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Characterization of Bean (*Phaseolus vulgaris* L.) Ecotype "Fagiolo Occhio Nero Di Oliveto Citra" Using Agronomic, Biochemical and Molecular Approaches

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Abstract: Common bean (*Phaseolus vulgaris*) is the most important grain legume and plays a significant role in human nutrition being a major source of dietary protein and representing a rich source of minerals and certain vitamins. Several large germplasm collections have been established, which contain large amounts of genetic diversity, including wild and domesticated species. In this study agronomic, biochemical and molecular characterization of landrace bean named "Fagiolo occhio nero di Oliveto Citra" (*Phaseolus vulgaris* L.), is described. Seeds were obtained by local farmers and field trials were carried out during years 2009-2010, in the typical cultivation site (Oliveto Citra, Salerno Province), using two different densities of investment. During 2011, in order to evaluate the performance in different environments, field trials were conducted in three localities (Battipaglia, Oliveto Citra and Controne). Data analysis shows good adaptability across locations and similar grain yield using two spacing's of seeds. Morphological characterization and molecular analysis, using AFLP and Minisatellite molecular markers, were performed on ten "biotypes" collected from local farmers. Seeds characterization showed variability on the violet area surrounding the hilum (named as eye) while markers have provided useful information on relationships between biotypes. Biochemical analysis, which includes the contents of protein, minerals and antioxidants, shows how the composition is consistent with respect to other landraces and commercial cultivars. The landrace under study revealed genetic stability and good adaptation to cultivated environment with best performance in the native area. In addition, the bio-agronomic characteristics are in accord with studies reported in literature.

Key words: Landrace, field trials, biotype, molecular markers

INTRODUCTION

Since the beginning of modern agriculture, legumes were considered essential, occupying the second rank in terms of planted area and production of grains. In the human diet, they are a valuable source of protein and are used as sources of edible oils and fodder plants for animals. The Leguminosae family, among the superior plants, is considered the third largest plant family on the earth containing more than 19.000 described species with 85% of the production occupied by the *Phaseolus vulgaris* species (common bean), that is considered as the most important for direct human consumption on all continents (Gepts *et al*., 2008). World production is concentrated mainly in Asia (India, Myanmar and China), South America (Brazil) and central east Africa.

In Italy, the common bean cultivation was introduced during 16th century. Thereafter, the farmers operated a selective pressure that produced myriad of landraces that have spread and adapted to the different climatic condition in each region (Piergiovanni and Lioi, 2010).

In this picture, given yield stability in low input agricultural system (Zeven, 1998), the locally adapted landraces could represent an economically valuable opportunity for farmers in marginal areas (Hawtin *et al*., 1997) and become the basis for plant breeders to develop new varieties (McCouch, 2004).

The landrace "Fagiolo occhio nero di Oliveto Citra" (*Phaseolus vulgaris* L.) is a grain legumes cultivated in the internal hilly area in Campania Region, appreciated locally for its particular organoleptic characteristics (Zaccardelli *et al*., 2004). This bean with indeterminate growth and climbing habits show white coloured and ovale-elongated seeds, with oval cross section and a

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typical black-violet area around hilum, with different shape and extension (from which the name "black eye"). The cultivation area is in the territory of the Municipality of Oliveto Citra, in the upper Sele Valley, in the Province of Salerno. This is a local production where the nutritional and organoleptic quality is closely linked to the territory. The cultivation techniques of this ecotype has been handed down locally for many years and often are still very archaic: in this case, the "Fagiolo occhio nero di Oliveto Citra" is often still cultivated using corn stem as a guardian, rather than plastic nets set to support poles (Zaccardelli *et al*., 2004).

Several methods were used to study the genetic diversity of ecotypes. Previously biochemical analysis based on phaseolin electrophoresis were widely used (Gepts *et al*., 1986), subsequently, molecular markers have greatly improved the possibility to investigate the genetic structure of common bean landraces. A variety of PCR-based molecular markers are useful tools for the study of genetic diversity; in particular AFLPs (Amplified Fragment Length Polymorphisms) (Vos *et al*., 1995) allow to detect polymorphism at a great number of loci, while the minisatellites provide the detection of short series of DNA bases and have been widely used in human species (Jeffreys, 2005).

The present study shows the results of tests carried out for three years in typical areas of cultivation. Agronomic traits were analyzed for two years in two different densities of investment and for one year in different localities. Moreover, morphological characterization of pods and seeds, biochemical and molecular analysis by PCR based markers, were performed.

MATERIALS AND METHODS

Plant material: Molecular analysis and morphological seed characterization were performed on ten biotypes (named as bio) collected from farmers located in the typical cultivation area (Oliveto Citra District, in Salerno Province) (Fig. 1). Agronomic characterizations were done on a selected biotype (bio-07).

Field trials and agronomic characterization: Three years experiments were performed. During 2009 and 2010 the "Fagiolo occhio nero di Oliveto Citra" bean was cultivated at an experimental farm in Oliveto Citra District (Salerno, Campania Region), located about 300 m above the sea level, in an internal hillside and marginal environment, characterized by annual mean rainfall of 1307 mm (70 year average data, www.abisele.it) and annual mean temperature of 15.7°C (30-years average data). Seeds were sowed in open field in middle July. Plants were grown in a completely randomized block experimental design with 3 repetitions each with an area of 14 m^2, adopting two different distances on the row (20 and 30 cm) and one distance among the rows (140 cm). Harvesting was performed by the end of October. During 2011 agronomic traits were recorded in two more experimental fields located in Southern Italy. The experimental farm of

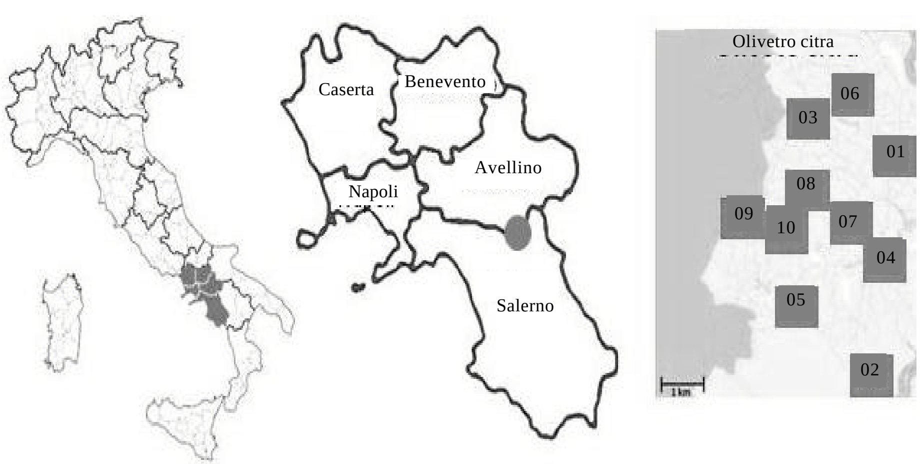

Fig. 1: Geographical position of campanian region in the Italian territory. The circle indicates the position of Oliveto Citra Municipality. The farms locations of the ten "Fagiolo occhio nero di Oliveto Citra" biotypes, are indicated by numbers: 1: S. sisto locality, 2: F. volpacchio locality, 3: Bosco locality, 4: Dogana locality, 5: Serrapiano locality, 6: Casale locality, 7: Chiusa locality, 8: Piano delle monache locality, 9: S. pietro locality and 10: Serra monica locality

CRA-ORT in Battipaglia (Sele Valley, Campania Region), was located in an intensive horticultural area near Tirreno sea coast, at 65 mt above the sea level, characterized by an annual mean rainfall of 947 mm and an annual mean temperature of 16.6°C (30-years average data). The experimental farm in Controne (Salerno, Campania Region), was located in an internal hillside and marginalenvironment at about 210 m above the sea level, characterized by annual mean rainfall of 1117 mm (70-years average data, http://www.abisele.it/) and annual mean temperature of about 16°C (30-years average data). All fields were characterized by a complete randomized block experimental design with 3 repetitions (surface area of 14 m^2) adopting distances of 20 cm on the row and 140 cm among the rows. Seeds were sowed in open field in middle July and harvestings were performed by the end of October. Productive traits analyzed included total grain yield (tons ha^{-1}) (GY), total pods number/plant (NP), Pod Seeds (PS) and weight of 1000 seeds (g) (SW).

Morphological pods and seeds characterization: Morphological analysis of pods and seeds include: pods shape expressed by a scale (1 = straight, 2 = slightly curved, 3 = curved, 4 = very curved), pods length, polar and equatorial pods width, seed length (mm), polar and equatorial seed width (mm), longitudinal seed section calculated expressed by a scale (1 = round, 2 = round oval, 3 = elongated oval, 4 = elongated oval with a flattened side, 5 = elongated oval with two flattened sides), average transversal seed diameter and "eye" dimension area expressed by a scale (1 = small area around hilum; 5 = large area around hilum).

Chemical compounds: Proteins and ash were determined by using the standard methods of the Association of the Analytical Chemists (AOAC, 1999); flours (<50 μm) were obtained from dry seeds using a grinder (Cyclotec 1093 Tecator) and proteins content (N×6.25) was done by the Kjeldahl method (AOA-C 1999). Protein digestibility was assessed by the multienzyme method of Bodwell *et al.* (1980). All enzymes: porcine pancreatic trypsin (type IX, 15310 BAEE IU mg^{-1} protein), bovine pancreatic chymotrypsin (type II, 51 IU mg^{-1} protein), porcine intestinal peptidase (P-7500, 115 IU g^{-1} solid) and bacterial protease (type XIV; 4.4 IU mg^{-1} solid) were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Liquid-Liquid Extraction of phenolic compounds was performed as described by Cardador-Martinez *et al.* (2002). Lyophilized ground seed coats (70 g) were placed in a flask and mixed with 100% methanol (1:50, w/v ratio). The flask was shaken for 24 h at 20°C while wrapped in aluminum foil to protect extract from light. The sample was then filtered using Whatman paper and methanol was removed under reduced pressure. The methanol extract was lyophilized and stored in the dark at-20°C until HPLC-MS analysis. Tannin extracts were obtained from legume flours by incubation in 100% ethanol (0.04 g of flour per ml ethanol) for 16 h at room temperature, under stirring. The extracts were centrifuged (4000 3 g, 10 min) and tannin content (catechin equivalents) was estimated according to Price *et al.* (1978).

Elemental contents were determined in dry ash by using Atomic Absorption Spectrophotometry (AAS-Perkin Elmer, Model analyst 800). The laboratory procedure for the preparation and determination of micro and macro elements were used as outlined by Radojevic and Bashkin (2006).

DNA extraction: Plant tissues were collected from bulks of ten "biotypes" cultivated on farms in the typical cultivation area (Fig. 1) (Oliveto Citra District, in Salerno Province). For two biotypes (bio-03 and bio-10) leaf tissue from 10 single plants were also collected. DNA extraction was performed using the miniprep protocol described in Doyle (1991). Concentration of DNA and its quality were measured by absorbance at 260 and 280 nm, respectively, using a Biophotometer (Eppendorf, AG, Hamburg). After extraction, DNA was stored at 4°C. Molecular analysis using AFLP and minisatellite markers was performed on bulks of the ten biotypes and, for biotypes 03 and 10 only, on samples of single plants. In addition, bulks of other two ecotypes cultivated in Campania Region, "Fagiolo di Controne" (cultivated in Salerno Province) and "Fagiolo di *S. antonio* Abate" (cultivated between Salerno and Napoli Province) were characterized too. The second ecotype is characterized by the typical black-violet area around hilum such as "Fagiolo Occhio nero di Oliveto Citra" ecotype.

AFLP reactions: Template preparation and AFLP fingerprinting were essentially performed as described in Vos *et al.* (1995). Adapters and the MseI and EcoRI primers used were as follows: EcoRI adapter (5'-CTCGTAGACTGCGTACC-3' and 5'-AATTGGTACGCAGTCTAC-3') and MseI adapter (5'-GACGATGAGTCCTGAG-3' and 5'-TACTCAGGACTCATC-3'), primers EcoRI (5'-GTAGACTGCGTACCAATTC-3') and primers MseI (5'-GATGAGTCCTGAGTAAC-3'). Selective amplification was performed at the same condition of the preamplification using the selective primers each elongated with two nucleotides (5'-sequence primer *Eco*RI+GA-3' and 5'-sequence primer *Mse*I+CT-3').

Primer combinations used were: Eco-ACC/Mse-CAC, Eco-ACC/Mse-CAT, Eco-AAC/Mse-CTG. Amplified fragments were separated on 5% denaturing polyacrylamide sequencing gel according to the method of Janssen *et al*. (1996); marker was the 100-bp DNA ladder of Gibco Brl. Run was performed at 70 V for 150 min. Amplification bands were revealed by silver staining as described in Zaccardelli *et al*. (1999).

PCR on minisatellite regions: The amplifications were performed in a volume of 25 μL containing 25 ng of DNA, 200 μM each of dNTPs, 3 mm of $MgCl_2$, 1 U of Taq DNA polymerase, 50 ng of primer and 2.5 μL of 10X reaction buffer. The primer (5'-GAGGGTGGCGGTTCT-3') was designed on the minisatellite sequence of the M13 phage (primer M13).

The amplification program was: 94°C for 30 seconds (1 min in the first cycle), 50°C for 1 min, 72°C for 1 min, for a total of 40 cycles and a final extension at 72°C for 6 min. Amplifications were performed using the Applied Biosystems thermo cycler. Taq DNA-polymerase, 10X reaction buffer, $MgCl_2$ solution and nucleotides (Ampli-Taq-Gold Set) were purchased from Applied Biosystems; the primers were purchased from Gibco Brl. Electrophoresis was performed at 90 V in 1.4% agarose gel with 1X TBE as running buffer. The amplification bands were revealed by ethidium bromide staining.

Data analysis: For all bio-agronomic traits, descriptive statistics were calculated, including Analysis of Variance (ANOVA) and Pearson correlation coefficient, adopting the statistical software JMP, Version 7. SAS Institute Inc., 2007. Means comparison were performed by Student's t. Molecular markers data analysis were performed by comparing the groups including biotypes with an identical pattern; one rectangular binary matrix (1 = presence of band, 0 = absence of band) for AFLP nalysis and one rectangular binary matrix for minisatellite analysis, were constructed to assess the gels. Using the software program Numeric Taxonomy Ntsys-pc (Numerical Taxonomy and Multivariate Analysis System) version 2.0 (Exeter Software, Setauket, NY, USA), a similarity triangular matrix was created from each rectangular matrix using the band-based Dice similarity coefficient (SD) (Sneath and Sokal, 1973). Once the similarity matrix was constructed, the unweighted-pair group method with average linkages (UPGMA) (Sneath and Sokal 1973) was used to cluster the patterns (Vauterin and Vauterin 1992).

Correlations: The correlations between traits in different years, were calculated from a regression of genotype mean values using Pearson coefficient.

RESULTS

The multidisciplinary approach used to characterize the landrace "Fagiolo occhio nero di Oliveto Citra" revealed genetic stability between biotypes obtained by different farms, stability was confirmed in most of the pods and seeds traits. Different trend was observed for the agronomic traits. The ecotype shows strong interaction with the typical cultivation environment giving yield twice greater than other environments. Yield was instead stable using two different sowing distances. Below is a description of the results.

Morphological characterization of pods and seeds: Pod morphological characterization revealed variability and statistically significant differences only in pod length (Table 1); the biotypes can be divided in three groups: bio-1, 2, 3, 4 and 7, with length equal or under 8.5 cm; bio-8, with length between 8.5 and 9.0 cm; bio 5 and 6, with length greater than 9.0 cm; average length was 8.4 cm. Pod shape was mostly slightly curved and homogeneous between biotypes as well as for polar and equatorial pod width.

Table 1: Morphological pod and seed characteristics of "Fagiolo occhio nero di Oliveto Citra" biotypes

Biotypes	Pod shape $^{\alpha}$	Pod lenght (cm)	Polar pod width (mm)	Equatorial pod width (mm)	Seed length (mm)	Polar seed width (mm)	Equatorial seed width (mm)	Average transversal diameter (mm)	Longitudinal seed section $^{\beta}$	"Eye" area dimension(1-5)$^{\gamma}$
Bio-1	2.00^{a}	8.50^{a}	1.20^{a}	0.90^{a}	11.80^{a}	9.10^{a}	7.40^{a}	8.20^{a}	2.7^{a}	1.80^{de}
Bio-2	2.00^{a}	8.10^{bc}	1.20^{a}	0.90^{a}	11.60^{a}	9.00^{a}	7.30^{a}	8.10^{a}	2.4^{a}	4.30^{a}
Bio-3	1.90^{a}	8.00^{bc}	1.30^{a}	0.90^{a}	11.80^{a}	9.10^{a}	7.40^{a}	8.20^{a}	2.5^{a}	1.90^{de}
Bio-4	1.80^{a}	8.30^{bc}	1.30^{a}	1.00^{a}	11.50^{a}	8.90^{a}	7.50^{a}	8.20^{a}	2.5^{a}	1.70^{e}
Bio-5	1.80^{a}	9.20^{a}	1.20^{a}	1.00^{a}	11.003	8.70^{a}	7.40^{a}	8.00^{a}	2.5^{a}	2.80^{bc}
Bio-6	2.10^{a}	9.00^{a}	1.20^{a}	0.90^{a}	10.80^{c}	9.00^{a}	7.50^{a}	8.20^{a}	2.7^{a}	2.40^{cd}
Bio-7	2.00^{a}	8.00c	1.40^{a}	0.90^{a}	11.80^{a}	9.20^{a}	7.50^{a}	8.30^{a}	2.9^{a}	1.50^{b}
Bio-8	2.00^{a}	8.80^{a}	1.20^{a}	0.90^{a}	11.70^{a}	9.30^{a}	7.40^{a}	8.30^{a}	2.6^{a}	3.303
Bio-9	2.00^{a}	8.10^{bc}	1.20^{a}	0.90^{a}	11.60^{a}	9.20^{a}	7.40^{a}	8.30^{a}	3.2^{a}	2.10^{de}
Bio-10	1.80^{a}	8.20^{bc}	1.30^{a}	0.90^{a}	11.30^{a}	9.00^{a}	7.70^{a}	8.30^{a}	2.6^{a}	1.90^{de}
Mean	1.90	8.40	1.30	0.90	11.49	9.05	7.40	8.21	2.6	2.37

Values marked by the same letters are not statistically different for $p<0.05$ (Student's test), $^{\alpha}$1: Atraight, 2: Slightly curved, 3: Curved, 4: Very curved, $^{\beta}$1: Round, 2: Round oval, 3: Elongated oval, 4: Elongated oval with a flattened side, 5: Elongated oval with two flattened sides, $^{\gamma}$1: Small area around hilum, 5: Large area around hilum

Seed morphological analysis for all traits except seed length and "eye" area dimension, revealed homogeneity between biotypes. Seed length showed a mean value of 11.49 mm, with minimum of 10.8 mm for bio-06 and maximum of 11.80 mm for the biotypes 01, 03 and 07; statistically significant differences were detected between biotypes (Table 1) using Student's test. No correlation was detected between pods and seeds length. Seed width average was 9.05 mm with minimum of 8.70 mm for bio-05 and maximum of 9.30 mm for bio-08. Shape index value, calculated as ratio between length/width, showed mean of 1.27 with ranging from 1.20 (bio-06) to 1.30 (bio-01). Transversal seed diameter showed homogeneous trend for the 10 biotypes characterized, with mean of 8.21 mm ranging from a minimum value of 8 mm for bio-05 and a maximum value of 8.30 mm for bio-07, 08, 09 and 10. Size of black-violet area around hilum, defined as "eye", showed the greater variability between biotypes, with mean value of 2.37 ranging from the minimum of 1.50 for bio-07 to the maximum of 4.30 for bio-02.

Grain yield and other agronomic traits: Mean phenotypic values and results of Student t-test for bio-agronomic traits are summarized in Table 2 and 3. Among the traits, grain yield across years showed the greatest variability. In fact, considering the typical area of cultivation (Oliveto Citra) and the typical distance used on the row (20 cm), mean grain yield was 1.03 t ha^{-1} in 2009, 2.68 t ha^{-1} in 2010 and 4.56 t ha^{-1} in 2011, with more than 300% of variability across three years. Fewer differences were detected on other two traits: weight of 1000 seeds with 30% of variability (341.17 grams in 2009, 446.17 grams in 2010 and 414.48 grams in 2011) and pod seeds with 20% of variability (5.41 in 2009, 5.62 in 2010 and 4.62 in 2011). The greatest influence on grain yield were given by the average number of pods per plant, which was found to be twice in 2011 compared to the previous two years (40.71, 21.79 and 19.68, respectively). Very small variability using different seed spacing were detected in both 2009 and 2010 (Table 2). In fact, according to ANOVAs analysis and t-test results, no statistical differences were found between two different seed spacing in the experiments conducted in Olivet Citra among the two years. Differences between environments were instead detected: grain yield was higher and statistically different between Oliveto Citra and the other two locations, as well as for weight of 1000 seeds, with statistically significant difference between Battipaglia and Controne. Number of pods per plant resulted to be different and statistically significant between Oliveto Citra and Controne (Table 3).

Table 2: Mean phenotypic values and standard deviations for the two distance experiments carried out in Oliveto Citra district on "Fagiolo occhio nero di Oliveto Citra" during 2009-2010

Oliveto citra	No. of replicates	GY	SW	PS	NP
2009					
Distance 20 cm.	3	1.03±0.49	341.17±11.95	5:41±0.26	19.68±4.67
Distance 30 cm.	3	0.95±0.2	337.87±11.89	5.73±0.16	27.91±5.68
2010					
Distance 20 cm.	3	2.68±0.6	446.17±2.76	5.62±0.39	21.79±4.19
Distance 30 cm.	3	3:17±0.84	460.61±2.49	5:47±0.3	30.07±8.84

GY: Grain yield (t ha^{-1}) at 13% of humidity, SW: Weight of 1000 seeds at 13% of humidity, PS: Pod seeds, NP: No. of pods plant^{-1}, Means comparison showed no statistical difference between distances

Table 3: Mean phenotypic values and standard deviations for the experiments carried out on "Fagiolo occhio nero di Oliveto Citra" in three different envirouments during 2011

Locality	GY	SW	PS	NP
Battipaglia	2.15±0.66	522.64±85.17*	4.08±0.65	25.65±4.62
Controne	1.91±0.59	367.99±54.40*	4.05±0.28	23.26±4.99*
Oliveto citra	4.56±0.33**	414.48±23.93	4.62±0.95	40.71±10.97

GY: Grain yield (t ha^{-1}) at 13% of humidity, SW: weight of 1000 seeds at 13% of humidity, PS: Pod seeds, NP: No. of pods plant^{-1}, Means marked with *,**Differ significantly (t-test, $p<0.05$, $p<0.01$, respectively)

Table 4: Pearson's correlation coefficients among four traits on "Fagiolo occhio nero di Oliveto Citra" in three years of experiments

	Year	SW	GY	PS
GY	2009	0.36		
	2010	0.59		
	2011	-0.02		
PS	2009	-0.14	-0.49	
	2010	-0.24	0.21	
	2011	-0.08	0.64	
NP	2009	-0.16	0.45	0.33
	2010	0.21	0.81*	0.02
	2011	0.01	0.75*	0.07

GY: Grain yield (t ha^{-1}) at 13% of humidity, SW: Weight of 1000 seeds at 13% of humidity, PS: Pod seeds, NP: No. of pods plant^{-1}, *Significant at $p<0.05$

Correlation between traits: Table 4 shows the correlation matrix among bio-agronomic traits in three years of experiment and calculated separately for each experiment. Grain yield and number of pods/plant were positively correlated in three years and significantly correlated to each other in 2010 and 2011. Only these two traits showed significant correlation between them. Seed weight showed negative correlation with pod seeds and positive correlation with grain yield and number of pods/plant, respectively in 2009-2010 and 2010-2011; negative correlation were, instead, obtained in 2011 and 2009, respectively with grain yield and number of pods/plant. Pod seeds showed positive correlation with number of pods/plant in all years and positive correlation with grain yield in 2010-2011, while negative correlation with this last trait were detected in 2009.

Table 5: Chemical and biochemical contents and standard deviation of "Fagiolo occhio nero di Oliveto Citra"

Protein content (N×6.25)	Ash	Humidity	*In vitro* protein digestibility	Total polyphenols	Free polyphenols	Tannins
22.84±0.74	4.78±0.08	12.81±0.01	69.17±2.26	0.31±0.02	0.18±0.00	0.12±0.01

Table 6: Mineral contents standard deviation of "Fagiolo occhio nero di Oliveto Citra"

Potassium (K)	Magnesium (Mg)	Calcium (Ca)	Sodium (Na)	Phosphorus (P)	Iron (Fe)	Zinc (Zn)	Manganese (Mn)	Copper (Cu)
13434.5±774.3	1546.5±58.7	1033.5±9.2	nd*±2.26	3757±66.5	18.3±0.4	23.4±1.6	10±0.2	8.8±9.0

*nd: Not determinable

Biochemical analysis: Chemical and biochemical contents, including total protein, ash, humidity, *in vitro* digestibility, polyphenols and tannins, are shown in Table 5. Micro and macro elements contents are shown in Table 6.

Bean resulted to have 12.81% of humidity; after drying 4.8 grams were represented by ash, while protein content (N×6.25) resulted to be almost 23 on 100 grams of an edible portion. Of the total polyphenols (0.31 g/100 g), almost 60% were represented by free polyphenols (0.18 g/100 g), while tannins were 0.12 g/100 g. *In vitro* protein digestibility resulted to be 69.17 g. Macro and microelements analyzed were potassium, magnesium, calcium, sodium, phosphorus, iron, zinc, manganese, copper. Greater amount of potassium were detected (13434 ppm), followed by phosphorus (3757 ppm), magnesium (1546 ppm) and calcium (1033 ppm). Microelements were between 23.4 ppm for zinc and 8.8 ppm for copper. No trace of sodium was detected.

Molecular analysis: In order to see any similarities, molecular analyses were also performed on two other southern Italian ecotypes typical of Campania Region: "Fagiolo di Controne" and "Fagiolo di S. Antonio Abate". Analysis with minisatellite M13-PCR markers (Fig. 2), showed uniformity between the single plants of the biotypes 10 and little genetic variation were detected between plants in the biotype 03. This trend was confirmed with AFLP markers (Fig. 3) where the small genetic variability detected is more evident into biotype 03. Overall, the ten biotypes collected and analyzed from different farmers were almost identical each to other, with only some biotypes different with respect to the major part of the biotypes. For example, the biotype 02 resulted to be different from the biotype 10, as well as for the biotype 07, compared to the biotype 06. Moreover, AFLP markers showed how the ecotype "Fagiolo di S. Antonio Abate" resulted indistinguishable in comparison to the ecotypes "Fagiolo Occhio Nero di Oliveto Citra.

DISCUSSION

Molecular and agronomic analysis including seed characterization, show genetic stability of the bean ecotype under study, as well as adaptation to environments different from the origin and correlation between markers and seed traits. The variability detected with the Minisatellite/AFLP marker analysis could be directly correlated with the variability in seed morphology. In fact, the seeds characterization showed how the lower value of "eye" area (i.e. bio-03, bio-07) is associated to high value in morphology traits (length and width) and no true is on the contrary. This means that the size of the "eye" area didn't depend by the morphology of the seeds. Combining markers and seeds traits data, is possible to see how the biotype with the greatest value in "eye" area (biotype 02) is genetically distant from one of the biotype with the lower value (biotype 10); the same is for biotypes 07 (with the lowest value) and 06 (Table 1, Fig. 1, 2). This trend isn't seen with all markers. This could be explained by the quantitative nature of this trait and, therefore, by the interaction with the environment (Blair *et al*., 2006). Moreover, the molecular analysis of two other Southern Italian ecotypes "Fagiolo di Controne" and "Fagiolo di S. Antonio Abate", reveal how "Fagiolo occhio nero di Oliveto Citra" and "Fagiolo di S. Antonio Abate" are genetically identical, this probably because the last landrace, cultivated at Angri and Castellammare, was originated from Oliveto Citra area. Comparing seed morphological traits to almost a hundred Spanish common bean accessions evaluated in the Mediterranean environment (Casquero *et al*., 2006). The "Fagiolo occhio nero di Oliveto Citra" turns out to be more round, decreasing in seed length and increasing in seed width. According to what reported in Foschiani *et al*. (2009), the ecotype could be classified as intermediate between Borlotto and Cannellino type.

Agronomic traits show no significant variability in grain yield for the two seed spacing adopted, due to a balance between distance and number of pods for each plant. In fact, closer plants produced fewer pods with the same number and weight of the seed and this result may be related to competition between plants, giving the possibility to reach the same level of production adopting a broader sixth of plant.

Tests of three years of experiments show, instead, strong effect of time/environmental condition on the productivity (Fig. 4), in according with the results

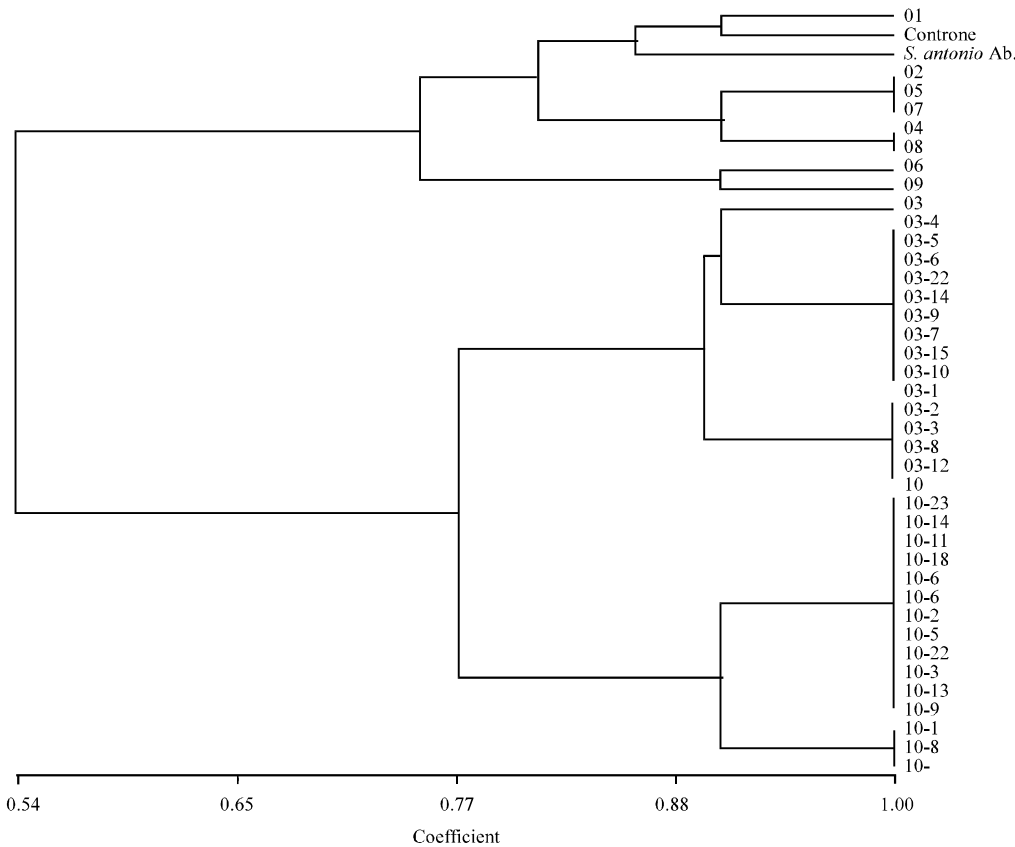

Fig. 2: Dendrogram obtained with M13 minisatellite marker showing relationship among ten bean biotypes of "Fagiolo occhio nero di Oliveto Citra" and the two landraces "Fagiolo di Controne" and "Fagiolo di Sant"Antonio Abate". On the x-axis is shown the coefficient of similarity, on the y-axis are indicated the biotypes used in the analysis and the two mediterranean landraces "Fagiolo di Controne" and "Fagiolo di S. Antonio Abate"

reported in other studies (Escribano *et al*., 1994; Casquero *et al*., 2006), where different response of Spanish bean landraces to the different environmental conditions were observed; interactions between Genotype and Environment (GE) showed how the best genotype for one environment is not the best for another (Falconer and McKay, 1996). In this study, among the three different environments, the ecotype gave his best performance in Oliveto Citra, where grain yield was two fold up than the other locations. No great differences were found between Battipaglia and Controne. Considering that the environment of Oliveto Citra is more similar to Controne than Battipaglia, the best performance in the typical cultivation area could be explained as the adaption of the landrace with the environments (i.e., optimal temperatures during flowering) and the interaction with the ecosystem. In fact, increase in yield is not related to bean seeds weight but rather direct consequence of the number of pods/plant, which means best flowering and fruit set. Moreover, in the two other locations, the bean ecotype showed good adaptability for the agronomic traits analyzed with no differences in the pod seeds and higher average weight of the seeds in Battipaglia. In a previous study (Casquero *et al*., 2006), yield traits were evaluated on ecotypes grown in a Mediterranean environment. Pod seeds agreed with what reported (4.97) while number of pod per plant resulted to be higher in the Oliveto Citra environment, ranging from 27.91 to 40.71 (Table 2, 3) compared the average of 25.1 reported by Casquero *et al*. (2006).

The weight of the seeds was found to be lower to what described previously (Casquero *et al*., 2006, Limongelli *et al*., 1996) and about half of the bean commercial types (Foschiani *et al*., 2009).

Seed protein amount agreed with those described in other studies involved common bean landraces and commercial varieties (Perazzini *et al*., 2008; Pinheiro *et al*., 2010): considering that protein content in dry beans ranges between 20 and 30% (Shellie-Dessert and Bliss, 1991), the "Fagiolo occhio

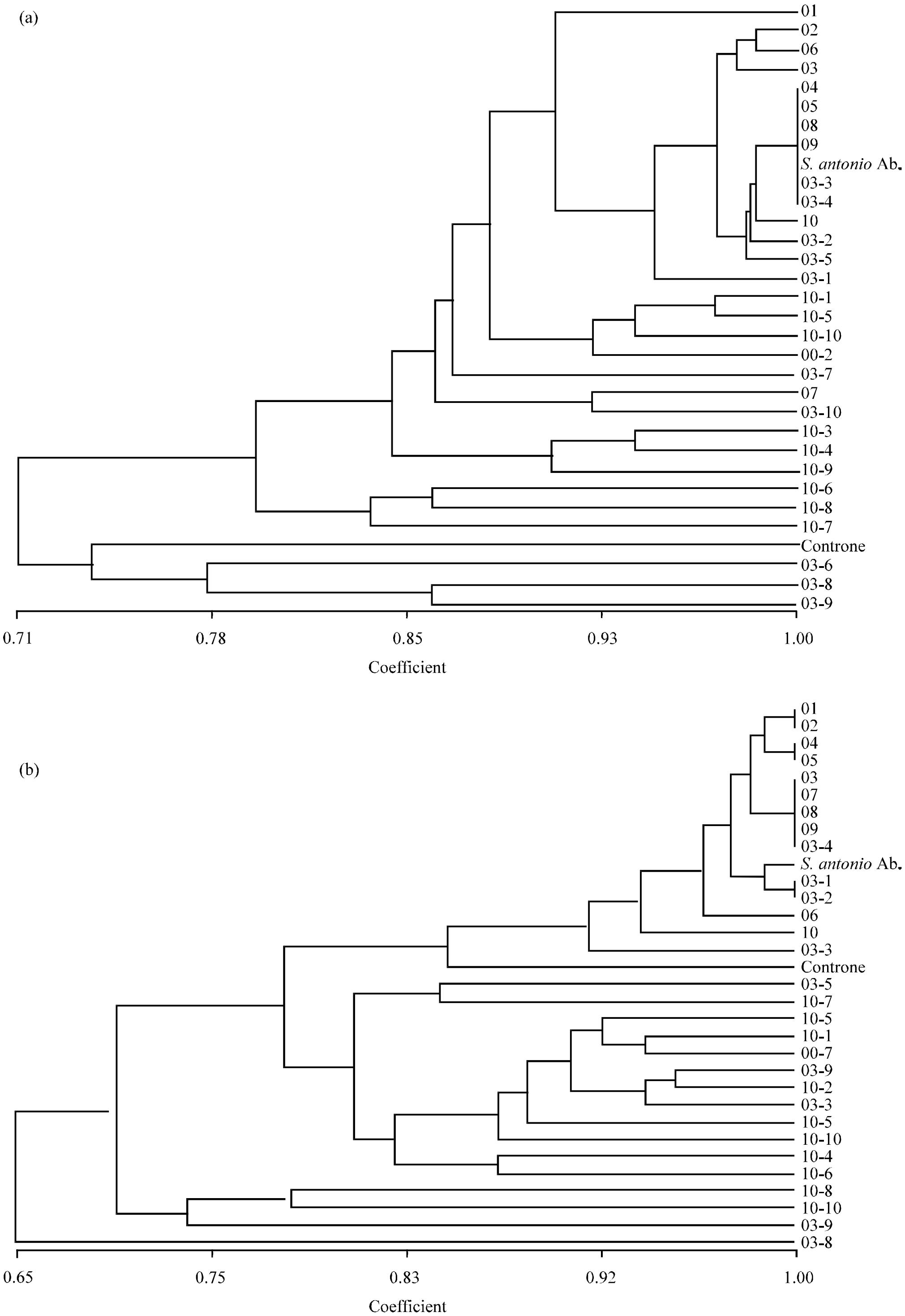

Fig. 3(a-b): Dendrograms obtained with two AFLP marker combination showing relationship among ten bean biotypes of "Fagiolo occhio nero di Oliveto Citra" and the two landraces "Fagiolo di Controne" and "Fagiolo di Sant"Antonio Abate (a) AFLP primer combination Eco-AAC/Mse-CTG and (b) AFLP primer combination Eco-ACC/Mse-CAT. On the x-axis is shown the coefficient of similarity, on the y axis are indicated the biotypes used in the analysis and the two mediterranean landraces "Fagiolo di Controne" and "Fagiolo di *S. antonio* Abate"

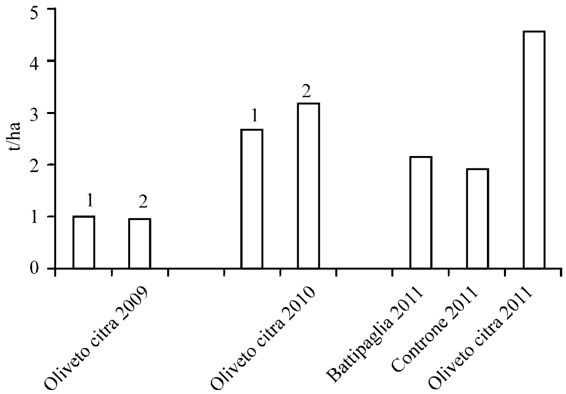

Fig. 4: Grain yield of "Fagiolo occhio nero di Oliveto Citra" in three years of experiments 1: 20 cm of distance on the row, 2: 30 cm of distance on the row

nero di Oliveto Citra" can be considered in line with the required parameters of nutritional quality. Other chemical value involving ash, humidity and protein digestibility are consistent to other studies (Yanez *et al.*, 1995). Seed mineral concentrations in common bean as well polyphenolics contents show variability among wild, cultivated landraces and modern cultivars (Moraghan and Grafton 1997; Pin-heiro *et al.*, 2010; Dinelli *et al.*, 2006), while tannins contents is due to seeds coat color (Caldas and Blair, 2009). Considering other studies (Pinheiro *et al.*, 2010; Paredes *et al.*, 2009; Caldas and Blair 2009), the "Fagiolo occhio nero di Oliveto Citra" is in line with the average contents of macroelements, polyphenols and tannins while, for the microelements, shows iron deficiency. Whereas the variation in polyphenolic contents is more related to the genotype than to the seed coat color (Espinosa-Alonso *et al.*, 2006), differences in eye area dimension should not cause variation in terms of antioxidant contents between biotypes.

CONCLUSION

The present study argued about the performance, variability and adaptation of the bean ecotype "Fagiolo occhio nero di Oliveto Citra". The work demonstrates how the landrace can be adapted in different environments than the natural habitat and how wide seeds spacing allow to save seeds, having the same yield production. Moreover, good level of elements and biochemical compounds have been detected and genetic stability was found between different lots of seeds obtained from several farmers in Oliveto Citra. This study shows the relevant importance of the "Fagiolo occhio nero di Oliveto Citra" as slow food and its potentiality as source for breeding programs.

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