Plant Physiology and Biochemistry 231 (2026) 110988

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

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

Improving yield of a bean ecotype using biostimulants: focus on bean amino acid profiles and plant responses

Emily Rose Palm [a,b], Gaia Santini [a], Alberto Niccolai [a], Marzia Vergine [c,*], Carmine Negro [c], Werther Guidi Nissim [a,b,d], Leonardo Sabbatini [a], Raffaella Balestrini [e], Maria Concetta de Pinto [f], Gholamreza Gohari [g,h], Vasileios Fotopoulos [h], Stefano Mancuso [a,i], Andrea Luvisi [c], Luigi De Bellis [c,d], Liliana Rodolfi [a], Federico Vita [a,f]

[a] *Department of Agriculture, Food, Environment and Forestry, University of Florence, 50121, Florence, Italy*
[b] *Department of Biotechnology and Biosciences, University of Milano-Bicocca, 20126, Milano, Italy*
[c] *Department of Biological and Environmental Sciences and Technologies, University of Salento, 73100, Lecce, Italy*
[d] *National Biodiversity Future Center, 90133, Palermo, Italy*
[e] *Institute of Biosciences and Bioresources, National Research Council, 70126, Bari, Italy*
[f] *Department of Bioscience, Biotechnology and Environment, University of Bari "Aldo Moro", 70121, Bari, Italy*
[g] *Department of Horticultural Science, Faculty of Agriculture, University of Maragheh, 97HF+498, Maragheh, Iran*
[h] *Department of Agricultural Sciences, Biotechnology and Food Science, Cyprus University of Technology, 3036, Limassol, Cyprus*
[i] *Fondazione per il Futuro Delle Città (FFC), 50121, Florence, Italy*

ARTICLE INFO

Keywords:
Phaseolus vulgaris
Methionine
Arthrospira
Molecular barcoding
Productivity

ABSTRACT

Biostimulants have emerged as having the potential to sustainably enhance crop performance as well as yield quantity and nutritional quality. Although naturally rich in lysine, beans are generally deficient in sulfur-containing amino acids like methionine and cysteine. Improving the nutritional imbalance in beans is highly desirable, especially in those with cultural and economic value, like Fagiolo di Sorana, a high-quality Protected Designation of Origin (PDO) bean variety from Pistoia, Italy. A spirulina-based (1 g/L and 3 g/L) and a commercially available (MC EXTRA; 1 g/L) biostimulant were applied as foliar sprays for two consecutive years to Fagiolo di Sorana plants grown under both open field and semi-controlled greenhouse conditions. Productivity was higher in treated plants: a 7 % increase (*p*-value, 0.036) was found in whole pod weight in the first year of the trial with 3 g/L and in the second year trial (*p*-value, 0.020) for MC EXTRA compared to the control. Improved amino acid composition of the beans were found, specifically an increase of 200 % (*p*-value, 0.040) and 400 % (*p*-value, 0.053) in methionine content with 3 g/L spirulina and MC EXTRA, respectively, compared to the control, thus addressing the bean's typical deficiency in sulfur amino acids. Bean digestibility increased 3 % (*p*-value, 0.013) with the higher concentration (3 g/L) of the spirulina-based biostimulant relative to the control-grown plants. Molecular barcoding identified genetic differences within a collection of ten Tuscan bean landraces, including the Fagiolo di Sorana variety, thus offering a first attempt at the genetic characterization essential for preserving landrace germplasm. These genetic data were then coupled with the assessment of protein digestibility to identify differences within the landrace collection. Thus, the use of biostimulants presents an opportunity to further enhance the yield and nutritional profile of this PDO without compromising its environmental integrity.

1. Introduction

Widespread acceptance of biostimulants in agriculture is on the upward trend, helping reduce farmers' dependence on high-priced fertilizers and making crops more tolerant to water scarcity and salinity (Koleška et al., 2017; Roche, 2024). Biostimulants are increasingly applied in organic farming as they fit the concept of environmental sustainability (Gerhards et al., 2021; Shawky et al., 2023). They can be applied to plants or soil, enhancing plant growth, nutrient uptake and development, and plant tolerance to environmental stresses (du Jardin,

---

* Corresponding author.
E-mail address: marzia.vergine@unisalento.it (M. Vergine).

https://doi.org/10.1016/j.plaphy.2025.110988
Received 30 July 2025; Received in revised form 20 December 2025; Accepted 22 December 2025

2015; Halpern et al., 2015). While biostimulants do not provide plants with essential nutrients like (bio)fertilizers, they are rich in many biologically active compounds. These include plant growth stimulating hormones such as auxin and cytokinins, and amino acids like proline that aid in buffering osmolarity imbalances induced by abiotic stresses (Johnson et al., 2024; Skylas et al., 2022), leading to improved nutrient uptake and translocation (Ma et al., 2022), enhanced growth rates and crop yields (tomatoes and maize) (Adoko et al., 2021; Francesca et al., 2021). Thus, the application of biostimulants could increase the fitness and yield of important staple crops.

Biostimulants can be made from various sources, including seaweed, microorganisms, humic and fulvic acids, amino acids, and plant extracts (Rouphael and Colla, 2020). Among the most well-studied algal-based biostimulants are those extracted from the brown seaweed *Ascophyllum nodosum*; its stimulating effects on plant growth and abiotic stress resistance are well documented (Shukla et al., 2019). The most promising candidates of biostimulants are those belonging to the prokaryotic cyanobacteria group, which include N_2-fixing microbes and species like spirulina (*Arthospira* spp.) (Vaishampayan et al., 2001; Singh, 2016). It is known that cyanobacteria excrete bioactive substances that act as signalling molecules, promoting plant growth (Colla et al., 2017) and the synthesis of phytohormones (Žižková et al., 2017), which influence diverse physiological processes in plants (Santini et al., 2021) and help protect plants from environmental stress such as heavy metals (Gharib and Ahmed, 2023) and salinity (Bauenova et al., 2024; Xu et al., 2023; Selem, 2018). At the molecular level, the upregulation of several genes involved in the primary and secondary metabolic pathways is expected (Barone et al., 2019). A recent study showed dramatic upregulation of stress response genes following the application of *Spirulina platensis* extract in wheat cultivated under drought conditions (Ibrahim et al., 2024), especially those of enzymes involved in detoxification of endogenous metabolic byproducts and those regulating metabolic processes. Current climate projections for 2050 indicate that drought conditions are likely to increase in both the amount of affected area and their intensity in northern (Baronetti et al., 2022) and southern Italy (Critto et al., 2016). Therefore, biostimulants may be an especially valuable tool in the production of PDO crops (Colla et al., 2017; Godlewska et al., 2019) by ensuring their beneficial effects are exerted without the application of chemical fertilisers, pesticides, or soil improvers (du Jardin, 2015).

The common bean *Phaseolus vulgaris* L. is an important species and a significant source of dietary protein throughout Latin America and Eastern Africa (Graham and Ranalli, 1997). Its introduction to the Italian peninsula in 1532 was well-accepted, given *P. vulgaris*' resemblance to cowpea (*Vigna unguiculata*), a widely cultivated and consumed legume in the Mediterranean (Piergiovanni and Lioi, 2010). Its cultivation quickly spread, with farmers throughout the peninsula exerting selective pressure for various organoleptic traits, leading to a wide variety of landraces (Piergiovanni et al., 2000). The different environments of the Italian peninsula have led to the development of many ecotypes, currently poorly studied and characterized due to their limited distribution areas (Dinelli et al., 2006) and unsuitable production characteristics (*e.g.*, a high number of seeds per pod), which negatively influence their commercial value (Piergiovanni et al., 2000). The genetic basis of this ecotypic variation can be explored through the many landraces that have been cataloged by germplasm centers. This approach facilitates the identification of genetic markers that are both linked to abiotic and biotic stress tolerance and to desirable organoleptic traits, which can be further explored phylogenetically through recent technologies like molecular barcoding. Some landraces have been designated as having unique characteristics and are especially tied to the local edaphic conditions and culture. For example, in 2002, the Sorana bean (“Fagiolo di Sorana”) received the Protected Designation of Origin (PDO) from the European Union with strict production rules (Reg. CE n. 1018 del 13.06.02). An ecotype of *P. vulgaris*, the Sorana bean grows in a restricted area of Pistoia showing unique organoleptic properties linked to its site of origin's microclimate and pedo-chemical conditions and cultivated under nearly organic agricultural practices. It has nutritional properties such as variegated vitamin content (thiamin, riboflavin, niacin, vitamin, vitamin B6 and folic acid) and minerals including potassium (K), calcium (Ca), magnesium (Mg), zinc (Zn), copper (Cu) and iron (Fe) (Celmeli et al., 2018). The immense variation that exists among ecotypes of *P. vulgaris* provides an ideal context in which to investigate the genetic basis of organoleptic and nutritional properties in a globally relevant staple crop.

Despite their being among the most important staple crops globally, *Phaseolus vulgaris* seeds are poor in methionine, an essential amino acid that contains sulfur and is required for protein synthesis and overall nutrition. This deficiency is significant for diets where beans are the main source of protein (Adedeji et al., 2016; Skylas et al., 2022; Skylas, 2024). The inability of humans to synthesize certain essential amino acids and the reduced content of methionine in legumes has long triggered scientific interest in increasing the levels of these essential amino acids in crop plants, including the testing of recombinant inbred lines with the goal of increasing levels of Cys and Met (Viscarra-Torrico et al., 2021). A few studies have shown that biostimulants can increase the amino acid content of plants to which they are applied. The biostimulant *Arthrospira platensis* (spirulina) not only enhanced growth parameters but also increased the levels of essential amino acids in lettuce plants (Mógor et al., 2018). In the legume *Cicer arietinum* L. (chickpea), the application of chestnut wood distillate as a biostimulant boosted the free amino acid content of seeds, especially valine, isoleucine and tyrosine (Fedeli et al., 2023). It is clear from the literature that yield and qualitative traits of *P. vulgaris* can be improved through the application of biostimulants. For instance, *Ecklonia maxima* has been proven to enhance the yield, protein flavonoids and carotenoid content as well as nutritional and nutraceutical properties in several common bean cultivars (Kocira et al., 2017, 2018, 2020; Nowak et al., 2023). However, a specific effect on amino acid production, especially those found at low levels in *P. vulgaris*, has rarely been investigated.

The main goal of this study was to improve the growth of the ecotype “Fagiolo di Sorana” and enhance the quality and quantity of harvestable product under its ideal edaphic conditions. It was hypothesized that both cyanobacteria and algal-based biostimulants would have a positive effective on plant biomass and yield due to evidence from earlier studies. However, the observed effect of spirulina-based biostimulants on amino acid production and digestability in plants was expected to improve specifically this aspect of the yield quality in Fagiolo di Sorana. For this purpose, the bean plants were treated with a foliar application of a biostimulant based on *Arthrospira* extracts and a commercial biostimulant and evaluated using a multidisciplinary approach. Open-field and greenhouse experiments were conducted over two growing seasons to assess the effects of biostimulant treatment on plant physiology and yield. Bean seed amino acid profiles and digestability were evaluated to determine if biostimulants have a direct impact on these properties. A molecular barcoding approach was used to elucidate the genetic relationships between different Tuscan landraces, focusing specifically on the trait of seed digestibility to explore the potential of this approach to select for specific organoleptic traits in staple crops.

2. Materials and methods

2.1. Experimental set-up

2.1.1. Plant material

Sixty common bean (*Phaseolus vulgaris* L.), ecotype ‘Fagiolo di Sorana’, plants (n = 15 per treatment) were used to test two biostimulants. Seeds for the molecular barcoding and digestibility analyses of ten Tuscan landraces were obtained from the bean germplasm bank of the Department of Agriculture Science and Technology, Food, Environment and Forestry at the University of Florence, Italy.

2.1.2. Growing conditions

Open field experiments were performed for two consecutive years (2019–2020) in Sorana, province of Pistoia, Italy (43°58′24.5″N 10°42′22.2″E). Environmental data related to temperatures and moisture were obtained from the Regional Service of Tuscany Region (SIR) website and reported as supplementary data (TOS11000091, www.sir.toscana.it). Seeds were sown directly in the field in mid-June in both years of the trial, and plants were grown following the standard protocol outlined in the production disciplinary. The same experiment was also repeated on another set of 60 plants in a greenhouse at the University of Florence (Italy) (lat. 43°48′58.6″ N, long. 11°11′58.1″ E) for two years (2019–2020). Seeds were germinated in mid-June of both years of the trial on moist filter paper at 23 °C and then transplanted to 15 cm diameter black polyvinyl pots filled with a 60:40 w/w mix of commercial potting soil and 8–16 mm expanded clay, respectively. Plants were cultivated under semi-controlled conditions (automatic irrigation twice a day), with natural, non-supplemented lighting (average 500 μmol m^{-2} s^{-1} PAR). Temperature and moisture data were continuously collected using probes (greenhouse experiment) or downloaded from the SIR Toscana website (TOS11000091 station, Sorana). Meteorological data collected over the two years for both trials are reported in the Supplementary material (Figs. S2 and S3).

For the barcoding analysis, seeds from each of the eleven landraces (Sorana included) were germinated on moistened filter paper in the dark. Once germinated the seedlings were transferred to a growth chamber with 250 μmol m^{-2} s^{-1}, 16L:8D light cycle, day:night temperatures of 23 °C:18 °C and relative humidity of 70 %. Seedlings were allowed to grow until the first true leaves had appeared before sampling.

2.1.3. Biostimulant treatments

Two different biostimulants were used in this study: one based on spirulina (*Arthrospira platensis* F&M-C256) dry mass, and the other, MC EXTRA, a commercial product from Valagro® (Syngenta Global, Basel, Switzerland) based on *Ascophyllum nodosum* extracts. Spirulina biomass was cultivated in Zarrouk medium (Zarrouk, 1966) in semi-batch mode and harvested by filtration. Biomass was then washed with tap water to remove excess bicarbonate and dried at low temperature (33 °C) for 20 h. Further details, as well as data related to biomass, biochemical composition, and ash content, are reported in Niccolai et al. (2019). The spirulina-based biostimulant was tested at two different concentrations (1 and 3 g/L, SP1 and SP3, respectively). These concentrations were selected based on preliminary, unpublished experimental trials conducted by our research group, which demonstrated their efficacy in eliciting positive physiological responses without causing phytotoxicity. MC EXTRA was applied at a concentration of 1 g/L, in accordance with the manufacturer's recommended dosage for foliar application. In both conditions (i.e., open field and greenhouse), both stimulants were diluted at the concentrations described above with distilled water and were applied in four foliar treatments. The first application (T1) occurred when the plants had at least four fully expanded sets of trifoliates, roughly one month from the time seeds were sown. Treatments were repeated three more times, every 15 days (T2, T3 and T4). At each treatment application, the volumes of biostimulant applied were increased to ensure complete wetting of the growing leaf surface. Control plants (CTRL) were treated simply with distilled water.

Leaf samples were collected from plants grown in open field conditions at the end of the experiments to measure leaf biochemical parameters - leaf pigments and polyphenolic contents. Sampling for physiological, genetic and biochemical parameters described below were performed at T1-T4, as indicated.

2.2. Physiological and productivity parameters

Gas-exchange measurements were performed 24 h after the foliar application of the biostimulants at four different times (i.e. T1, T2, T3, T4) during both growing seasons in both open field and greenhouse conditions. Six plants from each treatment group were used for the measurements. The LI-6400XT Portable Photosynthesis System (LICOR Biosciences; Nebraska, USA) was used to measure gas exchange on young, fully expanded leaves from each plant. The analyses were performed from 8:00 a.m. to 1:00 p.m. Carbon assimilation rate (A_n), and stomatal conductance (g_s) rates were measured with the following LICOR chamber settings: CO_2 concentration of 400 μmol CO_2 mol^{-1}, block temperature of 30 °C, light intensity of 1000 μmol m^{-2} s^{-1} PAR, and relative humidity between 50 and 60 %. The chamber area was 2 cm^2; however, if the considered leaf area was smaller than the chamber, the new area was determined and used to adjust photosynthetic parameters.

2.3. Pigments and polyphenols content

Spectrophotometric analyses to evaluate leaf pigment concentrations and polyphenolic content were carried out on 10 mg of fresh leaves from six plants (n = 6) for each treatment. Leaf samples were collected from plants grown in open field conditions following gas exchange measurements at each application of the biostimulants (T1-T4). The leaves were sampled, frozen in liquid nitrogen, and then finely ground to a powder. Subsequently, 1 mL of cold methanol was added to the ground leaves, followed by shaking for 30 min and centrifugation at 10,000 *g* for 10 min. The resulting supernatant was utilized for absorbance readings at 665, 652, and 470 nm to determine the levels of Chlorophyll *a* (Chl*a*), *b* (Chl*b*), and carotenoids, respectively. Absorbance readings were performed using a TECAN spectrophotometer with a 96-well black multiplate reader. Pigment quantification was conducted based on the equations provided by Wellburn (1994). Total polyphenol content was determined according to Ainsworth and Gillespie (2007). Briefly, 20 mg of leaf tissue was frozen in liquid nitrogen and homogenized. 2 ml of ice-cold 95 % (vol/vol) methanol was added, and samples were incubated at room temperature for 48 h. Samples were then centrifuged at 13,000 *g* at room temperature, and the supernatant was collected. 200 μl of 10 % (vol/vol) Folin-Ciocalteu reagent was mixed with 100 μl of supernatant and 800 μl of 700 mM Na_2CO_3. Finally, 200 μl were used for absorbance readings at 765 nm using a TECAN spectrophotometer with a 96-well black multi-plate reader.

2.4. Yield parameters

To compare plant productivity, we evaluated the number of pods per plant and the number of seeds per pod in both treated and non-treated plants at harvest from both open field and greenhouse conditions. Seeds and pods were dried in an oven at 70 °C until constant weight to remove any residual moisture and weighed to obtain the total pod weight and shelled pod weight per plant.

2.5. Quantification of amino acids in bean flour

Dry beans (0.5 g) from plants grown in open field conditions in each treatment were ground to a fine powder. The resulting bean flour was used for amino acid determination. Subsamples of bean flour, approximately 10 mg each, were weighed into 10 mL headspace glass vials with a crimp cap and 3 mL HCl 6 M (with 0.1 % w/v phenol) was added. The vials were placed in a preheated oven at 110 °C for 24 h. After hydrolysis, the samples were allowed to cool to handling temperature and treated as described by Dahl-Lassen et al. (2018). Oxidation was performed before hydrolysis to protect samples from degradation during the heating phase to analyze the sulfur-containing amino acids. Performic acid was used as an oxidizing agent, and the samples were prepared as described by Dahl-Lassen et al. (2018). Sample characterization and quantification were performed using an Agilent 1200 HPLC DAD ESI/MS-TOF system (Agilent Technologies, Palo Alto, CA, USA) equipped with a standard autosampler and an analytical column (Agilent InfinityLab Poroshell 120 HILIC-Z, 2.7 μm, 2.1 × 150 mm, PEEK-lined, (p/n 673775-924)). The column temperature was maintained at 25 ± 2 °C and the volume injected on the column was 5 μL. Gradient elution was performed using 10 mM ammonium formate plus 0.1 % formic acid in water as eluent A and 10 mM ammonium formate plus 0.1 % formic acid in 90 % acetonitrile as eluent B. The flow rate was kept constant at 0.400 mL min^{-1}, as reported by Zhao et al. (2021). The system was controlled by Agilent MassHunter acquisition software version B.06.01 build January 6, 6157. An amino acid standard mix (part number 5061-3330) including alanine (Ala), arginine (Arg), aspartic acid (Asp), cysteine (Cys), glutamic acid (Glu), glycine (Gly), histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), proline (Pro), serine (Ser), threonine (Thr), tyrosine (Tyr) and valine (Val) was obtained from Agilent Technologies Inc. (Santa Clara, CA, USA); methionine sulfone, cysteic acid were obtained from Millipore Sigma, Inc. (St. Louis, MO, USA) as reported by Zhao et al. (2021). Data processing was performed with Agilent MassHunter quantitative analysis software version B.07.00 build 7.0.457.0.

2.6. *Digestibility assay*

Beans collected from the open field experiments were tested for dry matter digestibility to assess differences induced by biostimulants using a modified version of the protocol proposed by Boisen and Fernández (1997), which reproduces *in vitro* the chemical-enzymatic catalysis that occurs in the proximal tract of the monogastric digestive system. Beans were freeze-dried and ground to a fine powder. 1 g of ground material was transferred in a 250 mL Erlenmeyer flask and mixed with 25 mL of phosphate buffer (0.1 M, pH 6.0). 10 mL 0.2 M HCl was added, and pH was adjusted to a value of 2.0. Finally, 3 mL of a freshly prepared pepsin water solution containing 30 mg of porcine pepsin with an activity of 0.8 FIP-U/mg (Applichem, Darmstdat, Germany) was added. A blank was also prepared with the same reactives but without beans. Flasks were closed with a rubber stopper and incubated under stirring at 39 °C for 6 h. After this time, 10 mL of phosphate buffer (0.2 M, pH 6.8) and 5 mL of a 0.6 M NaOH solution were added to the samples and to the blank. The pH was adjusted to a value of 6.8 by the addition of HCl or NaOH (1 M solutions) followed by the addition of 10 mL of a freshly prepared pancreatin 50 %-ethanol solution containing 500 mg of porcine pancreatin with an activity of 42362 FIP-U/g (Applichem, Darmstadt, Germany). Flasks were further incubated for 18 h under the same conditions described above.

Undigested residues were then collected by centrifugation (Neya 8, Neya, India) at 4500 rpm for 30 min, washed with deionised water to remove any buffer salts, and then centrifuged with the same parameters mentioned above. Both pellets were then dried at 80 °C for 6 h and then at 45 °C, until constant weight and then weighed. The supernatant from the two centrifugations was filtered on 47 mm glass-fiber membranes with nominal porosity of 1.2 μm (FILTER-LAB, Barcelona, Spain), dried in the same conditions described above, and weighed. Weights of the residues on filter paper were added to the weights of the pellets to account for any residual biomass or undissolved reagent not sedimented during centrifugation, obtaining the overall weight of undigested material. The blank was treated following the same steps mentioned above.

The *in vitro* dry matter digestibility, expressed as a percentage ± standard error of the mean, was calculated as the difference between the weight of the starting material and the weight of the undigested material (both expressed in grams) from which the blank value was previously subtracted. The analysis was performed in triplicate. Digestibility data were integrated by analyzing eleven Italian bean landraces (Sorana included) to highlight specific landrace properties, according to Niccolai et al. (2019).

2.7. *Barcoding analysis*

DNA barcoding was performed by sequencing three genes (nuclear *ITS,* internal transcribed spacer and two chloroplast intergenic spacers, *trnL-trnF* and *trnH-psbA*) using primer sequences reported in the literature (Nicolè et al., 2011). Briefly, 100 mg of fresh leaf tissue was collected from landrace seedlings (Table 1), and DNA was extracted according to Allen et al. (2006). The amplicon sequences were obtained by assembling the forward and reverse sequence of one amplicon, according to Sanger-sequencing method (Eurofins, Ebersberg). Phylogenetic relationships were then established with *ITS*, *trnL-trnF* and *trnH-psbA* gene sequences, which were concatenated and analyzed *using* MEGA 11 software (Tamura et al., 2021). For the phylogenetic tree, Maximum Likelihood-based on the Tamura-Nei model was used with a number of Bootstrap Replications equal to 1000 (Tamura and Nei, 1993).

2.8. *Statistical analysis*

Data from amino acids, pigments and polyphenolic analyses were assessed for normal distribution through a Shapiro-Wilk test and homogeneity distribution of variance through Bartlett's test (Bartlett, 1937). Then, samples from pigment and polyphenolic analyses were analyzed using a two-way ANOVA followed by Dunnett's post *hoc* test ($p \leq 0.05$) (Dunnett, 1955). As biometric (yield) data do not follow a normal distribution, statistical analyses were carried out using the Kruskal-Wallis test (Kruskal and Wallis, 1952) coupled with Dunn's post-hoc test (Dunn, 1964). Analysis of amino-acid composition was assessed through one-way ANOVA coupled with Tukey HSD test ($p \leq 0.05$) (Tukey, 1949), followed by a Principle Component Analysis (PCA). The PCA results were graphically processed to highlight the contribution of each variable (compounds) and the differentiation of the observations (samples). PCA was computed using XLSTAT (version 2016.02.27444), whereas ANOVA analysis was computed using GraphPad Prism version 9.4.0 (GraphPad Software, San Diego, CA). The data from digestibility data of Sorana and landrace samples were analyzed according to one-way ANOVA coupled with Dunnett's test ($p \leq 0.05$) (Dunnett, 1955), within treatment groups (CTRL as reference, left side) and Tukey HSD test (p-value <0.05) (Tukey, 1949) for landrace analyses (Sorana bean as reference, right side).

3. Results

3.1. *Photosynthesis data*

Figs. 1 and 2 show the data corresponding to carbon assimilation rate (A_n) and stomatal conductance rate (g_s) in 2019 and 2020, respectively. A few significant trends were observed in the first year of analysis (Fig. 1), mainly at T3 and T4. An increase in both A_n and g_s at T3 for SP3 compared to CTRL, and an increase in A_n at T4 for SP1 compared to CTRL. However no discernible trend between the two spirulina concentrations and MC EXTRA was found in either year. In 2020, A_n at T4 was significantly higher in SP3 (14.04 μmol CO_2 m^{-2} s^{-1}; p-value, 0.0268) than in the other three treatments: CRTL (6.82 μmol CO_2 m^{-2}

Table 1
List of Tuscan bean landraces evaluated in the present work for digestibility and phylogenesis.

Name	Provenience
Fagiolo di Sorana	Sorana (PT)
Fagiola Fiorentina	Garfagnana (LU)
Piattella Pisana	Pisa (PI)
Fagiola di Venanzio	Murlo (SI)
Fagiolo dall'Occhio del Valdarno	San Giovanni Valdarno (AR)
Turco Grigio	Lucca (LU)
Fagiolo rosso di Lucca	Lucca (LU)
Zolfino	Pratomagno (AR)
Lupinaro di Lucca	Lucca (LU)
Fagiolo di Bigliolo due Facce	Bigliollo (MS)
Fagiolo Cannellino di Sorano	Sorano (GR)

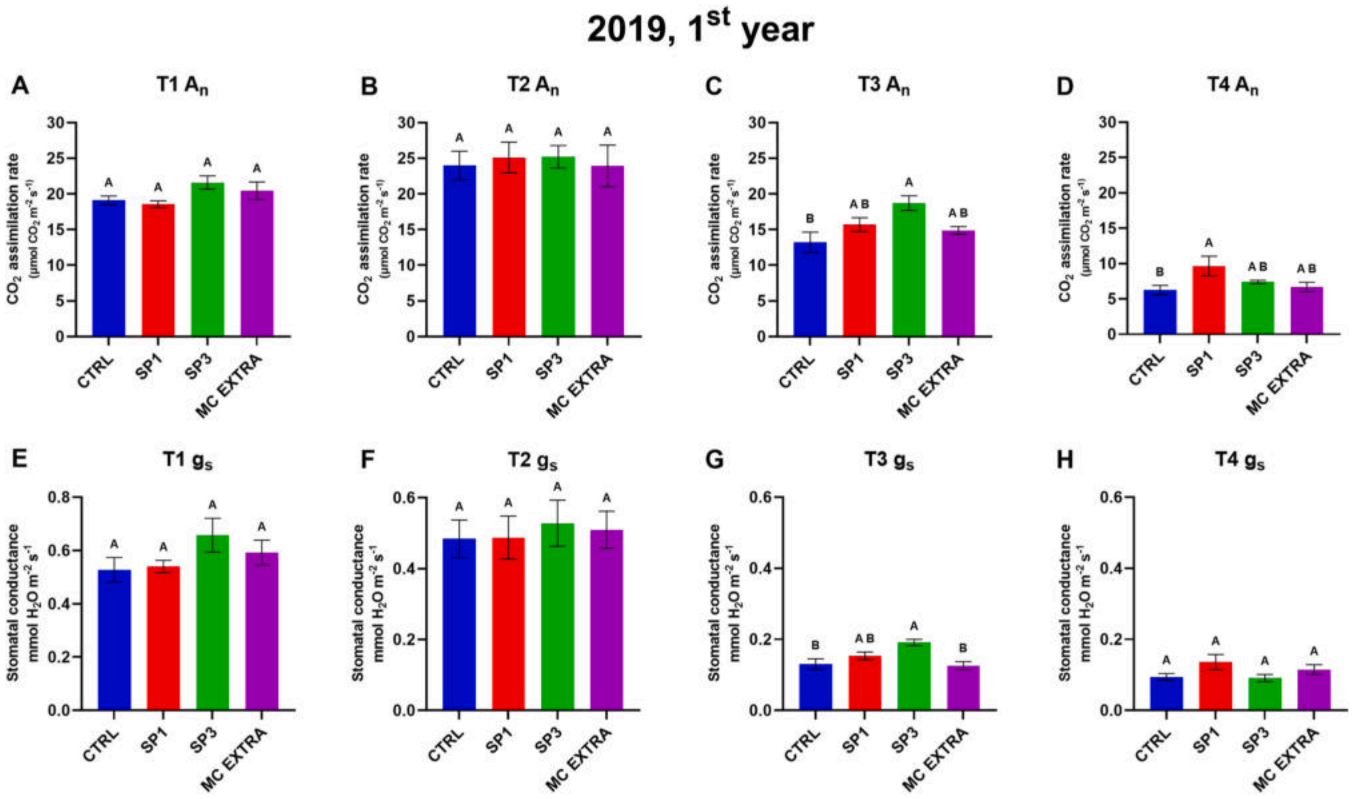


Fig. 1. Carbon assimilation rate (A_n) and stomatal conductance (g_s) data of the first year of analysis (2019) in open field conditions. Measurements were carried out on the first fully-expanded leaf from the apex of fagiolo di Sorana plants. Data are the mean values ± S.E.M, consisting of one leaf from six plants (n = 6) at four sampling times (T1-T4). The statistical significance of the difference between control (CTRL) and treatments (SP1, SP3, MC EXTRA) was assessed by one-way ANOVA followed by Tukey HSD test (p-value <0.05). **** <0.0001 *** < 0.001 ** <0.01 * < 0.05. A_n data, panels A–D; g_s data, panels E–H.

s^{-1}), SP1 (5.56 μmol CO_2 m^{-2} s^{-1}) and MC EXTRA (6.56 μmol CO_2 m^{-2} s^{-1}). Results from the greenhouse experiment (Figs. S4 and S5) indicate that differences were observed among treatments in both years. Stomatal conductance (g_s) data of the first year indicate significant differences in the CTRL-SP3 comparison at T1 and T2, showing an increase in stomatal conductance in the SP3 data compared to CTRL (T1: 0.133 mmol H_2O m^{-2} s^{-1} and 0.056 mmol H_2O m^{-2} s^{-1}, respectively (p-value, 0.0482); T2: 0.106 mmol H_2O m^{-2} s^{-1} and 0.066 mmol H_2O m^{-2} s^{-1}, respectively (p-value, 0.0367)). In the second year (Fig. S5), the A_n and g_s results were statistically significant in T3 and T4, especially in the comparisons SP1-CTRL (g_s: 0.238 mmol H_2O m^{-2} s^{-1} and 0.100 mmol H_2O m^{-2} s^{-1}, respectively (p-value, 0.003) and SP3-CTRL (A_n: 15.447 μmol CO_2 m^{-2} s^{-1} and 11.376 μmol CO_2 m^{-2} s^{-1}, respectively (p-value, 0.041) g_s: 0.247 mmol H_2O m^{-2} s^{-1} and 0.100 mmol H_2O m^{-2} s^{-1}, respectively (p-value, 0.0009)) at T3 and MC EXTRA-CTRL (A_n: 20.333 μmol CO_2 m^{-2} s^{-1} and 13.400 μmol CO_2 m^{-2} s^{-1}, respectively (p-value, 0.025) g_s: 0.244 mmol H_2O m^{-2} s^{-1} and 0.142 mmol H_2O m^{-2} s^{-1}, respectively (p-value, 0.029)) at T4.

3.2. *Pigments and total phenolic contents*

Pigment analyses from field-grown plants indicate that the application of spirulina-based biostimulant mainly affected the concentrations of Chl *a*, *b* and carotenoids during the first year of analysis (Fig. 3A–C,E). The values of all three parameters generally increased over time, and noteably, values for SP1 and SP3 were consistently greater than CTRL and MC EXTRA. Significant differences in Chl *a* concentrations between CTRL (0.822 μg/mg) and SP1 (1.316 μg/mg; p-value, <0.0001) and SP3 (1.377 μg/mg; p-value, <0.0001), were peaking at T3. At T3, Chl *a* concentrations in MC EXTRA were also significantly higher relative to CTRL (1.031 μg/mg and 0.822 μg/mg, respectively; p-value, 0.001) at T3, but MC EXTRA was lower than both SP1 and SP3. A similar trend was reported for Chl *b* data, where the biostimulant application lead to increased pigments in T3 in all the treatments (SP1, 0.489 μg/mg, p-value, <0.0001; SP3, 0.529 μg/mg, p-value <0.0001; MC EXTRA, 0.427 μg/mg, p-value 0.0394) compared to CTRL (0.038 μg/mg), whereas in T4, only the spirulina treatments (SP1, 0.387 μg/mg, p-value <0.0001; SP3, 0.351 μg/mg, p-value 0.0035) showed significant increase in Chl *b* content (CTRL, 0.291 μg/mg), even if the T4 overall data indicate a general decrease when compared with T3.

Total carotenoid concentrations significantly increased at T3 and T4 in all three treatment groups relative to CTRL (0.121 μg/mg, 100 %): 204 % relative to SP1 (0.247 μg/mg; p-value, <0.0001), 222 % for SP3 (0.269 μg/mg; p-value, <0.0001), and 160 % for MC EXTRA (0.194 μg/mg; p-value, <0.0001) at T3 and 314 % relative to SP1 (0.148 μg/mg; p-value, <0.0001), 268 % relative to SP3 (0.126 μg/mg; p-value, <0.0001), and 196 % relative to MC EXTRA (0.092 μg/mg; p-value, 0.0077) at T4 (CTRL, 0.049 μg/mg, 100 %). While the values for Chl *a*, *b* and carotenoid content declined in all treatment groups at T4, the relationships among samples remained unchanged. Conversely, biostimulant treatment did not significantly affect the pigment contents in the second year of analysis, although a general increase in treated plants compared to the CTRL (Fig. 3B–D,F) was observed with peak values again at T3. The treatment was a significant factor for Chl *b* content only (p-value, 0.0009), while the timing of the application and measurement was significant for all three pigments evaluated (Chl *a*: p-value, <0.0001; Chl *b*: p-value, <0.0001; carotenoids: p-value, <0.0001).

Two distinct patterns emerged regarding the relationships between the CTRL and treatment groups in terms of the total polyphenol content (Fig. 3G and H). In the first year (Fig. 3G), CTRL values were significantly higher compared to SP3 sample at T2, and significantly higher (CTRL: 2.132 mg GAE/g at T3; 3.887 mg GAE/g at T4) than all three

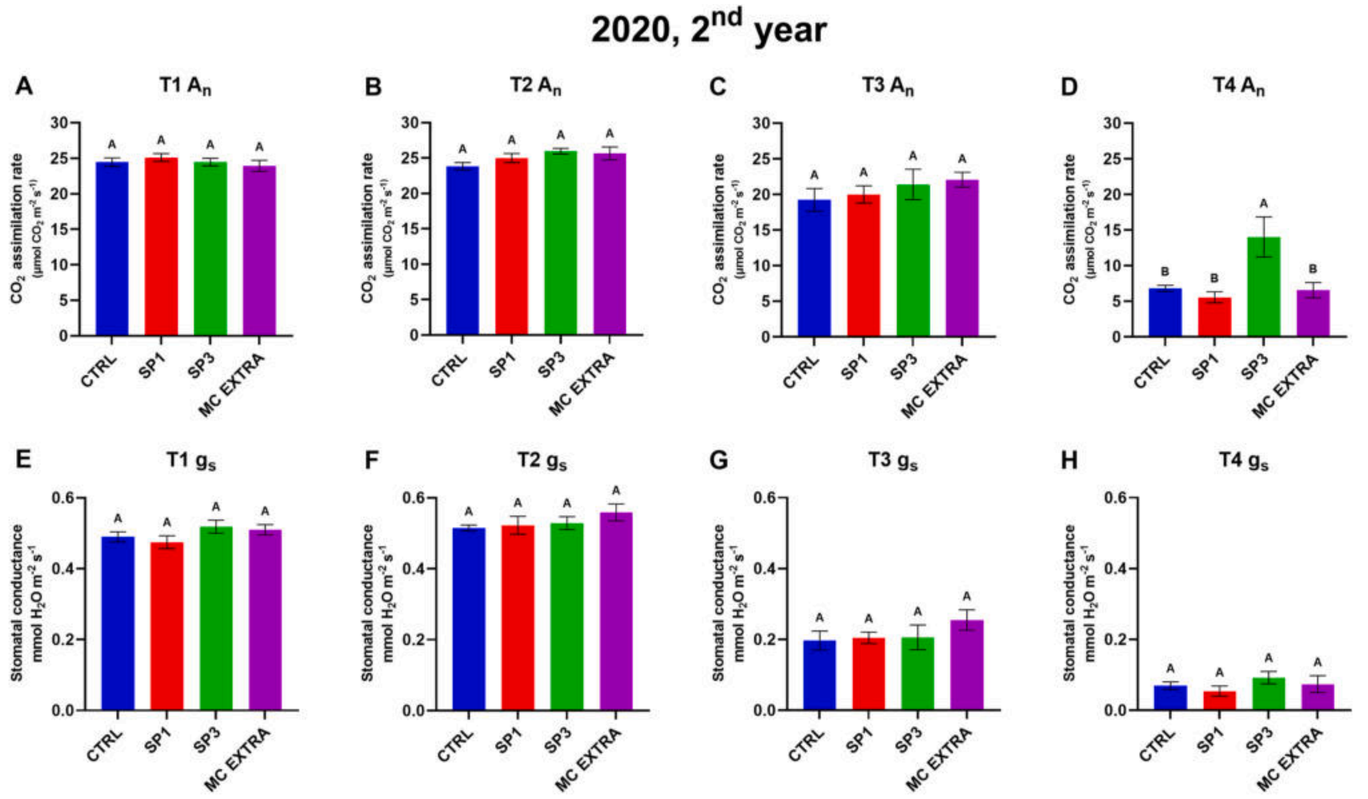


Fig. 2. Carbon assimilation rate (A_n) and stomatal conductance (g_s) data of the second year of analysis (2020) in open field conditions. Measurements were carried out on the first fully-expanded leaf from the apex of fagiolo di Sorana plants. Data are the mean values ± S.E.M, consisting of one leaf from six plants (n = 6) at four sampling times (T1-T4). The statistical significance of the difference between control (CTRL) and treatments (SP1, SP3, MC EXTRA) was assessed by one-way ANOVA followed by Tukey HSD test (*p*-value <0.05). **** <0.0001 *** < 0.001 ** <0.01 * < 0.05. A_n data, panels A–D; g_s data, panels E–H.

treatment groups (SP1: 1.544 mg GAE/g (*p*-value, 0.0003), 2.541 mg GAE/g (*p*-value, <0.0001), SP3: 1.656 mg GAE/g (*p*-value, 0.004), 2.427 mg GAE/g (*p*-value, <0.0001) and MC EXTRA: 1.719 mg GAE/g (*p*-value, 0.015), 3.227 mg GAE/g (*p*-value, <0.0001)) at T3 and T4, respectively. In contrast, MC EXTRA recorded the highest values at 2 out of 4 time points (T1 (3.728 mg GAE/g, *p*-value, 0.0002) and T2 (5.350 mg GAE/g, *p*-value, <0.0001) of the second year relative to CTRL (2.528 mg GAE/g, and 3.430 mg GAE/g at T1 and T2, respectively) (Fig. 3H). Both spirulina treatments had significantly greater polyphenol contents relative to CTRL (3.430 mg GAE/g) at T2, (SP1: 4.591 mg GAE/g (*p*-value, 0.0003), and SP3: 5.068 mg GAE/g (*p*-value, <0.0001)), with SP3 that also showed an increase in T4 (SP3: 6.352 mg GAE/g, *p*-value 0.1445) compared to CTRL (5.795 mg GAE/g) even if not statistical significant. At T3, a significant drop is reported, with CTRL having the highest values (4.735 mg GAE/g) relative to SP1 (3.304 mg GAE/g, *p*-value <0.0001) and SP3 (3.228 mg GAE/g, *p*-value <0.0001) and representing the only treatment that significantly increased its polyphenol contents in this time point.

3.3. *Yield parameters*

Yield production (average pod weight, whole and shelled) was evaluated for both experimental years in both open field (Fig. 4) and greenhouse trials (Figs. S6 and S7). Data distribution in Fig. 4A indicates that only SP3 significantly increased mean whole pod weight (1.586 g) relative to CTRL (1.469 g; *p*-value, 0.007) and mean shelled pod weight (SP3: 1.231 g; CTRL: 1.110 g; *p*-value, 0.0004) in the first year of the trial (Fig. 4C), despite the SP3 treatment positively affecting the mean bean weight compared to the CTRL sample and other treatments (Fig. 4B–D). In the second year, MC EXTRA had significantly higher values for both whole pod (1.781 g, *p*-value, 0.0410) (Fig. 4E and F) and shelled pod (1.331 g, *p*-value, 0.008) (Fig. 4G and H), relative to CTRL (1.664 g and 1.223 g for whole and shelled pod, respectively). In contrast to the first year of the trial, the average whole pod weight of SP3 (1.56 g; *p*-value, 0.022) was significantly lower than that of CTRL (1.664 g). Results from the greenhouse experiment show yield differences in both years when treated samples were compared to CTRL. The data from the first year clearly indicate that the mean shelled pod weight was statistically higher only when comparing MC EXTRA (1.053 g; *p*-value, 0.0053) with CTRL (0.866 g) (Fig. S6). Differences are even more pronounced in the second-year data (Fig. S7). All treated sample results for mean whole pod weight were significantly higher relative to CTRL (0.682 g), with the highest values reported for SP3 (0.890 g; *p*-value, 0.0083), followed by MC EXTRA (0.859 g; *p*-value, 0.0124) and SP1 (0.849 g; *p*-value, 0.0367). Lastly, SP1 (0.740 g; *p*-value, 0.0002) and MC EXTRA (0.661 g; *p*-value, 0.0104) mean shelled pod weights were significantly higher than CTRL (0.517 g).

3.4. *Amino acid content of bean flour*

The data presented in Figs. 5 and 6 show the amino acid profiles of bean flour prepared from dried beans of CTRL and biostimulant treated field-grown plants. Data were classified based on R-group properties. The nonpolar aliphatic amino acid group (Fig. 5A–F) showed statistically significant differences only with regard to methionine. Alanine (Fig. 5A) is predominantly found in the CTRL group, with lower concentrations in SP3, while SP1 and MC EXTRA showed values equal to SP3. Glycine levels (Fig. 5B) show an increase in MC EXTRA relative to CTRL, indicating a slight response to the biostimulant treatment. Interestingly, isoleucine levels (Fig. 5C) were lower in all biostimulant treated plants relative to CTRL. Leucine content was slightly increased in the treated plants (Fig. 5D), but in both cases, differences were not

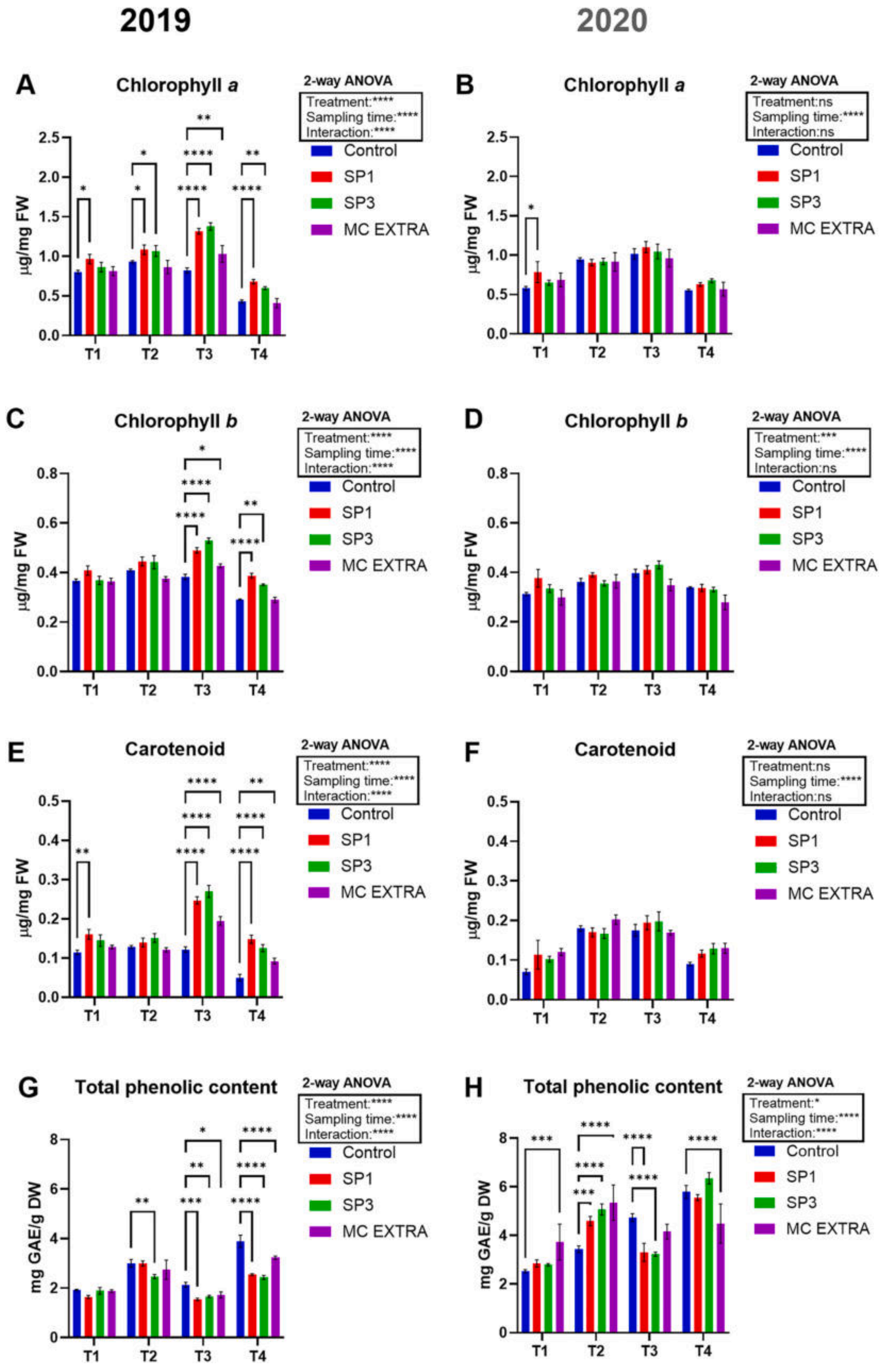


Fig. 3. Pigments (Chl *a*, *b* and carotenoids) and total polyphenolics data collected in two consecutive years (2019, 2020) from fagiolo di Sorana plants under biostimulant application in open field conditions. Data are the mean values ± S.E.M, consisting of one leaf from six plants ($n = 6$) at four sampling times within each year of analysis. 2-way ANOVA analyses (factors: treatment, sampling time) coupled with Dunnett's Post-hoc tests (p-value <0.05, reference sample, Control) were performed. **** <0.0001 *** < 0.001 ** <0.01 * < 0.05. First year data, panels A, C, E, G; second year data, panels B, D, F, H.

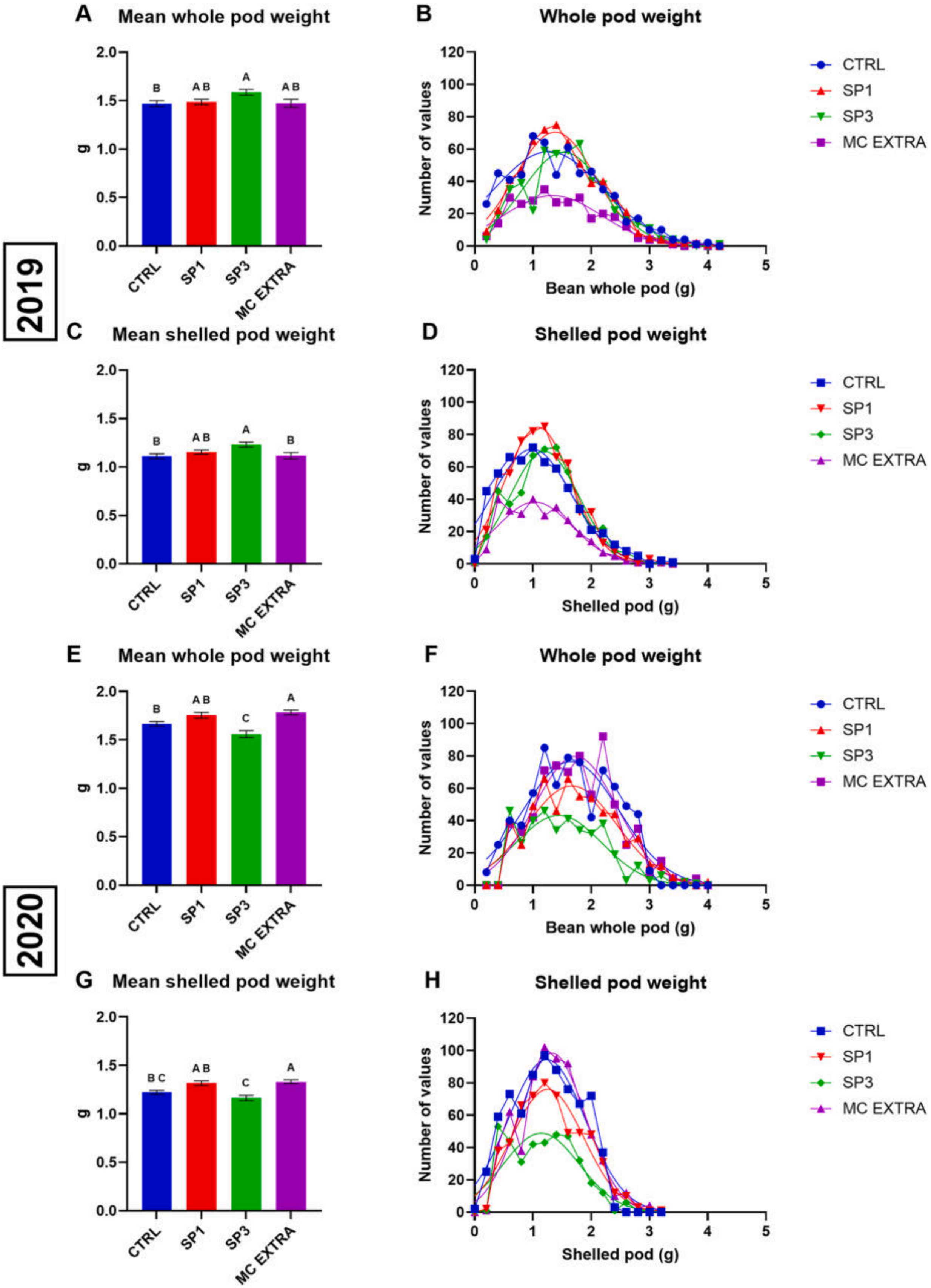


Fig. 4. Whole and shelled pod weight distribution data from the first (2019) and the second (2020) year of analyses of fagiolo di Sorana plants grown under open field conditions. (A, E) Whole pod distribution data of collected beans; (B, F) Mean whole pod weight data; (C, G) Shelled pod distribution data of collected beans; (D, H) Mean shelled pod weight data Leaf data. Statistical analyses of mean values (B, D, F, H) were performed according to one-way ANOVA coupled with Dunn's test (*p*-value <0.05). Values are mean ± S.E.M. (n = 500, minimum). **** <0.0001 *** < 0.001 ** <0.01 * < 0.05.

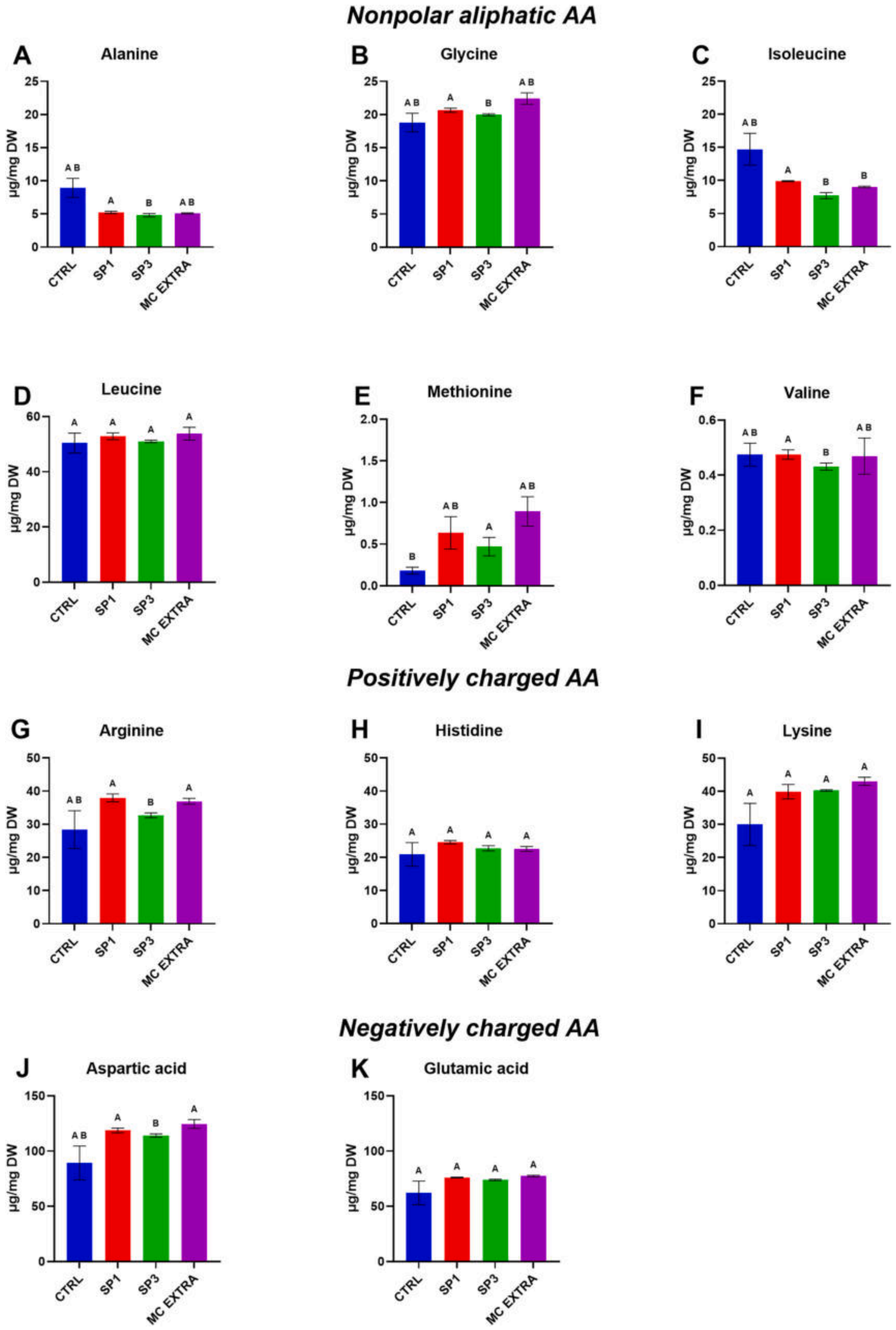


Fig. 5. Amino acid contents of beans collected in two years (2019, 2020) of fagiolo di Sorana plants grown under open field conditions. Data were grouped based on amino acid properties of side chains (R group). (A–F) Nonpolar aliphatic AA; (G–I) Positively charged AA; (J–K) Negatively charged AA. Statistical analyses were performed according to one-way ANOVA coupled with Tukey HSD test (p-value <0.05). Values are mean ± S.E.M. (n = 6). **** <0.0001 *** < 0.001 ** <0.01 * < 0.05.

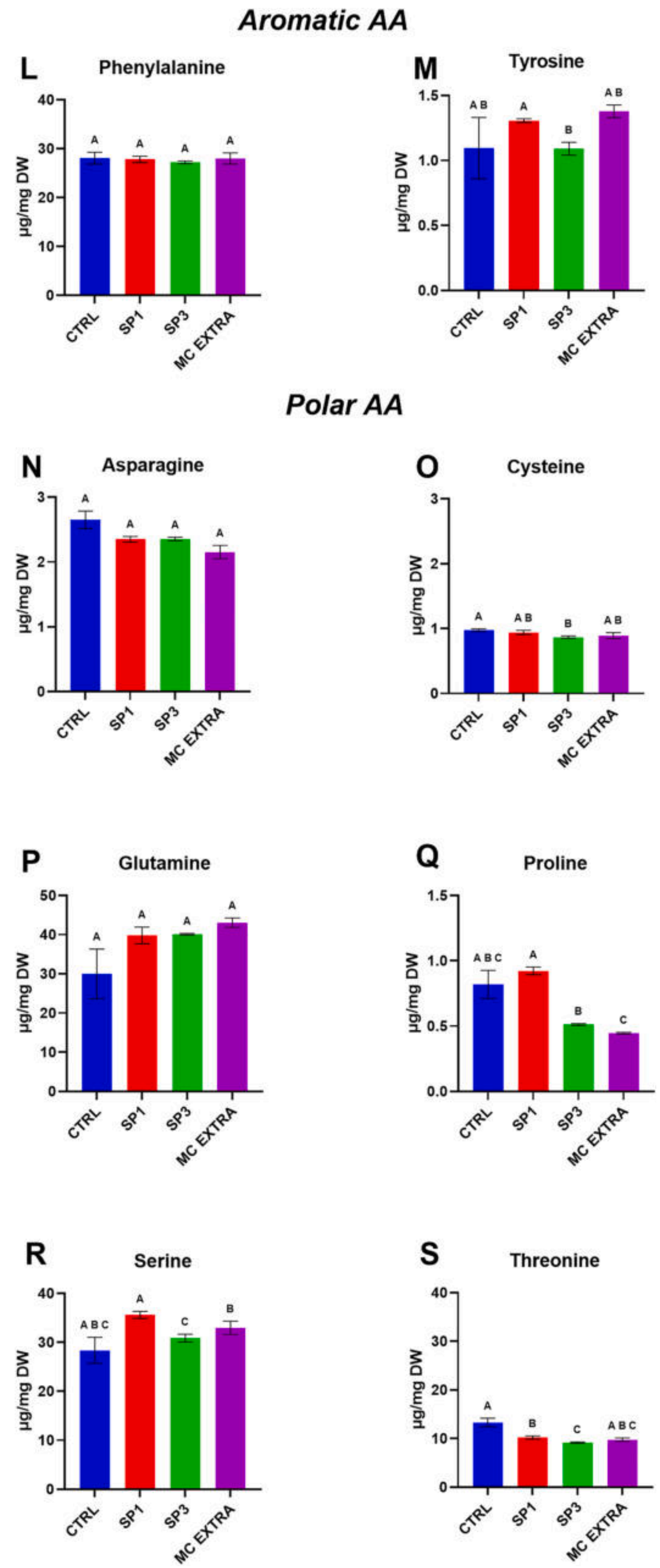


Fig. 6. Amino acid contents of beans collected in two years (2019, 2020) of fagiolo di Sorana plants grown under open field conditions. Data were grouped based on amino acid properties of side chains (R group). (L–M) Aromatic AA; (N–S) Polar AA. Statistical analyses were performed according to one-way ANOVA coupled with Tukey HSD test (*p*-value <0.05). Values are mean ± S.E.M. (n = 6). **** <0.0001 *** < 0.001 ** <0.01 * < 0.05.

significant. Methionine content is significantly affected by biostimulant application (Fig. 5E), with a general increase in treated samples compared to CTRL (0.181), reaching the highest values in MC EXTRA (0.892 μg/mg; *p*-value, 0.053), followed by SP1 (0.635 μg/mg; *p*-value, 0.092) and SP3 (0.470 μg/mg; *p*-value, 0.040). Conversely, the valine content in treated samples appears to be generally lower when compared to CTRL (Fig. 5F). Among the positively charged amino acid (AA) group (Fig. 5G–I), no significant differences were reported, despite a general increase in their contents in treated samples *vs* CTRL. The same trend was reported for the negatively charged AA (Fig. 5J and K) and aromatic AA groups (Fig. 6L and M), where biostimulant treatments did not significantly alter the aspartic and glutamic acid contents, or phenylalanine and tyrosine, respectively. Lastly, the polar AA group (Fig. 6N–S) showed a decrease in both asparagine and cysteine content in response to biostimulant applications (Fig. 6N and O), with cysteine levels significantly lower in SP3 plants (*p*-value, 0.0128). In contrast, no significant differences were reported for glutamine, where a general increase was observed relative to CTRL (Fig. 6P). An increase in proline content (Fig. 6Q) was found in SP1 plants, but a decrease with the SP3 (SP1 *vs* SP3, *p*-value, 0.0003) and MC EXTRA (SP1 *vs* MC EXTRA, *p*-value, 0.0001) treatments. Serine levels (Fig. 6R) are higher in all three treatment groups, highlighting a potentially noteworthy effect of these treatments on serine metabolism. In contrast, the threonine content (Fig. 6S) slightly decreased due to the treatments. Notably, this amino acid content decreased significantly in SP samples (SP1: 10.21 μg/mg; *p*-value 0.042; SP3: 9.17 μg/mg; *p*-value 0.025) compared to CTRL (13.30 μg/mg) (Fig. 6S).

The AA data were further analyzed using a PCA (principal component analysis) for both experimental years (Fig. 7), and their results were graphically processed to highlight the contribution of each variable (amino acid type) in the sample differentiation. The PCA distribution of samples (Fig. 7A) is shown along two primary axes, PC1 (which accounts for 55.21 % of the variance) and PC2 (19.34 %), thus describing 74.55 % of the total variance of the dataset. The CTRL and MC EXTRA groups display greater data dispersion, indicating higher variability within those treatments in the two years of analyses. In contrast, the SP1 group is tightly clustered, suggesting lower variability among its replicates, while SP3 shows moderate clustering. The distinct separation of the groups along PC1 and PC2 suggests differences in amino acid profiles. Specifically, the MC EXTRA and SP1 groups stand out as being the most different from the CTRL, thus implying that these treatments may significantly alter amino acid composition. On the other hand, SP3 overlaps more with the CTRL group, indicating less pronounced differences in amino acid levels. Data related to the correlation circle (Fig. 7B) describe how individual amino acids and metabolites contribute to sample distribution. Amino acids like alanine, threonine, proline, and histidine contribute strongly to positive values along PC1, suggesting they are present at higher concentrations in certain groups, likely CTRL. In contrast, methionine exhibits a strong negative association with PC1, implying that it is more abundant in the groups on the left side of the plot, MC EXTRA and SP3. Glycine and serine also strongly contribute to PC1, suggesting that they had higher levels in the SP1 and SP3 groups. Meanwhile, valine is positively correlated with PC2, while methionine is negatively associated. Other amino acids, including arginine, phenylalanine, and leucine, are more moderately distributed between PC1 and PC2, playing a less distinct role in grouping samples.

The PCA highlights clear differences in amino acid profiles between the experimental groups. MC EXTRA and SP3, in particular, are distinct from the CTRL, thus indicating that these treatments significantly affect amino acid metabolism. SP1, on the other hand, appears closer to the CTRL group in the multidimensional space of PCA. The analysis effectively underscores how different treatments influence specific amino acid contents, such as methionine, alanine, proline, and threonine.

3.5. *Digestibility data*

Samples from open-field experiments were analyzed to assess the digestibility of the total dry mass and determine how the treatment influenced bean organoleptic properties (Fig. 8A, left side). Furthermore, data were integrated by analyzing 11 Italian bean landraces (Fig. 8A, right side), including Sorana. Results reported in Fig. 6A indicate a slight increase in dry matter digestibility in SP3 (68.65 %; *p*-value 0.0131) compared to CTRL (65.68 %), while a lower increase is reported for SP1 (66.63 %) samples, even if not significant. Bean digestibility in MC EXTRA (64.79 %) treated plants was slightly lower compared to that of CTRL (65.68 %). By comparing dry matter digestibility data with data related to landrace collection listed in Table 1 (Fig. 8A, right side), it was observed that the Fagiolo di Sorana ecotype showed one of the lowest values (65.68 %), with the Lupinaro di Lucca representing the only landrace with a lower dry matter digestibility value (57.66 %). The two landraces with higher digestibility (Fagiolo dell'Occhio del Valdarno and Cannellino di Sorano, 88.70 % and 91.71 %, respectively) were 20 % more digestible than the Sorana bean.

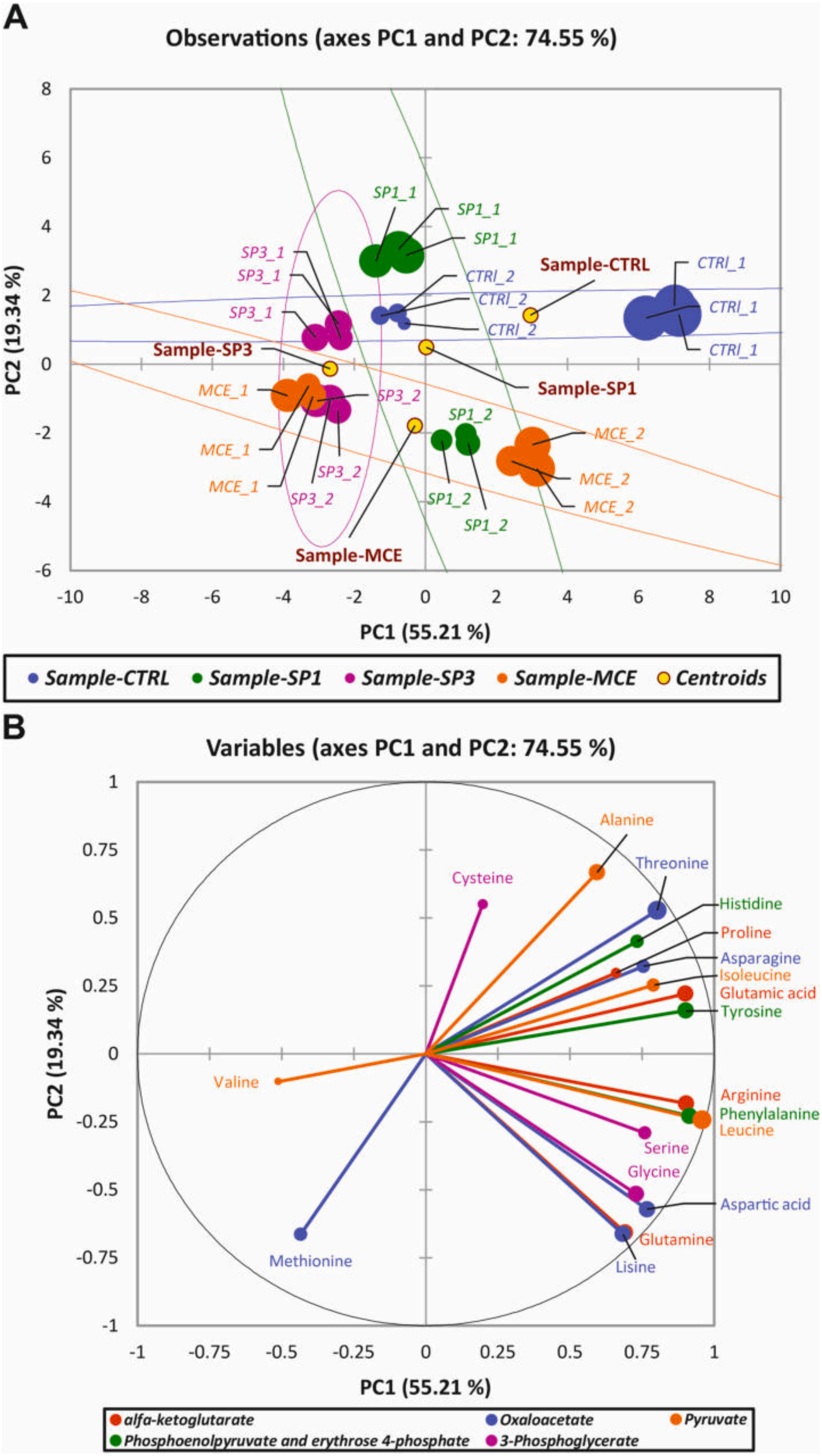
3.6. *Phylogenetic data*

The evolutionary history of the landraces collection was inferred using the MEGA11 software to build a phylogenetic tree. The concatenated sequence information from three genes analyses (*ITS*, *trnL-trnF* and the *trnH-psbA)* was used. The tree was made using the Maximum Likelihood method and the Tamura-Nei model. The tree with the highest log likelihood (−2382.25) is shown in Fig. 8B, with the Sorana sample highlighted in red. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were automatically obtained by applying Neighbor-Join and BioNJ algorithms to a matrix of estimated pairwise distances using the Tamura-Nei model, and then the topology with superior log likelihood value was selected. This landrace analysis involved 11 nucleotide sequences, with a total of 1312 positions in the final dataset (three concatenated genes). Branch lengths and bootstrap values for each node are reported. Fig. 8B shows that the only sample that clusters alone is represented by Fagiolo dall'Occhio del Valdarno, which belongs to a different species (*Vigna unguiculata*) rather than other landraces. By observing bootstrap data, the higher values are reported in two nodes (84, 93), which clustered most of the samples. In contrast, Fagiolo di Sorana did not cluster with other samples despite the value of its related node appearing to be lower (47). Also, Zolfino's placement in the tree indicates that it represents the closest sample to Fagiolo di Sorana.

4. Discussion

The use of biostimulants in agriculture has garnered significant attention due to their multifaceted benefits. Here the effects of two different biostimulants were tested on a *P. vulgaris* landrace Fagiolo di Sorana grown under its specific edaphic conditions. Though both stimulants (spirulina, a cyanobacteria and MC EXTRA, a brown algae extract) were expected to increase growth and productivity, the spirulina preparation was hypothesized to have a positive effect specifically on amino acid content and digestibility of seeds. Yield did increase with all treaments (SP1, SP3 and MC EXTRA) as did digestibility and concentrations of the amino acid methionine. Data from individual morphological and biochemical analyses point to specific targets of these two biostimulants, while the molecular barcoding data provide context for the physiological and organoleptic traits within the group of Tuscan bean landraces.

Although our results do not indicate strong differences in gas exchange parameters under either experimental condition (i.e., open field or greenhouse), an increase in yield and mean bean size was observed following the biostimulant application. These data could be explained in several ways, including the fact that the trial was conducted under the



(caption on next page)

Fig. 7. Principal component analysis (PCA) of amino acid contents identified in bean flour of fagiolo di Sorana plants grown under open field conditions using reference standard compounds. (**A**) According to results from multifactorial analysis, the observation factor map linked to the sample distribution. (**B**) Variable factor map related to the contribution of each polyphenol compound in the sample distribution. The different experimental groups are color-coded, with CTRL represented in blue, SP1 in green, SP3 in pink, and MC EXTRA in orange. The length of the vectors is correlated to their significance. The angle α formed between two vectors, or between a vector and an axis, indicates a positive correlation for $0 \leq \alpha < 90^\circ$ (r close to 1), a negative correlation for $90^\circ < \alpha \leq 180^\circ$ (r close to −1), and no linear dependence for $\alpha = 90^\circ$ (r close to 0). PC1, first dimension; PC2, second dimension.

specific edaphic conditions to which this ecotype is adapted. This ecotype is typically grown in soil rich in sand and gravel but lacking in nutrients such as calcium, nitrate and other minerals (Verreschi, 1994). Specific conditions include a high level of precipitation, leading to a unique microclimate. Results do indicate significant differences in photosynthetic performance during the first year of analysis, mainly in SP3 during the intermediate life cycle stage. However, no significant differences were observed during the second year. These findings were further supported by pigment analyses (Chl *a*, *b* and carotenoid), which showed increased levels of pigment content in the control group plants mainly during the first year. Discrepancies between the data from the two years could be attributed to the varying climatic and pedoclimatic conditions in the Sorana region during the two consecutive years of experiments, which may have influenced plant responses.

Environmental factors such as temperature and precipitation can significantly influence plant responses to biostimulants. In one study, variations in weather conditions during the growing season affected the biometric traits and nutritional properties of soybean cultivars treated with biostimulants, indicating that the effectiveness of such treatments can vary from year to year based on climatic factors (A. Kocira et al., 2018). This suggests that while spirulina-based biostimulants can improve growth and nutritional quality, their efficacy may be influenced by the prevailing climate conditions during cultivation. The effect of biostimulant application on plant metabolism may also be observed in the total polyphenolic content of leaves. Variability was found again between the two years of analysis, with higher values reported in the first year and increasing values recorded in treated plants (MC EXTRA in T2 and SP3 in T3) in the second year. A recent study found that spirulina biomass includes a range of phenolic compounds, which may contribute to the overall polyphenolic profile of the leaves of plants cultivated with spirulina supplementation (Papalia et al., 2019). Additionally, Al-Dhabi and Arasu (2016) highlighted that spirulina products are rich in polyphenols, which can enhance the antioxidant capacity of plant tissues.

The data on amino acid composition shows the alteration occurring in plants due to biostimulant application. The nonpolar aliphatic group indicates that glycine and methionine increased due to the treatment, whereas isoleucine decreased. The increase in methionine content is crucial because though common beans are rich in lysine (Añazco, 2023), but they are limited in sulfur amino acids, including methionine and cysteine (Saboori-Robat et al., 2019) This deficiency can impact the overall protein quality of bean-based diets, especially in populations that do not consume complementary protein sources rich in methionine, such as grains (Nosworthy et al., 2017). Surprisingly, no significant differences were reported for either charged AA groups, although a slight increase was observed for all the amino acids, including arginine, lysine, and aspartic acid. The aromatic amino acid group showed no significant difference, with consistent levels of phenylalanine across treatments and a slight increase in tyrosine content, particularly in SP1 and MC EXTRA. Lysine and aspartic acid are two important amino acids that play critical roles in the nutritional profile *P. vulgaris*. Lysine is particularly important as it is often the limiting amino acid in many plant-based diets, especially those that rely heavily on cereals, which usually lack sufficient lysine content (Añazco, 2023). Common beans are a rich source of lysine, making them a valuable protein source for vegetarians and vegans (Celmeli et al., 2018). While classified as a non-essential amino acid, aspartic acid is also abundant in common beans and plays a vital role in various metabolic processes, including synthesising other amino acids and neurotransmitters (Vronska and Demyd, 2019). These amino acids improve the overall protein quality of common beans, making them essential for muscle repair, growth, and metabolic health (Añazco, 2023). Lastly, the polar amino acid group, representing one of the most significant, showed a mixed trend. While asparagine, cysteine, and threonine contents decreased in treated samples compared to CTRL, glutamine and serine levels increased. These differences in amino acid compositions are linked to biostimulant treatment, thus affecting protein properties. For instance, Kocira (2019) demonstrated that biostimulant treatments in common beans resulted in reduced concentrations of asparagine and threonine, alongside enhanced levels of glutamine and proline, suggesting a selective modulation of the amino acid profile that could enhance nutritional quality (Yao et al., 2015). Similarly, Gharib and Ahmed (2023) reported that the foliar application of *S. platensis* led to decreased cysteine levels in rosemary plants while increasing glutamine and proline concentrations, indicating the potential of spirulina to positively influence the amino acid composition (Rosa-Sibakov et al., 2016). Furthermore, Abbas et al. (2022) found that biostimulants significantly reduced asparagine levels in faba beans, while glutamine and proline levels were notably increased, underscoring the ability of biostimulants to enhance the nutritional profile of legumes through targeted modulation of amino acid levels (Souza et al., 2020). The multivariate analysis (PCA) indicates that samples can be separated based on treatments and analysis year. Spirulina is recognized for its high protein content and rich amino acid profile, which includes essential amino acids such as lysine and methionine (Marjanović et al., 2024). In fact, the direct incorporation of spirulina into food products, such as gluten-free bread, has demonstrated a significant increase in protein content and essential amino acids. This indicates that spirulina can effectively augment the nutritional profile of common beans when used in food formulations (Figueira et al., 2011), resulting in higher levels of threonine, methionine, isoleucine and leucine. Conversely, our biostimulant applications (spirulina and MC EXTRA) through foliar applications only led to an increase in methionine, with a decrease in the aforementioned amino acids. Taken together, the application of spirulina during pre-and post-production of beans (*i.e.* during plant growth as a biostimulant and in food formulations, respectively) could result in significant increases of amino acids that would otherwise be lacking in conventionally grown beans.

The biostimulant treatment also seems to affect the protein content of beans, as shown by digestibility data, with SP3 emerging as the most effective treatment. Sorana beans, when compared to other tested landraces, exhibited lower digestibility. Studies have shown that there is significant variability in the nutritional composition among bean landraces, which can make them easier or harder to digest. For instance, Bosmali et al. (2023) suggest that the nutritional content of bean landraces is affected by microclimate conditions and genetic factors, as there are variations in nutritional components that may affect protein digestibility, such as trypsin inhibitors. This data suggests that genetic factors like landrace pedigree and the environmental conditions of cultivation areas can influence dry matter digestibility. Moreover, differences in landrace protein content, with protein content values ranging from 16.6 % to 26.2 %, may lead to potential differences in dry matter digestibility (Celmeli et al., 2018).

A recent study highlights the genetic diversity and population structure of common bean landraces in the Lazio region of Italy, using molecular markers to differentiate between various landraces based on their phaseolin patterns and growth habits (Catarcione et al., 2023).

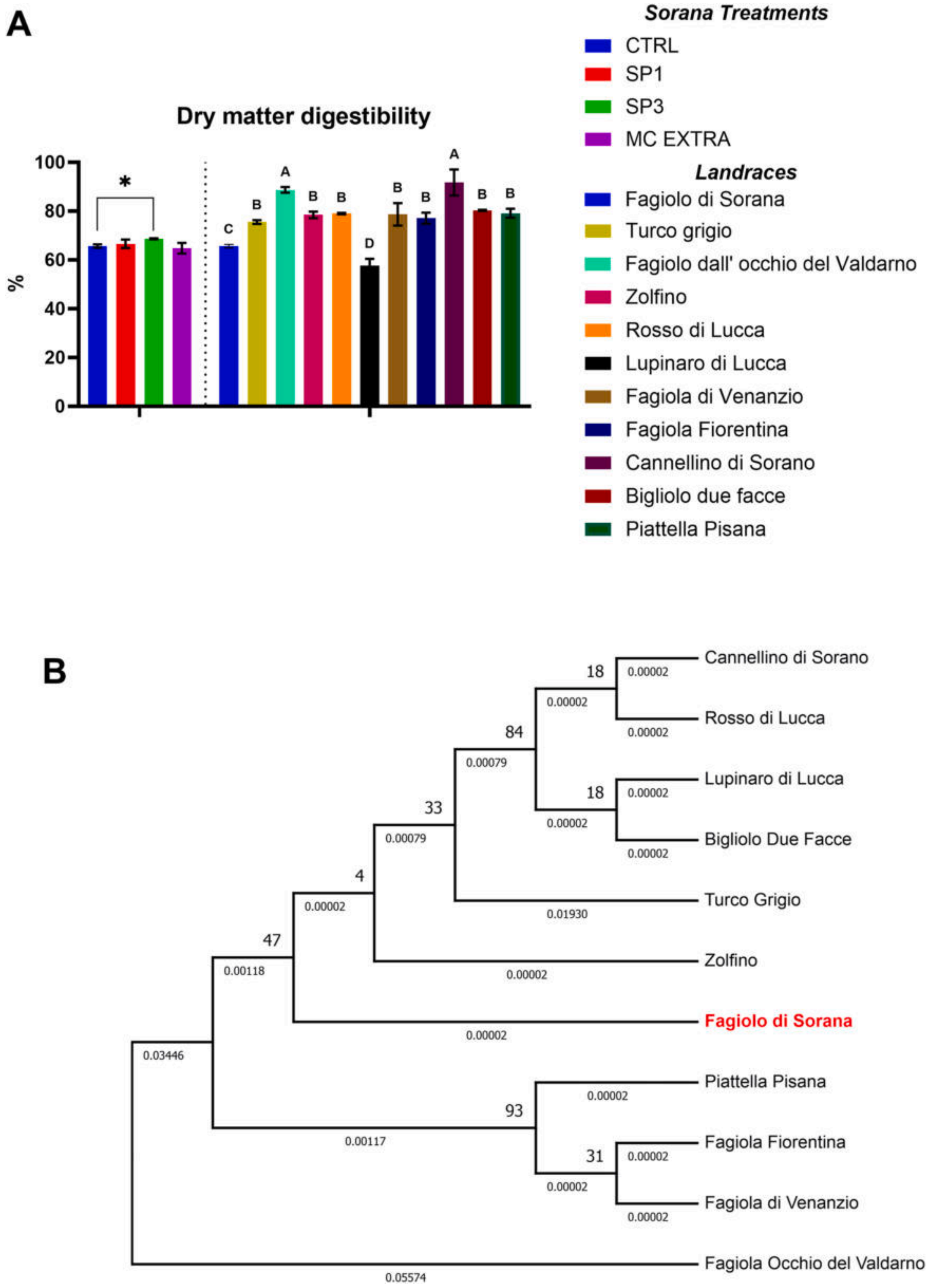


Fig. 8. Dry matter digestibility and phylogenetic tree data. Results are related to samples from the open field experiment of fagiolo di Sorana and landrace collection (A) and phylogenetic tree based on concatenated gene sequencing (*trnL*, *trnH*, *ITS*) on a bean landrace collection (B). Statistical analyses of Sorana and landrace samples were performed according to one-way ANOVA coupled with Dunnett's test (p-value <0.05) within treatment groups (CTRL as reference, left side, panel A) and Tukey HSD test (p-value <0.05) for landrace analyses (Sorana bean as reference, right side, panel A). Values are mean ± S.E.M. (n = 6). **** <0.0001 *** < 0.001 ** <0.01 * < 0.05. For phylogenetic tree analyses, the evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model (Tamura and Nei, 1993). The estimated branch lengths (the distance between nodes or tips in a phylogenetic tree) and frequencies are reported in the tree figure.

This approach not only facilitates the identification of distinct genetic groups but also aids in resolving instances of homonymy and synonymy among landraces, thereby enriching the understanding of their genetic relationships. Our phylogenetic analysis confirms Fagiolo di Sorana's peculiarity, providing an explanation for its amino acid composition and growth trends. Distinctive features were, in fact, identified through molecular markers, representing a step forward in deciphering landrace relationships. Digestibility data indicate that significant differences occur among landraces. Integrating genetic markers with morphological traits further strengthens the analysis, leading to a comprehensive understanding of the genetic landscape of landraces.

5. Conclusion

The present study demonstrates that the application of algae-based biostimulants to the Tuscan bean landrace Fagiolo di Sorana increases bean methionine concentrations and digestibility when grown under its restricted PDO protocol. This landrace, known for its highly prized product, was tested under open-field and greenhouse conditions over two consecutive years of experimentation. Results from the multidisciplinary approach indicate that treatments applied (the commercial product MC EXTRA and the extract obtained from *A. platensis* F&M-C256) affected the yield and amino acid profile of the pulses, especially methionine, thus indicating that foliar applications may have an important nutritional effect. An increase in amino acids such as methionine through the application of biostimulants would have dramatic impacts on the nutritional properties of a staple crop, and increase the market and cultural value of PDO lines like Fagiolo di Sorana. The increase in methionine content is particularly interesting due to its limited content in non-treated samples. Additionally, the barcoding analyses identified specific features that distinguish Fagiolo di Sorana within a Tuscan landrace collection, providing new insights into its genetic relationships. In conclusion, the present results indicate that biostimulants could represent an important alternative to traditional manure, leading to improved quality and nutritional properties of products from organic agriculture.

CRediT authorship contribution statement

Emily Rose Palm: Writing – review & editing, Writing – original draft, Investigation, Mario R. Tredici, Resources, Conceptualization. **Gaia Santini:** Writing – review & editing, Investigation, Formal analysis. **Alberto Niccolai:** Writing – review & editing, Investigation, Formal analysis. **Marzia Vergine:** Writing – review & editing, Writing – original draft, Formal analysis. **Carmine Negro:** Writing – review & editing, Formal analysis. **Werther Guidi Nissim:** Writing – review & editing, Validation, Investigation, Formal analysis. **Leonardo Sabbatini:** Methodology, Formal analysis, Data curation. **Raffaella Balestrini:** Writing – review & editing, Writing – original draft, Validation, Supervision. **Maria Concetta de Pinto:** Writing – review & editing, Visualization. **Gholamreza Gohari:** Writing – review & editing, Writing – original draft, Validation. **Vasileios Fotopoulos:** Writing – review & editing, Writing – original draft, Supervision. **Stefano Mancuso:** Writing – review & editing, Supervision, Project administration. **Andrea Luvisi:** Writing – review & editing, Visualization, Resources. **Luigi De Bellis:** Writing – review & editing, Validation, Funding acquisition. **Liliana Rodolfi:** Writing – review & editing, Resources, Data curation. **Federico Vita:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Investigation, Conceptualization.

Funding

This study was supported with funds from: i) Fondazione Caripit, Grant/Award Number: 2018.0527 to FV and SM; ii) PRIN 2022 funded by the European Union-Next-Generation EU, CUP, H53D23003320001, grant number 2022RYTHE3 to FV.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Editorial Board menbers: Gholamreza Gohari and Vasileios Fotopoulos. Given their role as editorial board members, they had no involvement in the peer review of this article and had no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to another journal editor. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We extend our heartfelt thanks to Roberto Dongacci, chairman of the association “Il Ghiareto Onlus”, for his invaluable support and insightful guidance regarding bean cultivation in Sorana (Province of Pistoia), Italy.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2025.110988.

Data availability

Data will be made available on request.

References

Abbas, M.S., Badawy, R.A., Abdel-Lattif, H., El-Shabrawi, H.M., 2022. Synergistic effect of organic amendments and biostimulants on faba bean grown under sandy soil conditions. Sci. Agric. 79. https://doi.org/10.1590/1678-992x-2020-0300.

Adedeji, A.A., Joseph, M., Plattner, B., Alavi, S., 2016. Physicochemical and functional properties of extruded sorghum-based bean analog. J. Food Process. Eng. 40. https://doi.org/10.1111/jfpe.12401.

Adoko, M.Y., Sina, H., Amogou, O., Agbodjato, N.A., Noumavo, P.A., Aguégué, R.M., Assogba, S.A., Adjovi, N.A., Dagbénonbakin, G.D., Adjanohoun, A., Baba-Moussa, L., 2021. Potential of biostimulants based on PGPR rhizobacteria native to Benin's soils on the growth and yield of maize (*Zea mays* L.) under greenhouse conditions. Open J. Soil Sci. https://doi.org/10.4236/ojss.2021.113010.

Ainsworth, E.A., Gillespie, K.M., 2007. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. Nat. Protoc. 2, 875–877.

Al-Dhabi, N.A., Arasu, M.V., 2016. Quantification of phytochemicals from commercial *Spirulina* products and their antioxidant activities. Evid.-Based Complement. Altern. Med. https://doi.org/10.1155/2016/7631864, 2016.

Allen, G.C., Flores-Vergara, M.A., Krasynanski, S., Kumar, S., Thompson, W.F., 2006. A modified protocol for rapid DNA isolation from plant tissues using cetyltrimethylammonium bromide. Nat. Protoc. 1, 2320–2325. https://doi.org/10.1038/nprot.2006.384.

Añazco, C., 2023. Common beans as a source of amino acids and cofactors for collagen biosynthesis. Nutrients 15, 4561. https://doi.org/10.3390/nu15214561.

Barone, A.M., Grappi, S., Romani, S., 2019. “The road to food waste is paved with good intentions”: when consumers' goals inhibit the minimization of household food waste. Resour. Conserv. Recycl. 149, 97–105. https://doi.org/10.1016/j.resconrec.2019.05.037.

Baronetti, A., Dubreuil, V., Provenzale, A., Fratianni, S., 2022. Future droughts in northern Italy: high-resolution projections using EURO-CORDEX and MED-CORDEX ensembles. Clim. Change 172, 22.

Bartlett, M.S., 1937. Properties of sufficiency and statistical tests. Proc. R. Soc. London. Ser. A-Mathematical Phys. Sci. 160, 268–282.

Bauenova, M.O., Sarsekeyeva, F.K., Sadvakasova, A.K., Kossalbayev, B.D., Mammadov, R., Token, A.I., Balouch, H., Pashkovskiy, P., Leong, Y.K., Chang, J.-S., Allakhverdiev, S.I., 2024. Assessing the Efficacy of Cyanobacterial Strains as *Oryza sativa* Growth Biostimulants in Saline Environments. Plants 13, 2504. https://doi.org/10.3390/plants13172504.

Boisen, S., Fernández, J.A., 1997. Prediction of the total tract digestibility of energy in feedstuffs and pig diets by in vitro analyses. Anim. Feed Sci. Technol. 68, 277–286.

Bosmali, I., Giannenas, I., Christophoridou, S., Ganos, C., Papadopoulos, A., Papathanasiou, F., Kolonas, A., Gortzi, O., 2023. Microclimate and genotype impact on nutritional and antinutritional quality of locally adapted landraces of common bean (*Phaseolus vulgaris* L.). Foods 12, 1119. https://doi.org/10.3390/foods12061119.

Catarcione, G., Paolacci, A.R., Alicandri, E., Gramiccia, E., Taviani, P., Rea, R., Costanza, M.T., Lorenzis, G.D., Puccio, G., Mercati, F., Ciaffi, M., 2023. Genetic diversity and population structure of common bean (*Phaseolus vulgaris* L.) landraces in the Lazio region of Italy. Plants 12, 744. https://doi.org/10.3390/plants12040744.

Celmeli, T., Sari, H., Canci, H., Sari, D., Adak, A., Eker, T., Toker, C., 2018. The nutritional content of common bean (*Phaseolus vulgaris* L.) landraces in comparison to modern varieties. Agronomy 8, 166.

Colla, G., Cardarelli, M., Bonini, P., Rouphael, Y., 2017. Foliar applications of protein hydrolysate, plant and seaweed extracts increase yield but differentially modulate fruit quality of Greenhouse tomato. Hortscience 52, 1214–1220. https://doi.org/10.21273/hortsci12200-17.

Critto, A., Torresan, S., Ronco, P., Zennaro, F., Santini, M., Trabucco, A., Marcomini, A., 2016. Assessing hydrological drought risk for the irrigation sector in future climate scenarios: lessons learned from the Apulia case study (Italy). In: EGU General Assembly Conference Abstracts. EPSC2016–7813.

Dahl-Lassen, R., van Hecke, J., Jørgensen, H., Bukh, C., Andersen, B., Schjoerring, J.K., 2018. High-throughput analysis of amino acids in plant materials by single quadrupole mass spectrometry. Plant Methods 14, 1–9. https://doi.org/10.1186/s13007-018-0277-8.

Dinelli, G., Bonetti, A., Minelli, M., Marotti, I., Catizone, P., Mazzanti, A., 2006. Content of flavonols in Italian bean (*Phaseolus vulgaris* L.) ecotypes. Food Chem. 99, 105–114. https://doi.org/10.1016/j.foodchem.2005.07.028.

du Jardin, P., 2015. Plant biostimulants: definition, concept, main categories and regulation. Sci. Hortic. (Amsterdam) 196, 3–14. https://doi.org/10.1016/j.scienta.2015.09.021.

Dunn, O.J., 1964. Multiple comparisons using rank sums. Technometrics 6, 241–252.

Dunnett, C.W., 1955. A multiple comparison procedure for comparing several treatments with a control. J. Am. Stat. Assoc. 50, 1096–1121.

Fedeli, R., Vannini, A., Celletti, S., Maresca, V., Munzi, S., Cruz, C., Alexandrov, D., Guarnieri, M., Loppi, S., 2023. Foliar application of wood distillate boosts plant yield and nutritional parameters of chickpea. Ann. Appl. Biol. 182, 57–64.

Figueira, F.d.S., Crizel, T.d.M., Silva, C.R., Salas-Mellado, M.d.l.M., 2011. Pão sem glúten enriquecido com a microalga *Spirulina platensis*. Brazilian J. Food Technol. 14, 308–316. https://doi.org/10.4260/bjft2011140400037.

Francesca, S., Barone, A., Rigano, M.M., 2021. One plant-based biostimulant stimulates good performances of tomato plants grown in open field. https://doi.org/10.3390/iecag2021-09703.

Gerhards, R., Ouidoh, F.N., Adjogboto, A., Avohou, V.A.P., Dossounon, B.L.S., Adisso, A. K.D., Heyn, A., Messelhäuser, M., Santel, H., Oebel, H., 2021. Crop response to leaf and seed applications of the biostimulant ComCat® under stress conditions. Agronomy 11, 1161. https://doi.org/10.3390/agronomy11061161.

Gharib, F.A.E.L., Ahmed, E.Z., 2023. *Spirulina Platensis* improves growth, oil content, and antioxidant activitiy of rosemary plant under cadmium and lead stress. Sci. Rep. 13. https://doi.org/10.1038/s41598-023-35063-1.

Godlewska, K., Michalak, I., Pacyga, P., Baśladyńska, S., Chojnacka, K., 2019. Potential applications of Cyanobacteria: *spirulina platensis* filtrates and homogenates in agriculture. World J. Microbiol. Biotechnol. 35. https://doi.org/10.1007/s11274-019-2653-6.

Graham, P.H., Ranalli, P., 1997. Common bean (*Phaseolus vulgaris* L.). F. Crop. Res. 53, 131–146. https://doi.org/10.1016/S0378-4290(97)00112-3.

Halpern, M., Bar-Tal, A., Ofek, M., Minz, D., Muller, T., Yermiyahu, U., 2015. The use of biostimulants for enhancing nutrient uptake. Adv. Agron. https://doi.org/10.1016/bs.agron.2014.10.001.

Ibrahim, M.I.M., Ahmed, A.T., El-Dougdoug, K.A., El Nady, G.H., 2024. Harnessing Spirulina extract to enhance drought tolerance in wheat: a morphological, molecular genetic and molecular docking approach. Egypt. Acad. J. Biol. Sci. H. Bot. 15, 73–94.

Johnson, R., Joel, J.M., Puthur, J.T., 2024. Biostimulants: the futuristic sustainable approach for alleviating crop productivity and abiotic stress tolerance. J. Plant Growth Regul. 43, 659–674.

Kocira, A., Świeca, M., Kocira, S., Złotek, U., Jakubczyk, A., 2018. Enhancement of yield, nutritional and nutraceutical properties of two common bean cultivars following the application of seaweed extract (*Ecklonia Maxima*). Saudi J. Biol. Sci. 25, 563–571. https://doi.org/10.1016/j.sjbs.2016.01.039.

Kocira, S., 2019. Effect of amino acid biostimulant on the yield and nutraceutical potential of soybean. Chil. J. Agric. Res. 79, 17–25. https://doi.org/10.4067/s0718-58392019000100017.

Kocira, S., Kocira, A., Kornas, R., Koszel, M., Szmigielski, M., Krajewska, M., Szparaga, A., Krzysiak, Z., 2017. Effects of seaweed extract on yield and protein content of two common bean (*Phaseolus vulgaris* L.) cultivars. Legum. Res. - An Int. J. https://doi.org/10.18805/lr-383.

Kocira, S., Szparaga, A., Kocira, A., Czerwińska, E., Wójtowicz, A., Bronowicka-Mielniczuk, U., Koszel, M., Findura, P., 2018. Modeling biometric traits, yield and nutritional and antioxidant properties of seeds of three soybean cultivars through the application of biostimulant containing seaweed and amino acids. Front. Plant Sci. 9. https://doi.org/10.3389/fpls.2018.00388.

Kocira, S., Szparaga, A., Treder, K., Findura, P., Bartoš, P., Filip, M., 2020. Biochemical and economical effect of application biostimulants containing seaweed extracts and amino acids as an element of agroecological management of bean cultivation. Sci. Rep. 10. https://doi.org/10.1038/s41598-020-74959-0.

Koleška, I., Hasanagić, D., Todorović, V., Murtić, S., Klokić, I., Parađiković, N., Kukavica, B., 2017. Biostimulant prevents yield loss and reduces oxidative damage in tomato plants grown on reduced NPK nutrition. J. Plant Interact. 12, 209–218. https://doi.org/10.1080/17429145.2017.1319503.

Kruskal, W.H., Wallis, W.A., 1952. Use of ranks in one-criterion variance analysis. J. Am. Stat. Assoc. 47, 583–621.

Ma, Y., Freitas, H., Dias, M.C., 2022. Strategies and prospects for biostimulants to alleviate abiotic stress in plants. Front. Plant Sci. 13, 1024243.

Marjanović, B., Benković, M., Jurina, T., Sokač Cvetnić, T., Valinger, D., Gajdoš Kljusurić, J., Jurinjak Tušek, A., 2024. Bioactive compounds from *Spirulina* spp.—Nutritional value, extraction, and application in food industry. Separations 11, 257.

Mógor, Á.F., de Oliveira Amatussi, J., Mógor, G., de Lara, G.B., 2018. Bioactivity of cyanobacterial biomass related to amino acids induces growth and metabolic changes on seedlings and yield gains of organic red beet. Am. J. Plant Sci. 9, 966–978.

Niccolai, A., Zittelli, G.C., Rodolfi, L., Biondi, N., Tredici, M.R., 2019. Microalgae of interest as food source: biochemical composition and digestibility. Algal Res. 42, 101617.

Nicolè, S., Erickson, D.L., Ambrosi, D., Bellucci, E., Lucchin, M., Papa, R., Kress, W.J., Barcaccia, G., 2011. Biodiversity studies in phaseolus species by DNA barcoding. Genome 54, 529–545.

Nosworthy, M.G., Neufeld, J., Fröhlich, P., Young, G., Malcolmson, L., House, J.D., 2017. Determination of the protein quality of cooked Canadian pulses. Food Sci. Nutr. 5, 896–903. https://doi.org/10.1002/fsn3.473.

Nowak, R., Szczepanek, M., Błaszczyk, K., Kobus-Cisowska, J., Przybylska-Balcerek, A., Stuper-Szablewska, K., Pobereżny, J., Hassanpouraghdam, M.B., Rasouli, F., 2023. Impact of the farming system and amino-acid biostimulants on the content of carotenoids, fatty acids, and polyphenols in alternative and common barley genotypes. Agronomy 13, 1852. https://doi.org/10.3390/agronomy13071852.

Papalia, T., Sidari, R., Panuccio, M.R., 2019. Impact of different storage methods on bioactive compounds in *Arthrospira platensis* biomass. Molecules 24, 2810. https://doi.org/10.3390/molecules24152810.

Piergiovanni, A.R., Cerbino, D., Gatta, C.D., 2000. Gli agro-ecotipi di fagiolo (*Phaseolus vulgaris* L.) della Basilicata. Sementi elette 46, 25–30.

Piergiovanni, A.R., Lioi, L., 2010. Italian common bean landraces: history, genetic diversity and seed quality. Diversity 2, 837–862. https://doi.org/10.3390/d2060837.

Roche, D., 2024. Moving towards a mechanistic understanding of biostimulant impacts on soil properties and processes: a semi-systematic review. Front. Agron. 6. https://doi.org/10.3389/fagro.2024.1271672.

Rosa-Sibakov, N., Heiniö, R.L., Cassan, D., Holopainen-Mantila, U., Micard, V., Lantto, R., Sözer, N., 2016. Effect of bioprocessing and fractionation on the structural, textural and sensory properties of gluten-free faba bean pasta. LWT 67, 27–36. https://doi.org/10.1016/j.lwt.2015.11.032.

Rouphael, Y., Colla, G., 2020. Editorial: biostimulants in agriculture. Front. Plant Sci. 11, 1–7. https://doi.org/10.3389/fpls.2020.00040.

Saboori-Robat, E., Joshi, J., Pajak, A., Solouki, M., Mohsenpour, M., Renaud, J., Marsolais, F., 2019. Common bean (*Phaseolus vulgaris* L.) accumulates most S-methylcysteine as its $γ$-glutamyl dipeptide. Plants 8, 126.

Santini, G., Biondi, N., Rodolfi, L., Tredici, M.R., 2021. Plant biostimulants from cyanobacteria: an emerging strategy to improve yields and sustainability in agriculture. Plants 10, 643.

Selem, E., 2018. Physiological effects of Spirulina platensis in salt stressed *Vicia Faba*. L. Plants. Egypt. J. Bot. https://doi.org/10.21608/ejbo.2018.3836.1178, 0, 0.

Shawky, A.A., Khalifa, G.S.A., Hegazi, A.M., ElSherif, M., 2023. Growth, productivity, and essential oil content of fennel plants treated with *Spirulina platensis* extract and compost tea under low nitrogen doses. Gesunde Pflanz. 75, 2899–2908. https://doi.org/10.1007/s10343-023-00870-z.

Shukla, P.S., Mantin, E.G., Adil, M., Bajpai, S., Critchley, A.T., Prithiviraj, B., 2019. Ascophyllum nodosum-based biostimulants: sustainable applications in agriculture for the stimulation of plant growth, stress tolerance, and disease management. Front. Plant Sci. 10, 462648.

Singh, K., 2016. Microbial and enzyme activities of saline and sodic soils. L. Degrad. Dev. 27, 706–718. https://doi.org/10.1002/ldr.2385.

Skylas, D.J., 2024. Dry fractionation of Australian mungbean for sustainable production of value-added protein concentrate ingredients. Cereal Chem. 101, 720–738. https://doi.org/10.1002/cche.10774.

Skylas, D.J., Johnson, J.B., Kalitsis, J., Richard, S., Whiteway, C., Wesley, I.J., Naiker, M., Quail, K., 2022. Optimised dry processing of protein concentrates from Australian pulses: a comparative study of Faba bean, yellow pea and red Lentil seed material. Legum. Sci. 5. https://doi.org/10.1002/leg3.161.

Souza, E.J.D. de, Pereira, A.M., Fontana, M., Vanier, N.L., Gularte, M.A., 2020. Quality of gluten-free cookies made with rice flour of different levels of amylose and cowpea beans. Br. Food J. 123, 1810–1820. https://doi.org/10.1108/bfj-09-2020-0860.

Tamura, K., Nei, M., 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Biol. Evol. 10, 512–526.

Tamura, K., Stecher, G., Kumar, S., 2021. MEGA11: molecular evolutionary genetics analysis version 11. Mol. Biol. Evol. 38, 3022–3027.

Tukey, J.W., 1949. Comparing individual means in the analysis of variance. Biometrics 99–114.

Vaishampayan, A., Sinha, R.P., Hader, D.-P., Dey, T., Gupta, A.K., Bhan, U., Rao, A.L., 2001. Cyanobacterial biofertilizers in rice agriculture. Bot. Rev. 67, 453–516.

Verreschi, V., 1994. I fagioli di Sorana. SP 44 Editore, Firenze.

Viscarra-Torrico, R.C., Pajak, A., Soler-Garzón, A., Zhang, B., Pandurangan, S., Diapari, M., Song, Q., Conner, R.L., House, J.D., Miklas, P.N., Hou, A., Marsolais, F., 2021. Common bean (*Phaseolus Vulgaris* L.) with increased cysteine and methionine concentration. Legum. Sci. 3. https://doi.org/10.1002/leg3.103.

Vronska, L.V., Demyd, A.Y., 2019. Amino acid profile of *Phaseolus Vulgaris* pods and dry extract prepared of them. ФарМӓевтичний Часопис 40–48. https://doi.org/10.11603/2312-0967.2019.1.9949.

Wellburn, A.R., 1994. The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. J. Plant Physiol. 144, 307–313. https://doi.org/10.1016/S0176-1617(11)81192-2.

Xu, X., Guo, L., Wang, S., Wang, X., Ren, M., Huang, Z., Jia, H., Wang, J., Lin, A., 2023 Dec 20. Effective strategies for reclamation of saline-alkali soil and response mechanisms of the soil-plant system. Sci Total Environ 905, 167179. https://doi.org/10.1016/j.scitotenv.2023.167179.

Yao, D.N., Kouassi, K.N., Erba, D., Scazzina, F., Pellegrini, N., Casiraghi, M.C., 2015. Nutritive Evaluation of the Bambara Groundnut Ci12 Landrace [*Vigna Subterranea* (L.) Verdc. (Fabaceae)] Produced in Côte D'Ivoire. Int. J. Mol. Sci. 16, 21428–21441. https://doi.org/10.3390/ijms160921428.

Zarrouk, C., 1966. Contribution a l'etude d'une Cyanophycee. Influence De Divers Facteurs Physiques Et Chimiques Sur La Croissance Et La Photosynthese De *Spirulina Mixima*. Thesis. Univ., Paris. Fr.

Zhao, H., Postl, D., Amburgh, M. Van, Ross, D., Ortega, A.F., Lapierre, A., 2021. Quantitation of amino acids in soy flour, dried cow's milk powder, and corn silage by Triple Quadrupole LC/MS/MS. Food Test. Agric.

Žižková, E., Kubeš, M., Dobrev, P.I., Přibyl, P., Šimura, J., Zahajská, L., Drábková, L.Z., Novák, O., Motyka, V., 2017. Control of cytokinin and auxin homeostasis in Cyanobacteria and algae. Ann. Bot. 119, 151–166. https://doi.org/10.1093/aob/mcw194.