YIELD, SEED QUALITY AND RESIDUAL BIOMASS CHEMICAL COMPOSITION OF ORGANIC FABA BEAN AS AFFECTED BY FARMING SYSTEM AND PLANTING TIME

CANTITATEA, CALITATEA SEMINȚELOR ŞI COMPOZIȚIA CHIMICĂ A BIOMASEI REZIDUALE DE BOB ECOLOGIC OBȚINUT ÎN FUNCȚIE DE SISTEMUL DE CULTURĂ ŞI EPOCA DE SEMĂNAT

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Abstract. *The effects of two farming systems (open field, greenhouse) and two transplanting times (11 October, 8 November) on plant growth, fresh pods yield, seed quality, and residual biomass quality for fuel production were assessed. The research was carried out in the years 2011-13 on faba bean in southern Italy. Crop dry weight, LAI and pods yield were higher in open field than in greenhouse and upon the 8 November transplant. Seeds to whole pod weight, yield and mean weight were better affected by the later planting time and by greenhouse, though farming system had no significant effect on yield. Seed fiber and proteins content attained higher values in greenhouse than in open field, whereas polyphenols and ascorbic acid showed the opposite trend; the later planting time better affected proteins and ascorbic acid, but polyphenols were more concentrated in earlier crops seeds. Residual biomass obtained in greenhouse and in earlier crops showed higher values of lignin and pectin, but lower cellulose, compared to open field.*
Key words: *Vicia faba* L., open field, greenhouse, plant growth, fiber, proteins, polyphenols, ascorbic acid.

Rezumat. *În studiul de față au fost evaluate efectele a două sisteme agricole (câmp deschis, seră) şi două epoci de înființare (11 X; 8 XI) privind creşterea plantelor, producția de păstăi, calitatea semințelor, precum şi calitatea biomasei reziduale pentru producerea de combustibil la bobul de grădină. Cercetarea a fost realizată pe perioada 2011-2013, pe bobul de grădină, din sudul Italiei. Greutate uscată, suprafața foliară şi producția de păstăi au fost mai mari în câmp deschis comparativ cu sera pentru epoca de semănat 8 noiembrie. Greutatea semințelor din total greutate păstăi, producția de păstăi şi greutatea medie a păstăilor au fost influențate de epoca de înființare mai târzie şi locul de cultură (seră), deşi sistemul de cultură nu a avut nici un efect semnificativ asupra producției. Conținutul total de fibre şi proteine au atins valori mai mari în seră decât în câmp deschis, în timp ce polifenolii şi acidul*

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ascorbic, au arătat o tendința opusă; epoca de înființare mai târzie afectează conținutul de proteine şi acidul ascorbic, dar în schimb polifenolii se concentrează în semințele obținute de la culturile timpurii. Biomasa reziduală obținută în seră şi în culturile timpurii au arătat valori mai mari de lignină şi pectină, celuloză mai redusă, în comparație cu câmpul deschis.

Cuvinte cheie: *Vicia faba* L., câmp deschis, seră, creşterea plantelor, fibre, proteine, polifenoli, acid ascorbic

INTRODUCTION

Faba bean (*Vicia faba* L.) is cultivated in many world areas, mainly located in China, Ethiopia, Mediterranean basin, central-northern Europe, Australia and South America, its surface extension accounting for 240,000 ha worldwide (FAOSTAT, 2014). Faba bean features fit a sustainable agriculture model (Nadal *et al*., 2003), since this species benefits from symbiosis with Rhizobium bacterium to fix nitrogen from the atmosphere.

Vicia faba is mostly grown in the field, but greenhouse environmental conditions may be suitable to organic vegetables, which are more susceptible to the environmental unbalances caused by a less intensive management (Maynard, 1994). Planting time also affects crop performances and, in this respect, Khalil *et al*. (2010) reported that in Pakistan the highest yield is favored by early October sowing, compared to late summer or mid-autumn planting.

Vicia faba seeds represent a remarkable energy source and they are rich in fiber, proteins, mineral nutrients, vitamins and antioxidants (Crépon *et al*., 2010). Notably, the high fiber concentration is beneficial for intestinal functions regulation as well as for blood glucose and cholesterol control (Macarulla *et al*., 2001) and polyphenols act as antioxidants, also protecting the plants against ultraviolet radiations (Jansen *et al*., 2001).

With the prospect of valorizing the whole crop system, the exploitation of residual lignocellulosic biomass for fuel production offers the advantage to use an added-value by-product (Schievano *et al.,* 2009).

In this research, we assessed the effects of farming system and planting time on crop growth,fresh pods and seeds yield, seeds quality, and residual biomass chemical composition.

MATERIAL AND METHOD

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, in 2011-2013 on a sandy-loam soil (87% sand, 11% silt, 2% organic matter). The highest mean temperature values were recorded in the first ten days of June (22.3 °C and 25.3 °C in open field and in greenhouse respectively) and the lowest in the first ten days of February (7.0 °C and 9.5 °C in open field and in greenhouse respectively).

Comparisons were made among four experimental treatments, obtained by the factorial combination of two farming systems (open field, greenhouse) and two planting times (11 October, 8 November). A split plot design with three replicates was

arranged, where each elementary plot was 6.20 m^2; plants were transplanted in single rows spaced by 0.85 m from each other and the spacing was of 0.13 m along the rows, with an areal density of 9 pt·m^{-2}.

Organic farming practices were performed in compliance with the EU regulation 834/2007. Each year the plants were supplied with 90 kg·ha^{-1} of N, 75 kg·ha^{-1} of P_2O_5 and 200 kg·ha^{-1} of K_2O. A 30% fertilizers dose was given just before transplanting and the remaining 70% on dressing, by using the Bioilsa 6-5-13 manure. Drip irrigation was activated when the soil available water capacity (AWC) decreased to 80%.

Fruit harvests were performed from mid-February to early June, as an average of the two research years.

General analytical methods. Plant samples were randomly selected 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; mean fruit weight on 30 unit samples; seeds weight on random samples including 20 pods. 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. After harvest, residual biomass samples were randomly collected in each plot and transferred immediately to the laboratory, where they were dried in an oven at 70°C under vacuum. The samples were, then, carefully milled andthe final material, composed of particles ≤ 1 mm diameter, was used for chemical analyses.

Seed quality analyses. Faba bean pods were randomly sampled in each plotin early April, then the seeds were extracted from the pods and the following laboratory determinations were performed.

Soluble solids, expressed as Brix at 20°C, were assessed using a Bellingham and Stanley digital refractometer, model RFM 81.

Fiber and proteins were determined using a chemometric method, applying near infrared spectroscopy (NIR) and principal component regression method (PCR). Spectra between 4000-10000 cm^{-1} were run in reflectance on a Perkin Elmer Frontier infrared spectrophotometer equipped with a NIRA accessory, acquired in Spectrum program and chemometrically processed by Quant+ software, both from Perkin Elmer.

The total polyphenols determination were performed with Folin Ciocalteu reagent (Singleton and Rossi, 1965), and determined in a Lambda 25 UV-Vis (Perkin Elmer) spectrophotometer at 760 nm, using gallic acid as standard.

Ascorbic acid was assessed according to Kampfelgen *et al.* (1995).

Lignin, cellulose, crystalline cellulose, hemicellulose and pectin in the crop residual biomass were assessed as previously described (Ercolano *et al.,* 2015).

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

RESULTS AND DISCUSSIONS

Plant growth and yield.There were no significant differences between years in the experiment, and only the effects of farming system and planting time are reported.

The highest leaf area index (LAI) was recorded at full fructification stage and it was not significantly affected by farming system;moreover, LAI showed

higher value under the transplant performed on11 October compared to the 8 November one (tab. 1). Different trends were recorded for plant dry matter, which attained higher value both with the later planting time and in open field conditions. In previous research (Confalone *et al.*, 2010), faba bean grown inopen field in north-western Spain showed a similar trend in LAI to that observed in the present work.

Table 1

Growth indexes and yield results of faba bean

Treatment	LAI ($m^2 \cdot m^{-2}$)	Dry weight ($g \cdot m^{-2}$)	Crop cycle duration (days)	Pods Yield ($t \cdot ha^{-1}$)	Number (per plant)	Mean weight (g)	Seeds Weight/ pod (%)	Yield ($t \cdot ha^{-1}$)	Mean weight (g)
Farming system									
Open field	4.4	1257.6	144.5	15.7	6.8	22.6	26.3	4.1	1.44
Greenhouse	4.3	1087.1	130.3	13.6	6.0	21.8	29.1	4.0	1.67
	n.s.	*	*	*	*	n.s.	*	n.s.	*
Planting time									
11 October	4.5	1041.3	133.0	11.2	5.4	20.5	26.0	2.9	1.42
8 November	4.1	1303.4	141.8	18.1	7.4	23.9	29.4	5.2	1.69
	*	*	*	*	*	*	*	*	*

n.s. no statistically significant difference; * significant difference at $p \leq 0.05$.

As shown in table 1, crop cycles were shorter both in greenhouse, by about two weeks compared to open field, and under the earlier transplant by about nine days. Pods yield attained a higher value in open field conditions than in protected environment and with the later planting time. These results were connected to the pods number per plant, which showed the same trends as those recorded for yield.The pod mean weight contributed to the production outcome only referring to the planting time effect, whereas no significant differences arose with regard to farming system. Both the ratio between seeds to pod weight and the mean seed weight were higher under protected environment than in open field and upon the later transplant. The latter better affected the seeds yield as well, but this variable did not change between the two farming system conditions.

Marcellos and Constable (1986) reported that sowing performed in early autumn in Mediterranean environments prolongs the crop cycle compared to later planting time, resulting in better growth and higher yields. In research carried out by Khalil *et al*. (2010) in open field in Pakistan (Peshawar region), the highest yield was obtained with early October sowing, compared both to late September and late October planting times. In south-western Australia, Mwanamwenge *et al.* (1999) reported that early planting time allows faba bean plants to avoid late spring high temperatures. 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.

In the sub-coastal area of Naples region, yield obtained from crop cycles transplanted on 11 October was adversely affected by temperatures exceeding faba bean tolerance threshold, which caused about 30 % plant mortality in greenhouse crops, compared to 15 % mortality recorded in open field. The phenological progress of the plants which survived was sub-optimal, as high temperature also caused earlier flowering and accordingly reduced pod set. The possible reason for this reduced number of pods is pollination deficiency, which is considered a major factor causing flower abortion (Chen *et al*., 2006). Conversely, Adisarwanto and Knight (1997) found that in Mediterranean environment sowing time has low effect on the time span between plant emergence and flowering beginning. However, temperature affects flowering progress which optimally happens at 22-23°C (Ellis *et al*., 1988), or even at lower temperatures for some cultivars.

Seed quality and antioxidants content. Dry residue and soluble solids of faba bean seeds were not affected by farming system (tab. 2), but the former variable attained a higher value upon the later planting time, whereas the soluble solids did not change between the two transplants. Moreover, farming system significantly affected fiber content, which was higher in greenhouse grown seeds than in the open field ones (tab. 2), whereas planting time did not have significant effect on this variable. Other authors reported lower fiber levels in faba bean seeds compared to those detected in our research (Hedley, 2001).

Table 2

Quality indicators and antioxidants in faba bean seeds

Treatment	Dry residue mg	Soluble solids °Brix	Fiber mg	Proteins mg	Polyphenols mg	Ascorbic acid µg
Farming system						
Open field	190	8.5	220	279	30.1	769
Greenhouse	193	8.9	238	293	25.4	489
	n.s.	n.s.	*	*	*	*
Planting time						
11 October	181	8.6	227	280	31.6	594
8 November	200	8.9	231	293	25.7	663
	*	n.s.	n.s.	*	*	*

Dry residue data are per g of fresh weight of seed tissue; other data are per g of dry weight of seed tissue.

n.s. no statistically significant difference; * significant difference at $p \leq 0.05$.

As shown in table 2, the seed protein content was higher under protected environment compared to open field and it increased with the planting delay. Protein values obtained in our research fall within the range reported by other authors (Ofuya and Akhidue, 2005).

Total polyphenols content (tab. 2) was higher in open field grown seeds than in the greenhouse ones and in the seeds obtained upon the first transplant. Polyphenols content in faba bean seeds obtained in our research are consistent with the values reported by Oomah *et al*. (2011).

Ascorbic acid concentration (tab. 2) was higher in faba bean seeds grown in open field than in the greenhouse and it increasedwith the planting time delay.

Residual biomass chemical composition. In order to exploit the residual biomass of faba bean crops for fuel production, we assessed its chemical composition (tab. 3). Greenhouse crops showed significantly higher lignin content, hemicelluloses and pectin than the open field ones. Open field grown biomass showed higher cellulose content, whereas no difference in crystalline cellulose was detected between the two farming systems.

The planting time delay resulted in a decrease of lignin and pectin content, an increase of cellulose (both total and crystalline) percentageand no significant effect on hemicellulose.

Table 3.

Chemical composition of faba bean residual biomass

Treatment	Lignin	Total cellulose	Crystalline cellulose (%)	Hemicellulose	Pectin
Farming system					
Open field	12.8	49.4	12.9	13.6	10.9
Greenhouse	14.3	47.6	12.7	15.0	11.8
	*	*	n.s.	*	*
Planting time					
11 October	15.0	46.4	12.0	14.5	12.3
8 November	12.2	50.9	13.7	14.1	10.6
	*	*	*	n.s.	*

n.s. no statistically significant difference; * significant difference at $p \leq 0.05$.

As the biomass quality for fuel production is mainly affected by cellulose, but hemicellulose also give an important contribution, in our research faba bean residual biomass showed valuable featuresfor biorefinery application (tab. 3).

Consistently with our results, other authors (Xuan *et al.,* 2015) reported the significant effect of planting time on biomass quality in sweet sorghum.

CONCLUSIONS

In the present research carried out on faba bean in Mediterranean area, open field crops benefited from more appropriate climate conditions than those grown in greenhouse and the transplant performed in mid-autumn resulted in better yield performances than the early autumn one. The latter led to higher pod ripeness precocity, as well as the protected environment conditions enhanced the plants phenological progress compared to the open field.

The open field climate conditions also enhanced seeds quality, in terms of higher antioxidants content and lower fiber percentage. As for crop residual biomass, open field growing resulted in the highest yield as well as the 8 November transplant and they both showed higher cellulose fraction, the latter being the main quality component in biorefinery application prospect.

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