POLISH SOCIETY FOR HORTICULTURAL SCIENCE

Folia Hort. 30(2), 2018, 375-385

DOI: 10.2478/fhort-2018-0033

FOLIA HORTICULTURAE

Published by the Polish Society
for Horticultural Science since 1989

ORIGINAL ARTICLE | Open access | www.foliahort.ogr.ur.krakow.pl

Production system effects on growth, pod yield and seed quality of organic faba bean in southern Italy

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ABSTRACT

A research was carried out in southern Italy in order to evaluate the effects of two farming systems (open field, greenhouse) and five transplanting times (27 September, 11 October, 25 October, 8 November, 22 November) on plant growth, harvest precocity, fresh pod yield and seed quality of organic faba bean. Crop biomass and root expansion were higher in open field than in greenhouse. The fourth planting time resulted in the highest crop biomass, whereas the second crop cycle showed the highest leaf area index. Greenhouse crops showed higher precocity than the open field ones by about two weeks, as well as the first transplant; the delay in harvest beginning increased from the second to the fourth planting time. Both fresh pod and seeds yield were significantly higher in open field than under protected environment with the third and fourth planting times. Seed fiber and protein content showed higher values in greenhouse compared to open field and increased with the transplant delay. Seed polyphenols attained higher concentration in open field and with the two earliest planting times. Overall, 25 October to 8 November planting times in open field best fitted the southern Italy growing conditions in terms of pod yield, but the 27 September to 11 October planting times resulted in the highest harvest precocity, remarkably enhanced under greenhouse growing, whereas seed quality was controversial.

Keywords: fiber, greenhouse, planting time, polyphenols, proteins, *Vicia faba* L.

INTRODUCTION

Faba bean (*Vicia faba* L.) is cultivated in many areas, mainly located in Mediterranean basin, western and eastern Europe, China, India, South America, Australia, its surface area accounting for: 240,000 ha worldwide (FAOSTAT, 2014); 58,688 ha in Italy, of which 7,553 ha are devoted to fresh pod yield (ISTAT, 2017). Faba bean shows features fitting a sustainable agriculture model (Nadal et al., 2003), benefiting from symbiosis with *Rhizobium* bacterium to fix nitrogen from the atmosphere. However, due to the slow initial establishment of the mutualistic relationship, a small N supply at planting is useful, even improving plant absorption of K, Mg and Ca (Sanchez-Chavez et al., 2010).

Faba bean devoted to fresh pod yield is mostly grown in the field, but greenhouse environmental conditions may allow for enhancing crop production earliness (Gallacher and Sprent, 1978) and it is also suitable to organic horticulture, which is more susceptible to the environmental unbalances caused by a less intensive management (Maynard, 1994). Moreover, since the higher market prices of the

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organic vegetables usually result in higher income for farmers, greenhouse cultivation of faba bean may be regarded as a cost effective alternative to traditional open field farming.

Planting time is one of the major factors affecting *Vicia faba* phenological development, as the turning phase depends on photothermal conditions and, indeed, temperature has a significant effect on flowering anticipation, similarly to photoperiod lengthening (Evans, 1959). In this respect, Ellis et al. (1988) found that optimal flowering progress occurs at 19.9-25.4°C, or even at lower temperature with some genotypes. Due to these requirements, faba bean crop cycle starts in autumn in mild climate areas and in spring in cooler ones, but the optimal planting time for maximizing earliness, yield and quality is strictly matched to the growing environment (Myers et al., 1982; Gomez et al., 2017). In this regard, in Pakistan, Khalil et al. (2010) reported that the highest yield is favored by early October sowing, compared to late summer or mid-autumn planting, and Fasheun and Balogun (1992) detected the significant correlation between dry matter production and radiation use efficiency as affected by planting time.

Moreover, *Vicia faba* seeds represent a remarkable energy source, providing 44 Kcal 100 g^{-1} of fresh seeds, and they are rich in fiber, lysine rich proteins, mineral nutrients, vitamins and antioxidants (Ofuya and Akhidue, 2005; Crépon et al., 2010). Notably, the high fiber concentration is essential for intestinal functions regulation as well as for blood glucose and cholesterol control (Macarulla et al., 2001). Polyphenols act as antioxidants as well as protective screens against ultraviolet radiations (Jansen et al., 2001) and they are highly present in the seed tegument (Chaieb et al., 2011). However, polyphenols excessive accumulation relevant to cell requirements can potentially damage lipids, proteins and nucleic acids (Cho and Kleeberger, 2010), promoting chronic diseases such as cancer, cardiovascular or neurodegenerative problems (Sas et al., 2007).

This research aimed to evaluate the effects of farming system and planting time on production earliness, fresh pod yield and seed quality indicators such as residues, fibers, proteins and antioxidants of faba bean grown in southern Italy environmental conditions.

MATERIAL AND METHODS

Plant material and growth conditions

Research was carried out on faba bean (*Vicia faba* L. major Hartz) cultivar Aguadulce supersimonia, grown under organic management in Naples, southern Italy (40°50' N, 14°15' E, 17 m a.s.l.), in Mediterranean area, from autumn 2011 to summer 2013 on a sandy-loam soil field (81% sand, 8% silt, 11% clay, 2% organic matter, 330 µS cm^{-1} EC). The ten-day means of temperature (day/night) and PAR recorded at the plant level are shown in Figure 1.

Comparisons were made between ten experimental treatments, obtained by the factorial combination of two farming systems (open field, greenhouse) and five planting times (27 September, 11 October, 25 October, 8 November, 22 November). A split plot design with three replicates was arranged, assigning the main plots to the farming

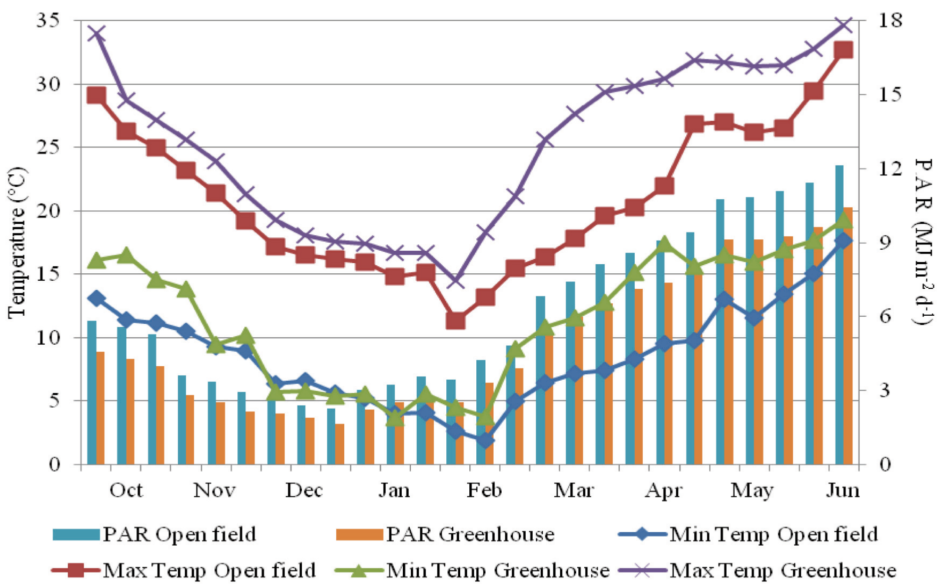

Figure 1. Ten-day means of temperature and PAR in Naples (Italy): means of 2011-12 and 2012-13

systems, and each elementary plot was 6.20 m^2; plants were transplanted in single rows 0.85 m apart, with 0.13 m spacing along the rows, achieving an areal density of 9 pt m^{-2}.

Greenhouse consisted of a three-span polytunnel, each span being 30 m long, 5 m wide, 2 and 3.5 m high at wall and roof respectively. Both in open field and greenhouse, organic farming practices were performed in compliance with the EC Regulation 834/2007 and related subsequent updates. Based on faba bean nutrient requirements per pod yield unit (Jensen et al., 2010), each year the plants were supplied with 60 kg ha^{-1} of N, 75 kg ha^{-1} of P_2O_5 and 200 kg ha^{-1} of K_2O; nitrogen was just given before transplanting, whereas phosphorus and potassium were split in a 30% dose prior to crop establishment (Bioilsa 6-5-13 manure) and the remaining 70% on dressing. Drip irrigation was activated when the soil available water capacity (AWC) decreased to 80%.

Harvests of fresh pods were performed from 8 February to 10 June in greenhouse and from 21 February to 16 June in open field, as an average of the two research years.

General analytical methods

Plant samples were randomly collected to assess the maximum leaf surface extension using a bench top LI-COR leaf area meter. Harvests of fresh pods were performed coinciding with maximum seed growth and determinations were made in each plot for: weight and number of undamaged pods classified as marketable; fruit mean weight on 30 unit samples; seeds weight on random samples including 20 pods. Precocity was expressed as the number of days from 1 January to the first harvest. Cumulative plant biomass was calculated as the sum of the above ground plant biomass at the end of each crop cycle plus the total fruit production from the beginning of the harvest period. Dry weight was assessed after dehydration of the fresh samples in an oven at 70°C under vacuum until they reached constant weight. At each plant sampling, soil was also sampled at 30 cm depth in order to perform determinations in the root apparatus, concerning root weight as well as length and diameter (Newman, 1966), allowing to calculate the root area expansion.

Root growth determinations

Fifty grams of air dried soil were suspended in 10% concentrated Calgon buffer (85% sodium hexametaphosphate and 15% sodium carbonate); roots were then separated after flotation, washing and wet sieving (0.04 mm^2). Next, the root length and diameter were determined (Newman, 1966).

Seed quality analysis

Faba bean pods were randomly sampled in each plot between 25 March and 29 April, the latter being a time point overlapping among the five crop cycles examined, and immediately transferred to the laboratory, where the seeds were extracted from the pods. From each seed sample, 50 g of seeds were randomly drawn out and homogenized with a blender in liquid nitrogen for the determinations of ascorbic acid in triplicate and of soluble solid content in duplicate. Other 50 g of seeds were frozen, lyophilized and then ground for the determinations of fiber, protein and total polyphenol contents in triplicates. Soluble solids, expressed as Brix at 20°C, were assessed using a Bellingham and Stanley digital refractometer, model RFM 81. Ascorbic acid was assessed according to Kampfenkel et al. (1995), total polyphenols as previously described (Caruso et al., 2014).

Fiber and proteins were determined using a chemometric method, with principal component regression method (PCR) applying near infrared spectroscopy (NIR). For PCR calibration parameters the fiber and nitrogen content were determined for 20 samples. Fiber was determined on the washed and dried residue from 1 g of lyophilized seeds extracted with petroleum ether and then hydrolyzed for 30 min in 100 mL H_2SO_4 0.5 M at boiling point; nitrogen was determined by HCNS analyzer (Fisons EA 1108) in triplicate. Spectra between 4,000-10,000 cm^{-1} of powdered seeds were obtained in reflectance with a NIRA accessory on a Perkin Elmer Frontier infrared spectrophotometer equipped with Spectrum software and chemometrically processed by Quant+ software, both from Perkin Elmer. The PCR parameters for fiber and proteins were, respectively, as follows: number of principal components, 5 and 4; R^2, 91.0 and 87.2; SEE, 1.706 and 1.395; SEP, 2.176 and 1.983; and means, 26.6 and 30.3. The protein content was obtained from nitrogen multiplied by 6.25.

Statistical analyses

Data were processed by analysis of variance and mean separations were performed through Duncan multiple range test at 0.05 probability level, using SPSS software version 21. Data expressed as percentage were subjected to angular transformation before processing.

RESULTS AND DISCUSSION

There were no significant differences between the two years of research and, therefore, only the effects of farming system and planting time as well as their interactions are reported.

Growth indexes and yield

The highest leaf area index (LAI) was recorded at full fructification stage, consistently with the results obtained in previous research carried out in temperate or Mediterranean environment (Lòpez-Bellido et al., 2005). Different trends were reported by other authors (Daur et al., 2011), who detected an increase of plant surface expansion from planting to flowering and then a decrease until seeds ripeness, due to gradual leaf senescence. In our research, root area reached the highest value when the first pods set and it was better affected by open field growing, the latter also showing higher values of plant root biomass compared to greenhouse ones (Tab. 1). Moreover, the second to fourth planting time (11 October to 8 November) resulted in the highest root surface expansion and the fourth transplant also in the highest root biomass.

Faba bean aerial biomass (Tab. 1) attained higher values in open field than in greenhouse; moreover, an increasing trend was recorded until the fourth planting time (8 November), though the latter was not significantly different from the third one (25 October), and a drop under the last transplant. Consistently with our results, other authors (Ellis et al., 1988) reported the increase of plant development rate with the temperature rise and a reverse trend when the latter exceeds the optimal threshold. Conversely to our findings, in previous research (Loss and Siddique, 1997) faba bean biomass grown in Australia showed a decreasing trend with the planting delay.

LAI was significantly affected by the interaction between farming system and planting time (Fig. 2). Open field grown plants showed higher leaf apparatus expansion than those managed in greenhouse with the fourth planting time, whereas the opposite trend was recorded with the fifth crop cycle. No significant differences were recorded between the two farming systems from the first to the third transplant. Moreover, in open field LAI did not significantly change under the transplants performed from 27 September to 8 November,

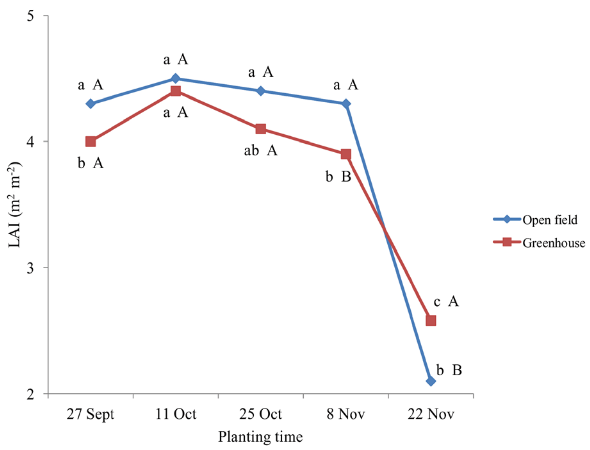

Figure 2. Interaction between farming system and planting time on faba bean leaf area index (LAI). Values followed by different letters are significantly different according to Duncan multiple range test at $p \leq 0.05$; capital letters refer to comparison between farming systems, lowercase letters between planting times

Table 1. Growth indexes of faba bean

	Growth indexes			
	Aerial parts		Roots	
	LAI (m^2 m^{-2})	Dry weight (g m^{-2})	Area (m^2 m^{-2})	Dry weight (g m^{-2})
Farming system				
Open field	3.9	1122.2	13.4	56.8
Greenhouse	3.8	951.7	9.8	38.7
	n.s.	*	*	*
Planting time				
27 September	4.2 bc	864.6 cd	11.1 b	47.1 bc
11 October	4.5 a	1041.3 bc	11.8 a	48.4 b
25 October	4.3 ab	1244.5 ab	12.1 a	47.5 b
8 November	4.1 c	1303.4 a	12.2 a	50.3 a
22 November	2.3 d	730.7 d	10.8 b	45.5 c

n.s. no statistically significant difference, *significant difference at $p \leq 0.05$. Within each column, values followed by different letters are significantly different according to Duncan multiple range test at $p \leq 0.05$

Table 2. Yield results of faba bean

Treatment	Harvest beginning	Marketable fresh pods			Seeds		
	Days from 1 January	Yield (t ha^{-1})	No. per plant	Mean weight (g)	Weight to pods (%)	Weight (t ha^{-1})	Mean weight (g)
Farming system							
Open field	80	13.2	5.7	22.4	26.2	3.5	1.42
Greenhouse	67	11.1	5.0	21.7	29.0	3.3	1.66
	*	*	*	n.s.	*	n.s.	*
Planting time							
27 September	45 d	6.0 c	3.1 c	18.7 c	24.4 c	1.5 d	1.30 c
11 October	58 c	11.2 b	5.4 b	20.5 b	26.0 b	2.9 b	1.42 b
25 October	74 b	17.2 a	7.2 a	23.6 a	29.3 a	5.0 a	1.70 a
8 November	94 a	18.1 a	7.4 a	23.9 a	29.4 a	5.2 a	1.69 a
22 November	99 a	8.4 c	3.5 c	23.5 a	28.9 a	2.4 c	1.61 a

n.s. no statistically significant difference; *significant difference at $p \leq 0.05$. Within each column, values followed by different letters are significantly different according to Duncan multiple range test at $p \leq 0.05$

but it just dropped with the latest planting time. Otherwise, greenhouse leaf apparatus attained the highest expansion in the second crop cycle and the lowest in the last one. Open field grown faba bean in north-western Spain (Confalone et al., 2010) showed a similar trend in LAI to that observed in the present work; in southern England, de Costa et al. (1997) reported a positive correlation between open field grown faba bean biomass and LAI, the latter being higher than that recorded in our research.

As shown in Table 2, the greenhouse crops showed higher precocity than the open field ones by about two weeks, as the higher temperatures recorded in the protected environment allowed the plants to reach earlier the needed thermal time for flowering, i.e. the degree-day sum of 1,000 to 1,100°C day, and accordingly the pod ripeness for harvesting. The values of degree-day sums detected in our research, calculated by taking into account the base-temperature of 1.7°C, are consistent with those reported for *Vicia faba* by Iannucci et al. (2008) in southern Italy and by other authors for some Mediterranean genotypes (Ellis et al., 1988, 1990).

As for the comparison between the planting times (Tab. 2), the first transplant of 27 September resulted in the earliest harvest beginning, which occurred on 8 February in greenhouse and on 21 February in open field. Notably, over mid-winter time the market prices of faba bean fresh pods are the highest in the year and the two-week harvest anticipation achieved under protected environment is even more profitable. A yield anticipation upon September early planting of greenhouse faba bean was also reported in previous research (Gallacher and Sprent, 1978).

Interestingly, the delay in harvest beginning progressively increased from the second (11 October) to the fourth transplant (8 November), though the third and fourth crop cycles were characterized by lower mean temperatures, i.e. enhanced vernalization, in the initial developmental phases as compared to the two earliest transplants. Indeed, the effect of vernalization on flowering anticipation is progressively increased as the temperature is lowered below 17°C (Evans 1959), but the thermal time connected with plant development optimal temperature is considered the most important contributor to this phenomenon in faba bean (Patrick and Stoddard, 2010). Moreover, the plant flowering of faba bean cultivar Aguadulce supersimonia, used in our research, was not affected by photoperiod in Naples environmental conditions, as this development stage was always reached within the photoperiod range of 9.2 to 14.7 hours. Consistently with our results, Stoddard (1993) reported that some Mediterranean cultivars flowered at much shorter photoperiod than 12 hours, whereas Iannucci et al. (2008) found that the Italian cultivars Manfredini and Vesuvio did not flower below 12 hours daylength.

As reported in Table 2, mean pod weight was not affected by farming system, whereas mean seed weight was higher in greenhouse grown crops; both parameters showed the highest values under the third to fifth transplant. In this respect, the light intensity increase associated to the transplant deferment from 27 September to 25 October positively affected the seed growth phase, which is very sensitive to photosynthesis rate (Toker, 2004) and, in fact, decreased levels of photoassimilates reaching developing seeds negatively impacts on their size (Borrás et al., 2004). The seed occurrence to pod weight, expressed as a percentage, was significantly higher in greenhouse grown crops than in the open field ones and under the third to fifth planting time.

The significant interaction between farming system and planting time on pod yield (Fig. 3) shows that open field yield was higher than greenhouse production both under the third and the fourth transplant. A reverse trend was recorded with the second and the fifth planting time, whereas no yield difference was detected in the first crop cycle. Moreover, open field production significantly increased from the first to the fourth planting time and then decreased with the last transplant. In

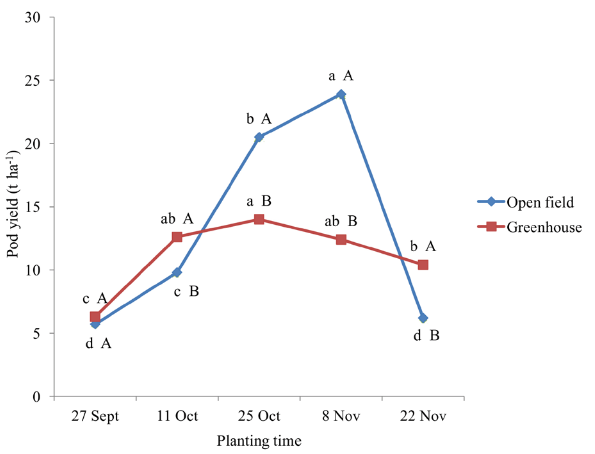

Figure 3. Interaction between farming system and planting time on faba bean pod yield. Values followed by different letters are significantly different according to Duncan multiple range test at $p \leq 0.05$; capital letters refer to comparison between farming systems, lowercase letters between planting times

greenhouse, there was an increase from the first to the second planting time, after which the yield was steady until the fourth transplant and finally showed a reduction in the last crop cycle. In open field there was no difference between the first and the fifth planting time, whereas in greenhouse the first crop cycle showed the lowest production value.

In previous research carried out in Mediterranean environment of south-east Australia (Marcellos and Constable, 1986), early autumn sowing prolonged the crop cycle compared to later planting times, thus resulting in better growth and higher yields. In a trial carried out by Khalil et al. (2010) in open field in the Peshawar region (Pakistan), the highest yield was obtained with early October sowing, compared both to earlier (late September) and later planting (second half of October). In our research, in open field conditions of southern Italy the best production results were achieved with transplant practiced two to four weeks later than the most effective ones performed in Australia or Pakistan. This outcome can be explained by the fact that faba bean thrives in cool and humid climate with evenly distributed rainfall from 650 to 1,000 mm per year (Gasim and Link, 2007); in this respect, in south-west Australia Mwanamwenge et al. (1999) reported that early planting time allows faba bean plants to avoid late spring high temperatures as well as aphid infestation.

In the sub-coastal area of the Naples region, yield obtained from the two crop cycles starting between late September and mid-October was adversely affected by both supra-optimal temperatures in the first phenological phases and by sub-optimal temperatures during the pod set and growth. Notably, high temperatures led to about 30% or 20% plant mortality in greenhouse or open field crops respectively, in the first month after transplant in the earliest crop cycle. The survived plants precociously reached the flowering phase in late autumn or early winter, under the first or the second planting time respectively, but this resulted in reduced pod set. The latter was presumably caused by sub-optimal temperatures for this phenological stage progress, which are also connected with pollination deficiency, considered as a major factor of flower abortion (Chen et al., 2006). Indeed, the optimal temperatures for flowering progress fall in the 19.9 to 25.4°C range (Ellis et al., 1988), though some cultivars are less demanding. The third (25 October) and fourth (8 November) planting times were the best yielding in open field, due to their later flowering which resulted in enhanced pod set under more favourable temperatures (Aguilera-Diaz and Recalme-Manrique, 1995), thus leading to a higher pod number per plant. The same transplants resulted in much lower yields in greenhouse, compared to open field, as a consequence of the excessive temperature increase along with the 17% average light reduction over the fructification period. Conversely to our findings, Adisarwanto and Knight (1997) reported that in Mediterranean environment, sowing time has low effect on the time span between plant emergence and flowering beginning.

The interaction between farming system and planting time also had a significant effect on pod

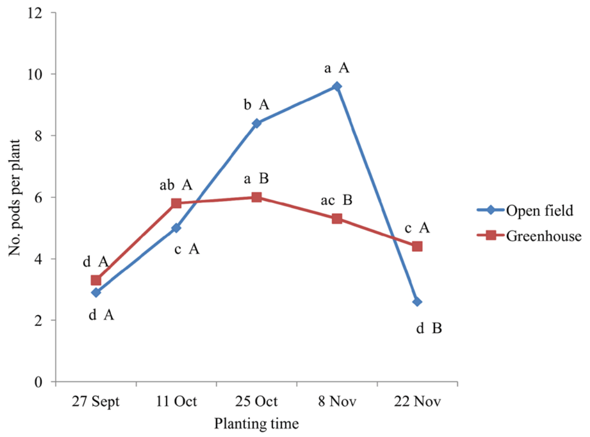

Figure 4. Interaction between farming system and planting time on faba bean pod number per plant. Values followed by different letters are significantly different according to Duncan multiple range test at $p \leq 0.05$; capital letters refer to comparison between farming systems, lowercase letters between planting times

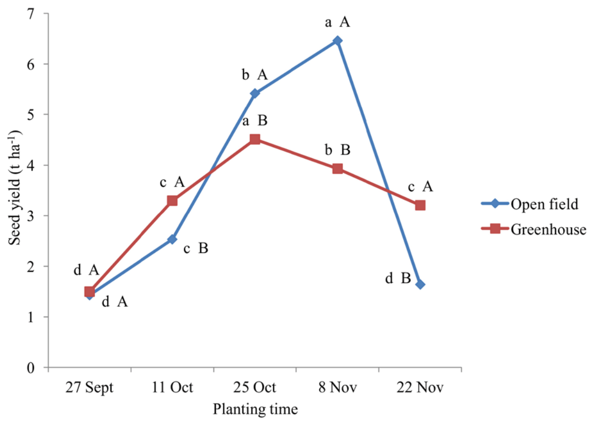

Figure 5. Interaction between farming system and planting time on faba bean seed yield. Values followed by different letters are significantly different according to Duncan multiple range test at $p \leq 0.05$; capital letters refer to comparison between farming systems, lowercase letters between planting times

number per plant (Fig. 4), whose trends are quite similar to those illustrated for yield; the latter was in fact positively related with the former variable. The lower pod number per plant recorded in the earliest and latest planting times or even in the intermediate transplants under greenhouse as compared to open field is connected with physiological stresses, which affect pod number rather than seed size or number per pod (Adisarwanto and Knight, 1997). However, Confalone et al. (2010) reported that in open field grown faba bean the trend of pods yield as a function of planting time depended on both fruit number and weight.

The interaction between farming system and planting time was also significant on seed yield (Fig. 5), which was higher in open field than in greenhouse under the third and the fourth planting time; in the second and in the fifth crop cycle the greenhouse crops showed higher values. Moreover, in open field an increasing trend was recorded up to the fourth transplant and a reduction in the latest crop cycle, whereas under protected environment a seed yield increase was observed until the third transplant and a subsequent drop in the two latest planting times.

Seeds quality and antioxidants content

Dry residue and soluble solids of faba bean seeds were not affected by farming system (Tab. 3). These variables increased from the first to the fifth planting time as well as from the first harvest of the sampled period (late March) to the latest harvest carried out thirty-five days later. Moreover, farming system significantly affected fiber content, which was higher in greenhouse grown seeds than in the open field ones (Tab. 3). Fiber value gradually increased from the first to the fifth planting time (221 to 240 mg g^{-1} d.w. respectively). Fiber content also increased with the progress of the fructification phase from 182 mg g^{-1} d.w. in early pods to 267 mg g^{-1} d.w. in the latest ones. Other authors reported lower fiber levels in faba bean seeds compared to those detected in our research (Hedley, 2001).

As shown in Table 3, the protected environment resulted in higher seed protein content than the open field, 304 and 270 mg g^{-1} d.w. respectively, these values falling within the range reported by other authors (Ofuya and Akhidue, 2005). Moreover, the protein content increased with the delay of both transplant (+16.2% with the last crop cycle compared to the first one) and harvest time (264 and 326 mg g^{-1} d.w. in the earliest and latest harvest, respectively).

Table 3. Quality indicators and antioxidants of faba bean seeds

Treatment	DR (mg)	SS (°Brix)	Fiber (mg)	TP (mg)	PP (mg)	AA (µg)
Farming system						
Open field	191	8.6	219	270	31.3	824
Greenhouse	194	9.0	241	304	26.4	524
	n.s.	n.s.	*	*	*	*
Planting time						
27 September	171 d	8.5 c	221 b	266 c	32.9 a	581 c
11 October	182 c	8.7 bc	223 b	281 bc	32.8 a	636 b
25 October	198 b	8.9 ab	230 ab	286 b	26.8 b	703 a
8 November	201 b	9.0 ab	236 a	294 ab	26.7 b	710 a
22 November	210 a	9.1 a	240 a	309 a	25.5 b	736 a
Harvest date						
25 March	152 e	8.2 c	182 e	264 d	40.9 a	528 d
4 April	170 d	8.4 bc	216 d	280 cd	29.2 b	585 c
11 April	191 c	8.7 b	236 c	291 bc	28.5 b	619 bc
18 April	212 b	9.1 a	254 b	304 b	23.1 c	669 b
29 April	235 a	9.3 a	267 a	326 a	17.7 d	755 a

DR data are per g fw of seed tissue; other data are per g dw of seed tissue. Abbreviations: DR, dry residue; SS, soluble solids; TP, total proteins; PP, polyphenols; AA, ascorbic acid. n.s. no statistically significant difference, *significant difference at $p \leq 0.05$. Within each column, values followed by different letters are significantly different according to Duncan multiple range test at $p \leq 0.05$

In our research, the seed protein content showed increasing trends with the temperature rise; indeed, protein biosynthesis in faba bean seeds is affected by the interaction among genetic, agronomic and climate factors (Hood-Niefer et al., 2011) and it is quite intensive during the storage phase of seed development. The latter is characterized by a switch in cotyledon metabolism from cell division to cell expansion and by endopolyploidization (Borisjuk et al., 1995). Notably, the beginning of protein accumulation is linked with a sucrose signal originating from the onset of sucrose/H+ symporter activity (Rosche et al., 2005). Within the two major storage protein classes, the synthesis of vicilin starts about 21-24 days after flower anthesis and ceases about 7-10 days later whereas the synthesis of legumin begins about 4 days after vicilin synthesis completion (de Pace et al., 1991).

In legume seeds there are structural linkages between proteins and fibers, affecting seeds nutritive value which is connected to the availability of proteins digested and absorbed in the digestive tract. In fact, a significant protein percentage (23 to 43% of total proteins) remains insoluble and less nutritionally available, as it is connected to the insoluble fiber fraction (Martin-Cabrejas et al., 2008). Indeed, the proteins linked to insoluble fiber components (cellulose, hemicellulose, and lignin) are not hydrolysed into lower molecular weight compounds such as small peptides and amino acids (Bravo, 1998).

Total polyphenols content (Tab. 3) was higher in open field grown seeds than in the greenhouse ones (31.3 vs 26.4 mg g^{-1} d.w. respectively). Moreover, polyphenols were more concentrated in the seeds obtained with the first and the second transplant (32.9 mg g^{-1} d.w. as an average), whereas no significant differences were observed in the later cycles (26.3 mg g^{-1} d.w. as an average). Harvest time also had a significant effect on polyphenols content, which showed a 53.6% reduction in faba bean seeds picked up in late April compared to the ones obtained in late March. Notably, in our research the seed polyphenols contents displayed opposite trends to the ones recorded for proteins and they fell in the range reported by Oomah et al. (2011). These compounds reportedly show a positive correlation with the antioxidant activity (Chaieb et al., 2011; Pastor-Cavada et al., 2011), whereas the correlation with temperature is controversial, as it resulted positive in banana and tomato (Rivero et al., 2001; Caamal-Velázquez et al., 2007) but adverse in lettuce and watermelon (Boo et al., 2011; Rivero et al., 2001). Phenylalanine ammoniumlyase (PAL) is considered the main enzyme in the phenyl-propanoid building pathway, catalyzing the L-phenylalanine turning into *trans-cinamic* acid, which is the intermediate compound in phenolic biosynthesis (Dixon and Paiva, 1995). This enzyme activity increases in response to thermal stress and it is considered one of the main cell acclimation symptoms against stress (Leyva et al., 1995). On the other hand, phenol oxidation is performed either by peroxidases (POD) or priority by polyphenols oxidases (PPO); the latter enzyme catalyses the oxidation of *o*-diphenols to *o*-diquinons, as well as monophenols hydroxylation (Martínez-Téllez and Lafuente, 1997). Both enzymes have been associated with physiological damage caused by thermal stress and, in fact, under high or low temperature stress these enzymes are activated, whereas the enzymes oxidizing the same compounds are inhibited (Leyva et al., 1995; Martínez-Téllez and Lafuente, 1997). Accordingly, soluble phenolic compounds can accumulate as a mechanism of acclimation to overcome high or low temperature stress (Rivero et al., 2001).

Ascorbic acid concentration (Tab. 3) was 57% higher in faba bean seeds grown in open field than in the greenhouse, increasing from the first to the third planting time (581 and 703 mg g^{-1} d.w. respectively), after which it remained steady up to the last crop cycle. Finally, ascorbic acid in seeds increased by 43% during the five spring harvests interval. Consistently with the results recorded in faba bean under conventional management (Amalfitano et al., 2018), in our research, conversely to polyphenols whose biosynthesis was activated by thermal stress, ascorbic acid was affected by light intensity, as its content was higher in open field and increased with the delay of both transplanting and harvest time. However, with regard to the connections between these two antioxidant compounds, Altunkaya and Gökmen (2009) reported the important role of polyphenols in preventing enzymatic degradation of ascorbic acid.

CONCLUSIONS

The present research was carried out in Mediterranean area (southern Italy), with the aim of evaluating the transplant anticipation feasibility of fresh pod-producing faba bean under open field or greenhouse conditions. In this respect, the earliest transplant performed on 27 September showed the highest pod ripeness precocity, which was even enhanced in greenhouse with the first harvest occurring on 8 February compared to 21 February in open field growing. This result fulfills both the high economic benefit goals from produce sale and the crop cycle reduction on behalf of a subsequent spring-summer cultivation. However, delaying the transplant to mid-autumn (25 October-8 November) enabled faba bean for achieving the highest pod yield under open field conditions, though seed quality appeared controversial.

ACKNOWLEDGEMENT

The authors wish to thank Mr. Roberto Maiello, Dr. Giuseppe Melchionna and Dr. Antonio Marra for their assistance with laboratory analyses as well as Mr. Rosario Nocerino and Dr. Vincenzo Cervinzo for their assistance with research facilities management.

FUNDING

This research was supported by individual funding provided by the Department of Agricultural Sciences of University of Naples Federico II.

AUTHOR CONTRIBUTIONS

C.A. – was involved in seed chemical analysis, data interpretation and manuscript writing; D.A. – performed seed chemical determinations and relevant processing; C.B. – was involved in agronomic determinations and seed chemical analysis; A.C. – contributed to agronomic determinations and sample preparation; G.M. – cooperated with field and laboratory determinations as well as data statistical processing; G.C. – conceived the investigation and was involved in data interpretation as well as in manuscript writing. All authors read and approved the final manuscript.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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Received May 28, 2018; accepted July 6, 2018