.5	<0.05	-	-	-	474.3	485.0	ns	478.24	481.1	ns	-	-	-	-	-	-
	TT	1.6	2.1	<0.01	-	-	-	1.9	1.8	ns	1.83	1.8	ns	-	-	-	-	-	-

C, cultivar; Y, year of sowing; T, time of sowing; S, seeding rate; ns, not significant; CP, crude protein; CF, crude fibre; TS, total starch; TT, total tannins; NS, not significant. *Other interactions are not reported as they are not significant. Means are expressed as g kg⁻¹ on dry matter basis. Probability (P) within a single variable and row indicates that the samples are statistically different, according to ANOVA procedure.

Table 5. Amino acids compounds of seeds in trial 2: effect of cultivar, time of sowing, seeding density and their respective interactions

Trial	C		P	T		P	S		P	Interaction* CxT
	<i>Pascià</i>	<i>Sultano</i>		Winter	Spring		70 (seeds m ⁻²)	110 (seeds m ⁻²)		
Essential a.a.	47.09	50.62	<0.001	50.28	47.43	<0.05	48.37	49.34	ns	-
Sulfur a.a.	1.02	1.11	ns	1.21	0.93	<0.05	0.93	1.21	ns	-
Aromatic a.a.	8.20	9.33	<0.05	9.53	7.95	ns	8.20	9.28	<0.05	<0.01

C, cultivar; T, time of sowing; S, seeding rate; ns, not significant *Other interactions are not reported as they are not significant. Means are expressed as g/kg on dry matter basis. Probability (P) within a single variable and row indicates that the samples are statistically different, according to ANOVA procedure.

ciated with a decrease in mean grain weight along with an increased per-grain total nitrogen content, thus leading to an overall increase in protein percentage (Motzo *et al.*, 2007). However, taking into account the total amount of protein gained per hectare, winter sowing proved to be the best choice.

With regard to the total tannins (TT) content of chickpea seeds, *Sultano* contained 25% more total tannins ($P < 0.01$) than large-sized *Pascià*, thus confirming the importance of genetic effect for this parameter and the higher TT content in smaller seeds (Nikolopoulou *et al.*, 2006).

Moreover, the finding that *Sultano* contained more TT than *Pascià* (less resistant to AB), agrees with results of Kumar *et al.* (2013), who stated that AB resistance is related to the accumulation of phenolic compounds in chickpea seeds of different genotypes. It is well known the role of phenolics in the resistance mechanisms of plants against fungal pathogens (Lattanzio *et al.*, 2006). Some variables such as rhizome inoculation (Abdalla *et al.*, 2013) and row spacing (Menga *et al.*, 2014) proved to be effective on the accumulation of phenols in chickpea. However, in our study neither the year nor the sowing date or sowing rate (only tested for Trial 2) affected TT content.

Conclusions

In summary, this study has shown that both environmental conditions and genetic factors affect not only grain yield but also the nutrient and anti-nutrient compositions of chickpea seeds, determining a considerable range in their qualitative characteristics. Time of sowing was found to affect strongly both yield and chemical composition of seeds. Winter sowing appeared to be the best choice in the Mediterranean environment when cultivating to maximise the grain yield. Moreover, even though delayed sowing improved CP content (+10%), the total amount of protein obtainable per hectare was higher for winter planting. The cultivar *Sultano* proved more productive than *Pascià* especially when climatic conditions were favourable for AB outbreak, emphasising the importance of selecting AB resistant genotype to improve the agronomic performance of this grain legume when sown in organic cropping systems. Plant density had relatively little effect on the considered parameters.

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