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RESEARCH

# Genotype and Plant Biostimulant Treatments Influence Tuber Size and Quality of Potato Grown in the Pedoclimatic Conditions in Northern Apennines in Italy

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## Abstract

In marginal mountain areas, farm management presents challenges, particularly the sustainable improvement of yield and quality. To ensure this agronomic result, it is crucial to select appropriate varieties and apply sustainable agricultural practices, such as the use of plant biostimulants. To address these challenges a two-year field study was conducted using three potato varieties (Désirée, Kennebec and Spunta) in the Tuscan-Emilian Apennines. These varieties were treated with two plant biostimulants: one based on hydrolyzed proteins from animal epithelial tissue (Fitostim®) and another based on seaweed extracts (FitostimAlga®). Agronomic and biochemical traits were used to evaluate the development of plants, yield and tubers quality. Significant interactions among factors were found, resulting in higher or lower efficiency of the plant biostimulant treatment depending on weather conditions and potato genotype. Furthermore, results demonstrated that plant biostimulant treatments increased the leaf chlorophyll content (+11.5%), the number of leaves per plant (+13.3%) and the height of potato plants (+6.5%), while no effects were observed on yield. The Désirée variety achieved the highest yield (0.54 kg plant$^{-1}$), whereas Kennebec was shown as the best variety to use for production of French fries due to a lower tuber quantity of reducing sugars, which were reduced also by plant biostimulants treatment (-18%). Moreover, Spunta tubers had the highest content of polyphenols, and the best value was achieved by Spunta variety treated with Fitostim® alga in the second year. Our finding have proven that plant biostimulant treatments can increase the quality of potato tuber without compromising yield.

**Keywords** Mountain agriculture · *Solanum tuberosum* · Morpho-physiological characterization · Qualitative characterization · Food composition

## Introduction

*Solanum tuberosum* L. is a perennial, herbaceous plant belonging to the Solanaceae family, native to the central Andean area of South America. After its introduction to Europe, it was adapted to high latitudes and became one of the most cultivated horticultural crops worldwide due to human consumption and industrial and zootechnical uses (Gutaker et al., 2019; Luthra et al., 2018). Potato is a key nourishment in the Mediterranean diet, ensuring an optimal intake of energy, due to its high starch content and high quality nutrients representing an important source of vitamins, e.g., ascorbic, folic, and pantothenic acids (Docimo et al., 2023), minerals, such as magnesium, phosphorous and potassium, and antioxidant compounds, including some phenolic components (e.g., chlorogenic, isochlorogenic, neochlorogenic and caffeic acids) (Andre et al., 2007, 2014; Choi et al., 2016; Ezekiel et al., 2013). Numerous factors, such as choice of the variety, agro-climatic conditions,

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physio-chemical properties of soil, and crop management practices (Galdón et al., 2012), can influence tuber nutritional composition. More than 5,000 cultivated varieties of potato are known worldwide, classified according to physical characteristics of tubers (e.g., shape, texture, flesh, and skin color), growth cycle or intended use (Burlingame et al., 2009; Ahmed et al., 2024). A large genetic variability has been created by natural and anthropic selections across different cultivation areas, allowing for good adaptability, productivity, and profitability of the different potato production systems worldwide (Fogelman et al., 2019).

Potato quality standards consider many characteristics, such as the nutritional composition of tubers (e.g., content of starch, vitamin C, proteins, and beneficial elements), tuber size and shape, absence of blemishes, flesh color, intended use (Stark et al., 2020). Desired quality parameters depend also on the intended use: the content of reducing sugars (glucose and fructose), in tubers destined for French-fries, should be lower than 0.2% of fresh weight, in order to avoid Maillard reaction and acrylamide production during fries' preparation at high temperature (Liyanage et al., 2021; Mottram et al., 2002).

To ensure food safety and quality, and to satisfy demanding and well-informed consumers, Governments and NGOs either imposed or adopted mechanisms that track product or ingredient from origin to customer (identity preservation-tracking), and systems that trace product/ingredient from shelf backward to origin/source (traceability) (Van Der Vorst, 2006). In other words, traceability allows to document/trace products (food, feed, and ingredients) and their history throughout the entire production chain, including distribution and sales (Charlebois et al., 2014). Traceability takes into account each phase of the farm-to-fork production cycle, as well as other elements such as the quality and acceptability of the final product, farming inputs, disease and pest management, and genetic identity (Yu et al., 2022). In particular, the latter determines the genetic 'fingerprint' of a product allowing to distinguish it quickly and uniquely from others (Kampan et al., 2022).

According to the National Institute of Statistics of Italy (ISTAT), the area cultivated with potato plants in 2020 amounted to approximately 47,000 ha, (ISTAT, 2020) and with a consumption over 550,000 tons (CSO Italy, 2020). Potato is commonly cultivated both in fertile plain territories and in disadvantaged mountain areas, in which agriculture supports the whole socio-economic system and potato cultivation may represent a way for alleviating depopulation (Fleury et al., 2008; Gentilcore, 2012; De Jong, 2016).

In these growing areas, there are various challenges to face, including the establishment of a sustainable system from a social, economic, and environmental point of view (Reisch et al., 2013). In addition, mountain environments are characterized by fragile geo-ecology, inaccessibility of field and limited resources (Subedi et al., 2016).

The mountainous regions of the Italian Northern Apennines are known for their wilderness, their charming landscapes and as a leisure destination for a great number of tourists. Despite their natural and historical heritage, mountainous areas of the Mediterranean, and among them the Apennine territories, the decline in the importance of agriculture has led to a significant reduction of activities in rural zones, with progressive depopulation of villages (Modica et al., 2017). The agroecological management of farms presents numerous challenges due to environmental variations across the landscape (Rueff et al., 2015). Despite physical difficulties, mountain agriculture has the potential to produce sustainable crops (Manta, 2023) and, potatoes are one of the few crops that can be grown in the northern Apennines, making them a suitable and versatile option for farmers in disadvantaged mountainous areas (Devaux et al., 2021). Not all potato varieties are suitable for cultivation in mountainous areas. To ensure qualitative and yield standards, it is important to select specific varieties based on their harvest season and to apply sustainable agriculture practices, such as the use of plant biostimulants. (Di Donato et al., 2020; Giupponi et al., 2020).

In Europe, plant biostimulants are included in fertilizing products Regulation (1009/2019) and categorized into two groups; microbial and non-microbial biostimulants, depending on their composition (Rouphael & Colla, 2020). The former group consists of beneficial bacteria and fungi, while the latter includes humic and fulvic acid, protein hydrolysates and other N-containing compounds, seaweed extracts and botanicals, chitosan and other biopolymers and other inorganic compounds intended ad beneficial elements (e.g., Al, Co, Na, Se, and Si) (Caradonia et al., 2019; Du Jardin, 2015). Plant biostimulants are able to modify plant physiological processes promoting plant growth and improving stress responses when applied in minute quantities (du Jardin, 2012). In particular, non-microbial biostimulants based on seaweed extracts and protein hydrolysates are gaining interest as novel sustainable agriculture technology (Xu & Geelen, 2018). Seaweed extracts contain the mixture of essential bioactive compounds such as polysaccharides, phenolic compounds, phytohormones and minerals (Raja & Vidya, 2023). A seaweed extract derived from *Ascophyllum nodosum* (Fitostim® Alga) was shown to increase the biomass of lavandin plants without altering the chemical composition of essential oils (Caccialupi et al., 2022), and to enhance the internal quality of cucumbers, in terms of amount of plastid pigments and tocopherols (Zamljen et al., 2024). On the other hand, products based on hydrolyzed proteins are composed by soluble peptides, free amino acids and organic nitrogen (Malécange et al., 2023). Their positive effect was observed in several crops such as lavandin, where it increased the fresh and dry weights of inflorescence of treated plants (Caccialupi et al., 2022), tomato, for which stimulated the growth of treated plants under drought stress was observed (Francesca et al., 2022), and in *Capsicum annuum.* As far as the use of plant biostimulants in potato crop cultivation is regarded, interesting results for different aspects of the production were observed. The application of extracts from *Artemisia vulgaris* L. increased the chlorophyll *a* and chlorophyll *b* content in potato leaves (Findura et al., 2020). Wadas and Dziugieł (2020) reported an increase in marketable yield after foliar application of *Ascophyllum nodosum* and *Ecklonia maxima* extracts. In this case, the plant biostimulant activity was influenced by weather, in fact, the effect was higher in warm and very wet growing conditions. The foliar application of different seaweed extracts increased yield (weight of potato tuber, tuber bulking rate and tuber yield), potato tuber quality (ascorbic acid and reducing sugar content) (Garai et al., 2021; Głosek-Sobieraj et al., 2022) and chlorophyll leaf content (Mystkowska, 2022). This suggests that seaweed extracts not only increase the yield but also nutritional value. Regarding the biostimulants based on protein hydrolysates, a field study on two potato varieties showed plant biostimulants based on amino acids and humic substances could increase plant growth and tuber quality and yield, however the effects of plant biostimulants was linked to potato genotype (El-Zohiri & Asfour, 2009).

In recent times, a relatively novel approach to enhancing agricultural output has been the creation of new plant biostimulants. These fertilized products have been studied as promising instrument to ensure high quantity and quality crop yield (for a recent review, see Zulfiqar et al., 2024). Plant biostimulants can thus be a tool to improve potato production. Potato is a crop that is particularly well-suited for cultivation in marginal lands and conditions. Biostimulant products have shown positive results in non-intensive agricultural systems that are characteristic of these areas. However, the application of plant biostimulants to potato crop in marginal areas, such as Tuscan-Emilian Apennines, has not been evaluated so far. Thereby, the aim of this study was to evaluate how biostimulants influence the potato production, in terms of quantity and quality, grown in the pedoclimatic conditions in Northern Apennines in Italy. To obtain these, this study evaluated: *i*) the agronomic and qualitative characteristics of Désirée, Kennebec and Spunta, three of the most cultivated potato varieties in the Tuscan-Emilian Apennines *ii*) the response of each variety to two plant biostimulants; based on hydrolyzed proteins from animal epithelial tissue (Fitostim®, SCAM s.p.a., Modena, Italy) and on seaweed extracts (Fitostim® Alga, SCAM s.p.a., Modena, Italy), respectively; *iii*) the genetic profiles for varietal characterization and future traceability. This work could be useful to implement a sustainable agricultural system for Northern Apennines pedoclimatic conditions, the adoption of suitable genotypes and the application of innovative fertilizers ensuring a profitable yield and production of a superior quality necessary to satisfy producers and consumers.

## Materials and Methods

### Plant Material

Three potato varieties were used in this study: Kennebec, Désirée and Spunta (Table 1). These varieties are among those indicated by the official production guideline issued by the Chamber of Commerce of Modena (Italy) to be cultivated in the Montese area, located at an altitude between 840 and 1,200 m above the sea level.

### Plant Biostimulants Treatments

Two non-microbial biostimulants: Fitostim® based on hydrolyzed proteins from animal epithelial tissue (composed of 8% organic nitrogen, 15% free ammino acid and 25.2 organic carbon); Fitostim® Alga based on brown seaweed extracts and yeast extracts (2% organic nitrogen, 10% organic carbon and 50% organic matter < 50 kD). Désirée, Kennebec and Spunta potato plants were nebulized with the products applied at concentration of 150 g $hL^{-1}$ using a hand pressure sprayer. When the plant reached the growth stage 3 - 'Main stem elongation' (Meier, 2001), three treatments were applied with 2 weeks intervals. Tap water was sprayed on control plants.

### Experimental Design

Field experiments were conducted in the municipality of Montese, Modena, Emilia Romagna (Italy) in a territory of 'Patata di Montese Producers' Association' (44°14'56"N, 10°56'15"E) (Fig. 1). Soil features and nutrients content are reported in Table 2.

**Table 1** Varieties, pedigree, and geographical origin of potato varieties used

| Variety | Skin color | Flesh color | Pedigree | Origin |
|---|---|---|---|---|
| Désirée | Red | Yellow | Urgenta × Depesche | The Netherlands |
| Kennebec | Yellow | White | (Chippewa x Kathadin) × (Earlaine x W-ras) | United States of America |
| Spunta | Yellow | Yellow | Bea × USDA × 96-56 | The Netherlands |

Seed tubers were planted on March 22, 2021, and April 6, 2022, respectively. Planting density was 5 tubers m$^{-2}$, with a spacing of 0.80 m between rows and a spacing of 0.25 m between plants within the row. The experimental design was a randomized block with 3 replications, 3 potato varieties (Désirée, Kennebec and Spunta) (first factor) and 3 biostimulant treatments (FITOSTIM®, FITOSTIM® Alga and control treated with tap water) (second factor). Each plot consisted of 24 plants occupying an area of 4.8 m$^2$, with a border row planted around each plot.

Fertilizers such as fermented cow manure (40 t ha$^{-1}$), straight phosphatic fertilizer (0.3 t ha$^{-1}$) ("ScorieK" (Scorie Thomas), AlFe, Mantova, Italy) were applied ahead of planting, according to the Potato of Montese Production Manual. During the two growing seasons, weed control was made manually, late blight was controlled with 2 treatments based on dimethomorph and zoxamide treatments (Presidium® One, Gowan, Faenza, Italy), and the possible infestation of the Colorado beetle was controlled with 1 treatment based on acetamiprid (Kestrel, Nufarm, Bologna, Italy). Fields were rainfed, therefore, no irrigation was done for both seasons. The crop rotation performed was bread wheat (one crop cycle) for both the growing seasons.

## Agronomic Traits Evaluation

At full bloom (92 and 90 days after planting in 2021 and 2022, respectively), plant height and leaves number *per* plant were recorded for 3 plants for plot in each block. Moreover, the chlorophyll content was measured in young leaves (3 leaves for each plant) using a Dualex 4 Scientific instrument (Dx4, FORCE-A, Orsay, France). Meanwhile, leaf samples were collected and pooled from different plants of each variety, stored in a portable cooler (4 °C), transported to the laboratory, and immediately processed for DNA extraction.

At harvest (August 24, 2021, and August 30, 2022), a set of agronomic traits, such as yield *per* plant, number of tubers *per* plant, average weight of tubers, dry weight of tubers *per* plant, tuber diameter, was recorded and/or calculated for 3 plants for plot in each block. Moreover, tuber samples (1 kg for each replicate) were used for subsequent qualitative analysis (content of glucose, fructose, proteins, starch, and polyphenols). All the varieties were harvested on the same day in both the growing seasons.

## Quality Analysis of Potato Tubers

Peeled potato tubers were diced and blended using a food processor (Pimmy 500 W, Ariete, Florence, Italy). Distilled water was added (1:2 weight/weight) to facilitate the mulching and promote the extraction. The homogenized mixtures were centrifuged twice for 20 min at 4000 rpm and 4 °C. The supernatant was collected and used for sugar and protein determinations. The quantification of glucose and fructose as well as of total starch was carried out using a spectrophotometric method by enzymatic determination with the Megazyme Assay Kit K-SUFRG and the Megazyme Assay Kit Total Starch Assay Kit AA/AMG (Megazyme International Ireland Ldt., Bray, Ireland), respectively, following the manufacturer's instructions (Lombardo et al., 2017).

Total proteins were determined using a spectrophotometric method following the Micro 2 mL assay protocol of the Bradford Reagent described in the technical bulletin (Sigma-Aldrich, Saint Louis, MO. Technical bulletin No. B 6916) (Bradford, 1976). Bovine serum albumin was used as standard protein.

The Folin-Ciocalteu assay (Singleton et al., 1999; Tagliazucchi et al., 2010) was used to quantify the total phenolic content (TPC). A solution (70% methanol, 28% distilled water, 2% formic acid) for extraction was prepared according to Martini et al. (2021). Peeled potato tubers were diced, and the extraction solution was added (30:50 weight/weight). Using an immersion blender (Pimmy 500 W, Ariete, Florence, Italy), a homogenized mixture was obtained. After stirring for one hour at 37 °C, each sample was centrifuged at 4.050 rpm for 20 min at 4 °C. The supernatant was collected for analysis to be done with a spectrophotometric method. Sample absorbance was measured at 760 nm, using an UV–vis spectrophotometer (UV-6300PC – Double Beam Spectrophotometer, VWR International s.r.l., Milan, Italy). Results were expressed as mg of gallic acid equivalent/100 g of potato.

## Statistical Analysis

The experiment was designed as a randomized complete block design with three treatments, three varieties and three replications representing the blocks. Since no block effects were found, data were subjected to analysis of variance (Three-way ANOVA) using GenStat 17th (VSN International, Hemel Hempstead, UK). Means were compared using the Duncan's post-hoc test at the 5% level.

DNA extraction and purification, PCR amplification and capillary electrophoresis.

Young leaves were collected from three plants for each variety and pooled together for DNA extraction using the CTAB method, as described by Doyle and Doyle (1987), with modifications. After its extraction and purification, DNA was quantified by a NanoDrop UV Visible Spectrophotometer (NanoDrop 1000, ThermoFisher Scientific, Waltham, USA). For simple sequence repeat (SSR) analysis, 19 primer pairs, selected from the literature, were used (Table 3). For each molecular marker two specific primers (forward and reverse) and a universal M13(-19) primer

**Fig. 1** Location of field experiment. (Source Image © 2024 Airbus GoogleLandsat / Copernicus Data SIO, NOAA, U.S. Navy, NGA, GEBCO)

**Table 2** Soil characteristics

| Soil characteristics | 2021 | 2022 |
|---|---|---|
| Sand (%) | 21.82 | 23.60 |
| Silt (%) | 49.33 | 41.20 |
| Clay (%) | 28.85 | 35.10 |
| pH (in water) | 7.80 | 7.70 |
| Electrical Conductivity (salinity) (µS/cm) | 167.00 | 381.00 |
| Limestone (%) | 10.32 | 18.10 |
| Active limestone (%) | 5.40 | 6.40 |
| Organic carbon (%) | 1.09 | 1.47 |
| Nitrogen total (%) (Kjeldahl method) | 0.14 | 0.21 |
| Organic matter (%) | 1.88 | 2.53 |
| Carbon-to-Nitrogen ratio | 8.10 | 7.10 |
| Cation Exchange Capacity (meq 100 $g^{-1}$) | 24.10 | 24.40 |
| Exchangeable Calcium (ppm) | 4532.50 | 4765.00 |
| Exchangeable Calcium (meq 100 $g^{-1}$) | 22.62 | 23.78 |
| Exchangeable Magnesium (ppm) | 168.75 | 78.00 |
| Exchangeable Magnesium (meq 100 $g^{-1}$) | 1.39 | 0.64 |
| Exchangeable Potassium (ppm) | 217.50 | 449.00 |
| Exchangeable Potassium (meq 100 $g^{-1}$) | 0.56 | 1.15 |
| Calcium-to-Magnesium ratio | 16.29 | 37.00 |
| Calcium-to-Potassium ratio | 40.66 | 20.70 |
| Magnesium-to-Potassium ratio | 2.50 | 0.60 |
| Assimilable Phosphorus (ppm) | 23.35 | 13.00 |
| Assimilable Phosphorus pentoxide (ppm) | 53.47 | 29.00 |

labelled with fluorescent dye FAM (6-carboxy-fluorescine) were used. The sequence-specific forward primers presented a common M13 tail at their 5′ end (5'-CAC GAC GTT GTA AAA CGA C-3'). Separate amplification for each SSR marker was performed in a 25 µL volume containing 20 ng of template DNA, 1x PCR buffer, 1.5 mM $MgCl_2$, 0.20 mM of each dNTP, 0.04 µM of forward primer with a M13 tail, 0.2 µM of reverse primer, 0.32 µM of fluorescent labelled M13 tail (FAM, ThermoFisher Scientific, Waltham, USA) and 1 unit of DreamTaq™ DNA polymerase (ThermoFisher Scientific, Waltham, USA) (Schuelke, 2000).

The reactions were carried out on a 2720 Thermal Cycler (ThermoFisher Scientific, Waltham, USA), with an initial denaturation temperature of 95 °C for 3 min, followed by 10 cycles consisting of three steps: 95 °C for 30 s, 65 °C for 30 s (with each cycle the annealing temperature decreased 1 °C) and 72 °C for 30 s. Products were amplified for other 30 cycles at 95 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s. After 30 cycles, the samples were subjected to a final extension step for 7 min at 72 °C.

The amplified fragments were initially checked on 1.5% agarose gel electrophoresis at 80 V in TBE buffer and, afterwards, a sizing analysis was performed using capillary electrophoresis. For agarose gel electrophoresis SYBR® Safe DNA gel stain (10,000X concentrate in DMSO, ThermoFisher Scientific, Waltham, USA) was used. Samples were prepared for capillary electrophoresis adding 1 µL of dye-labelled PCR products to a 12.5 µL of running mix (1 µL of GeneScan 500 LIZ size standard, ThermoFisher Scientific, Waltham, USA plus 11.5 µL of pure formamide). After denaturation (3 min at 95 °C), capillary electrophoresis was performed using the genetic analyzer ABI PRISM 310 Genetic Analyzer (ThermoFisher Scientific, Waltham, USA). The peak position and size of SSR alleles were inferred using GeneMapper software v4.0 (ThermoFisher Scientific, Waltham, USA).

## Results

### Weather Conditions

Figure 2 reports the weather conditions registered during the experiments and retrieved from Dexter system (Regional Agency for Environmental Protection-ARPA-, Emilia Romagna, Italy).

The weather conditions in the municipality of Montese, where the experiments were carried out, varied between the first (2021) and second year (2022) of the study. The monthly average temperatures (min and max) were lower in the former for most of the time. What is worth noting, during the tuber germination in June, the maximum average temperature was 12 and 14 °C in the former and latter, respectively. Total overall rainfall was higher in 2022 (+176.50 mm), with an exceptional peak of approximately 150 mm in August.

### Agronomic Traits

At full flowering, a significant effect of different factor (Year, Variety, Treatment) interactions was observed for some of measured traits: Treatment by Year and Variety by Year interactions produced different effects on leaf chlorophyll content, while Treatment by Variety and Variety by Year on plant height (Table 4; Fig. 3). The plant biostimulant treatments, in fact, were more efficacy in increasing the leaf chlorophyll content in the wetter-warmer year (2022) (+14.5%) in comparison to the colder-dryer year (2021) (+7.4%), and in increasing plant height in Spunta.

Considering only the effect of the treatments on plants, Fitostim® and Fitostim® Alga significantly increased the values of all the morpho-physiological traits compared to the control (leaf chlorophyll content ~+10%, number of leaves *per* plant ~+12% and plant height +5%) (Table 4).

Considering the two growing seasons, the values observed for leaf chlorophyll content and leaf number *per* plant were higher in the wetter-warmer year (+28% and +17.5%, respectively), with the highest variation between the two year observed in Desirée, whereas the plant height

**Table 3** Microsatellite markers used in this study, including their chromosome location, repeat motif, sequences of their specific primers (forward and reverse), and peak size range (bp)

| Marker ID* | Chr. | Motif | Forward (5' to 3') | Reverse (5' to 3') | Peak range (bp) | References |
|---|---|---|---|---|---|---|
| STM5127 | 1 | $(TCT)_5$ | FAM-TTCAAGAATAGGCAAAACCA | CTTTTTCTGACTGAGTTGCCTC | 248–291 | Villano et al., 2012 |
| 4026/4027 | 1 | $(CTAT)_n$ $(CTAG)_n$ | FAM-AACTTGCGGGAATAAGTGACG | ACTATACACACGTGCCCTGAAACTAG | 265–346 | Kishine et al., 2017 |
| 8242 | 2 | $(CTTT)_n$ | FAM-CGTCTTGGATGTCTTAGTTGTGG | GCAAAACCAGAAAGGCTAACAAAC | 191–218 | Kishine et al., 2017 |
| STM2022 | 2 | $(CAA)_3$ $(CAA)_3$ | FAM-GCGTCAGCGATTTCAGTACTA | TTCAGTCAACTCCTGTTGCG | 166–233 | Tillault & Yevtushenko, 2019 |
| 12,002 | 3 | $(ACAT)_n$ | FAM-CCATGAACCTGAAGTTTTTCTGC | TGGATATCTTGTGCCTACAAGCTAG | 209–235 | Kishine et al., 2017 |
| STI0012 | 4 | $(ATT)_n$ | FAM-GAAGCGACTTCCAAAATCAGA | AAAGGGAGGAATAGAAACCAAAA | 183–234 | Villano et al., 2012 |
| 16,410 | 4 | $(ATAC)_n$ | FAM-GTATGTTTGAGTAAAATCCTCCACCA | TTCTCTGCCCCCTTTTAATTTG | 258–354 | Kishine et al., 2017 |
| STI0032 | 5 | (GGA)n | FAM-TGGGAAGAATCCTGAAATGG | TGCTCTACCAATTAACGGCA | 127–148 | Villano et al., 2012 |
| STM0019 | 6 | $(AT)_7$ $(GT)_{10}$ $(AT)_4$ $(GT)_5$ $(GC)_4$ $(GT)_4$ | FAM-AATAGGTGTACTGACTCTCAATG | TTGAAGTAAAAGTCCTAGTATGTG | 167–235 | Reid et al., 2011 |
| STM3009 | 7 | (TC)13 | FAM-TCAGCTGAACGACCACTGTTC | GATTTCACCAAGCATGGAAGTC | 143–176 | Reid et al., 2011 |
| STM1016 | 8 | (TCT)9 | FAM-TTCTGATTTCATGCATGTTTCC | ATGCTTGCCATGTGATGTGT | 243–275 | Tillault & Yevtushenko, 2019 |
| STM1104 | 8 | (TCT)5 | FAM-TGATTCTCTTGCCTACTGTAATCG | CAAAGTGGTGTGAAGCTGTGA | 178–199 | Tillault & Yevtushenko, 2019 |
| STM3012 | 9 | $(CT)_4$ $(CT)_8$ | FAM-CAACTCAAACCAGAAGGCAAA | GTTTTTAGGCAGTTCTTGGGG | 165–212 | Reid et al., 2011 |
| 35,584 | 9 | $(GAAA)_n$ | FAM-AGTAAGTCAAACTCAACTCCAAGGTG | GTTCTAGATTATCTCACTCATGCCTTTC | 84–111 | Kishine et al., 2017 |
| STM1106 | 10 | (ATT)13 | FAM-TCCAGCTGATTGGTTAGGTTG | ATGCGAATCTACTCGTCATGG | 145–211 | Villano et al., 2012 |
| 43,016 | 11 | (ATCC)n | FAM-CAAGCTGCATGAAAGCCATC | TTTGCCTAAAAGTTTGTAGTGTGAGG | 184–227 | Kishine et al., 2017 |
| STM0037 | 11 | (TC)5 (AC)6 AA (AC)7 (AT)4 | FAM-AATTTAACTTAGAAGATTAGTCTC | ATTTGGTTGGGTATGATA | 87–133 | Tillault & Yevtushenko, 2019 |
| STM2028 | 12 | (TAC)5 (TA)3 (CAT)3 | FAM-TCTCACCAGCCGGAACAT | AAGCTGCGGAAGTGATTTTG | 286–408 | Reid et al., 2011 |
| 46,514 | 12 | (TATC)n | FAM-TGCTTTTTGTTTCCTTTTGTGTG | GGAATGAAACTAAGCCTTGCTCTG | 130–172 | Kishine et al., 2017 |

was higher in the colder-dryer year with exception of Spunta (Table 4; Fig. 3a2-b2).

Considering the vegetative habit of genotypes, Spunta plants had a pronounced "bushy" aspect, different than plants belonging to Désirée, which appeared slenderer. In addition, Spunta showed a reduced size, whereas Désirée showed the highest plants (Table 4).

At harvest, a significant effect of interaction of Variety and Year on yield plant$^{-1}$ was observed (Table 5; Fig. 4), resulting in an increase in yield plant$^{-1}$ values for Désirée variety (+ 18%) and an decrease for Spunta variety (-20%) in the wetter-warmer year compared to colder-dryer one. No significant differences in yield traits were found as an effect of treatments (Table 5), while a genotype effect was observed with the highest values of yield *per* plant and tuber dry weight *per* plant registered for Désirée. No significant effect of year was observed on these traits.

Several significant interactions among Treatment, Variety and Year affected tuber size and weight traits (Table 6; Fig. 5, Supplementary Figs. 1 and 2).

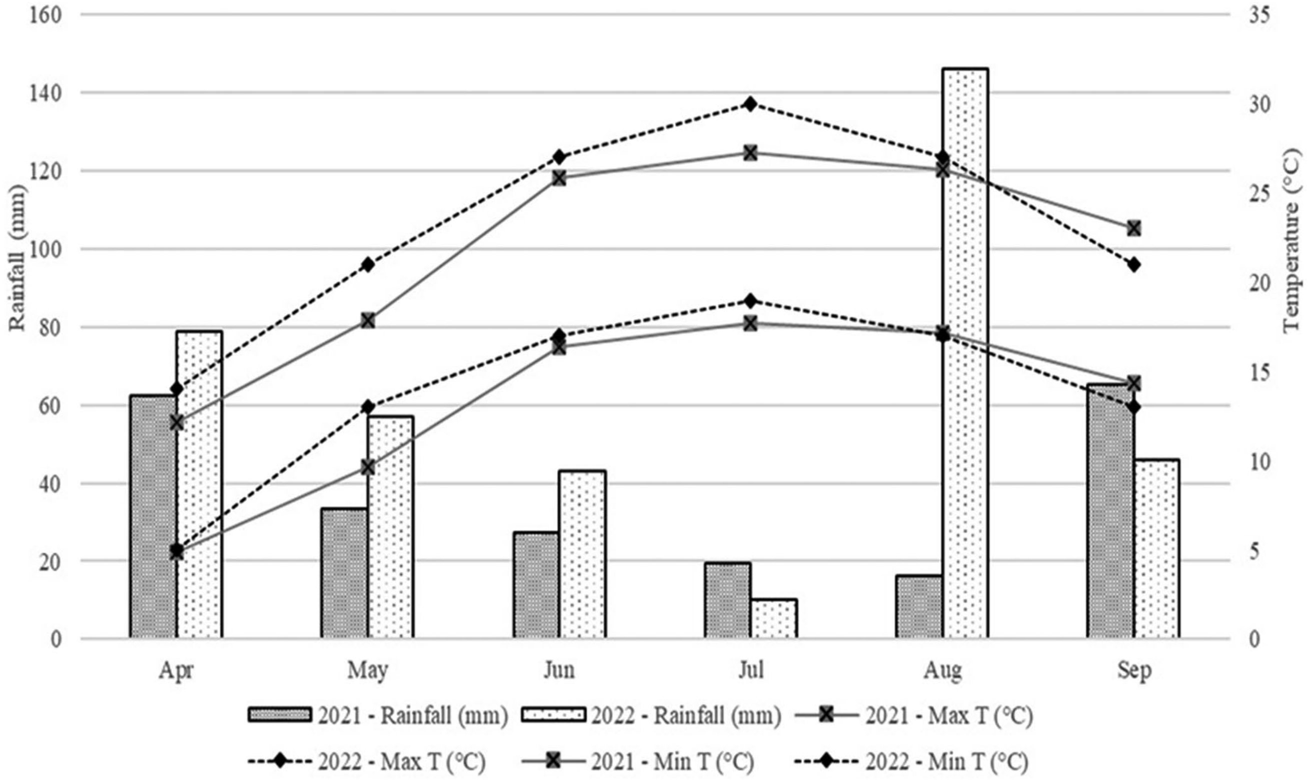

**Fig. 2** The weather conditions during the experiments retrieved from Dexter system (Regional Agency for Environmental Protection-ARPA-, Emilia Romagna, Italy)

**Table 4** Traits evaluated at full flowering

| | | Chl | | Leaf number plant$^{-1}$ | | Plant height (cm) | |
|---|---|---|---|---|---|---|---|
| Treatment (T) | Control | 38.00 | b | 46.00 | b | 60.67 | b |
| | Fitostim® Alga | 41.27 | a | 52.14 | a | 64.61 | a |
| | Fitostim® | 42.37 | a | 50.64 | a | 63.29 | a |
| | | | *** | | ** | | * |
| Variety (V) | Désirée | 41.97 | a | 50.44 | ns | 69.31 | a |
| | Kennebec | 42.26 | a | 50.56 | ns | 65.79 | b |
| | Spunta | 37.41 | b | 47.78 | ns | 53.47 | c |
| | | | *** | | ns | | *** |
| Year (Y) | 2021 | 33.87 | b | 45.06 | b | 64.98 | a |
| | 2022 | 47.23 | a | 54.13 | a | 60.73 | b |
| | | | *** | | *** | | *** |
| Treatment-by-Variety | | | ns | | ns | | * |
| Treatment-by-Year | | | * | | ns | | ns |
| Variety-by-Year | | | *** | | ns | | *** |
| Treatment-by-Variety-by-Year | | | ns | | ns | | ns |

Data is presented as mean (Treatments = mean of 27 plants; Variety = mean of 27 plants; Year = mean of 45 plants). Different letters indicate statistically significant differences among varieties; *significant at 0.05; **significant at 0.01; ***significant at 0.001; Chl = Chlorophyll content in leaves

The interaction between Variety and Year affected the number of tubers *per* plant, average weight of tubers, and the number of tuber with a size between 4 and 6 cm (Table 6; Fig. 5, e Supp Figs. 1 and 2) showing significant differences among variety in the colder-dryer year. In particularly, Désirée showed the highest number of tuber with and the lowest tuber weight, whereas an opposite behaviour was observed in Spunta.

The interaction among all factors affected the number of tubers *per* plant, the average weight of tubers, and the number of tuber with a size between 4 and 6 cm. Interactions among Treatment, Variety and Year showed no significative

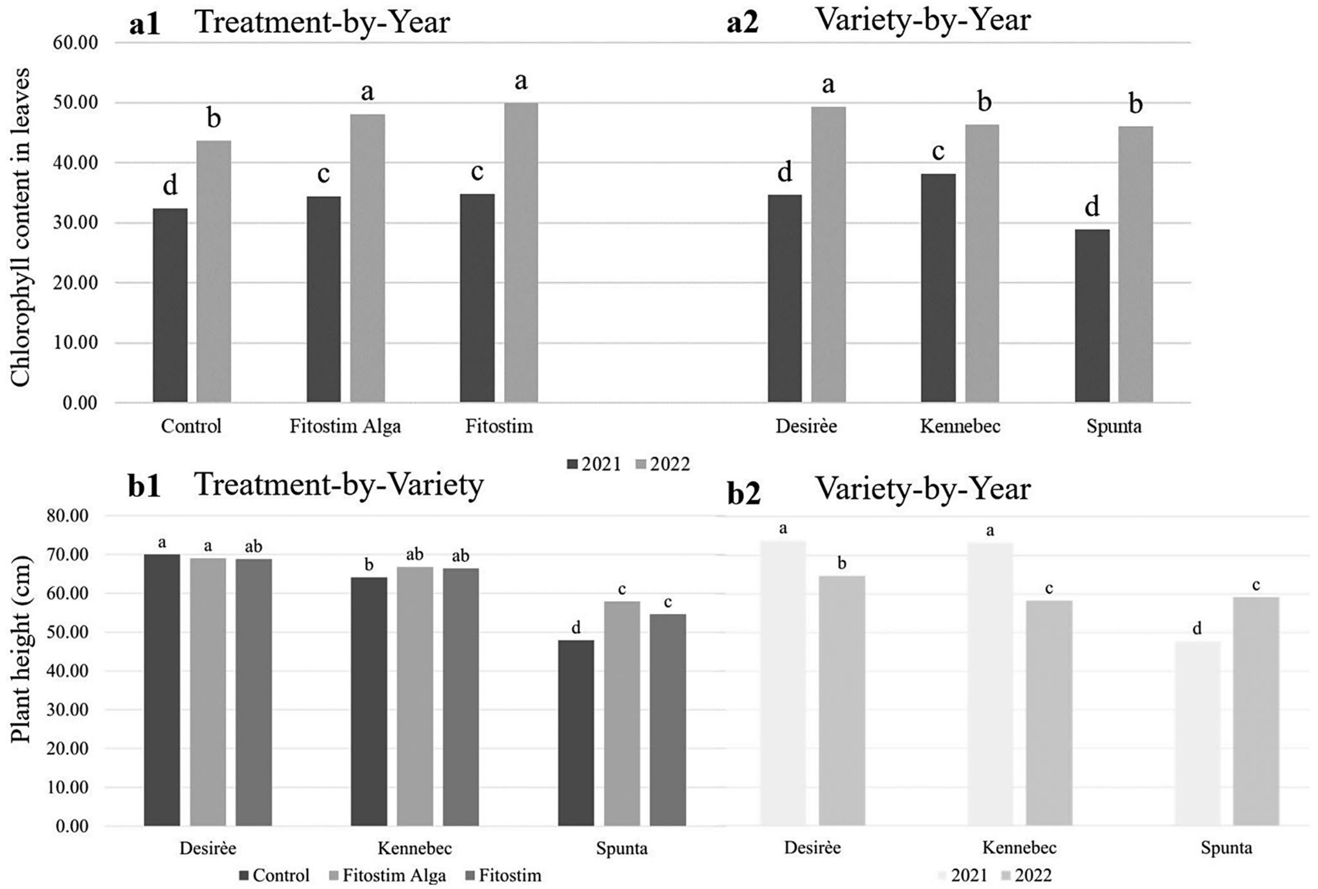

**Fig. 3** Interactions between factors for traits evaluated at full flowering. a – Treatment-by-year (* significant at 0.05) and Variety-by-Year (*** significant at 0.001) interactions for the chlorophyll content in leaves. b – Treatment-by-Year (* significant at 0.05) and Variety-by-Year (*** significant at 0.001) interactions for the plant height (cm)

**Table 5** Yield traits evaluated

| | | Yield plant$^{-1}$ (kg) | | Tuber dry weight plant $^{-1}$ (g) | |
|---|---|---|---|---|---|
| Treatment (T) | Control | 0.49 | ns | 113.50 | ns |
| | Fitostim® Alga | 0.48 | ns | 105.60 | ns |
| | Fitostim® | 0.52 | ns | 121.40 | ns |
| | | | ns | | ns |
| Variety (V) | Désirée | 0.54 | a | 123.60 | a |
| | Kennebec | 0.46 | b | 111.10 | ab |
| | Spunta | 0.49 | ab | 105.80 | b |
| | | | * | | * |
| Year (Y) | 2021 | 0.50 | ns | 110.20 | ns |
| | 2022 | 0.49 | ns | 116.80 | ns |
| | | | ns | | ns |
| Treatment-by-Variety | | | ns | | ns |
| Treatment-by-Year | | | ns | | ns |
| Variety-by-Year | | | ** | | ns |
| Treatment-by-Variety-by-Year | | | ns | | ns |

Data are presented as mean (Treatments = mean of 27 plants; Variety = mean of 27 plants; Year = mean of 45 plants). Different. Different letters indicate statistically significant differences among varieties. *significant at 0.05; **significant at 0.01; ***significant at 0.001

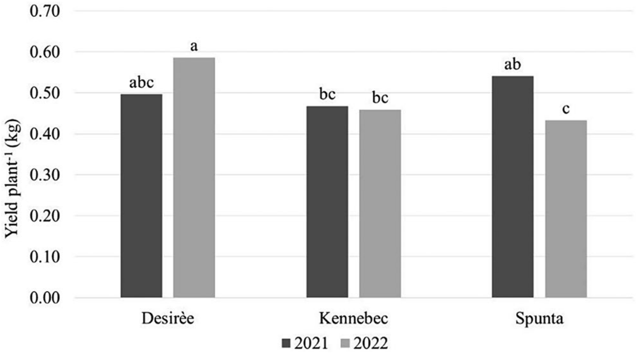

**Fig. 4** Interaction Variety-by-Year (**significant at 0.01) for yield plant$^{-1}$ (kg)

effects on Désirée. However, in Kennebec, there was an increase in number of tubers and a decrease in average weight of tubers in colder-dryer year linked to Fitostim® Alga treatment and, in the wetter-warmer year link to Fitostim® treatment. In Spunta, the same behaviour was observed only in colder-dryer year linked to Fitostim® treatment.

**Table 6** Tuber size and weight traits evaluated

| | | Number of tuber per plant | | Avarage weight of single tuber (g) | | Tuber number with diameter < 4 cm | | 4 cm < Tuber number with diameter > 6cm | | Tuber number with diameter > 6 cm | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatment (T) | Control | 4.06 | b | 136.70 | a | 0.09 | ns | 0.76 | ns | 3.21 | b |
| | Fitostim® Alga | 4.93 | a | 116.80 | ab | 0.12 | ns | 1.10 | ns | 3.71 | a |
| | Fitostim® | 4.80 | a | 114.30 | b | 0.07 | ns | 0.98 | ns | 3.74 | a |
| | | | * | | * | | ns | | ns | | * |
| Variety (V) | Désirée | 5.74 | a | 96.70 | b | 0.11 | ns | 1.33 | ns | 4.30 | a |
| | Kennebec | 4.33 | b | 115.30 | b | 0.13 | ns | 0.87 | ns | 3.33 | b |
| | Spunta | 3.72 | b | 155.80 | a | 0.04 | ns | 0.64 | ns | 3.03 | b |
| | | | *** | | *** | | ns | | ** | | *** |
| Year (Y) | 2021 | 4.07 | b | 147.00 | a | 0.19 | a | 1.17 | a | 2.71 | b |
| | 2022 | 5.12 | a | 98.2 | b | 0.00 | b | 0.73 | b | 4.40 | a |
| | | | *** | | *** | | ** | | ** | | *** |
| Treatment-by-Variety | | | ns | | ns | | ns | | ns | | * |
| Treatment-by-Year | | | ns | | ns | | ns | | ns | | ns |
| Variety-by-Year | | | * | | *** | | ns | | ** | | ns |
| Treatment-by-Variety-by-Year | | | * | | * | | ns | | ns | | * |

Data are presented as mean (Treatments = mean of 27 plants; Variety = mean of 27 plants; Year = mean of 45 plants). Different letters indicate statistically significant differences among varieties. *significant at 0.05; **significant at 0.01; ***significant at 0.001

Considering the effects of factors, Désirée and both plant biostimulant treatments yielded the highest number of tubers *per* plant and the lowest average weight of tubers (Table 6).

A significant difference was noted between tubers produced in colder-dryer year and those produced in the wetter-warmer one in terms of size and weight: tubers in the wetter-warmer year were larger and more uniform than in the colder-dryer one, resulting in no undersized tubers (< 4 cm in diameter) being found during the grading process (Table 6; Figs. 5, 6 and 7). The number of tubers with a diameter > 6 cm increased after both the plant biostimulant treatments.

## Tubers Quality Analysis

The content of proteins, starch, reducing sugars (glucose and fructose) and polyphenols were evaluated for a nutritional characterization of the three potato varieties used in the experiments and for the evaluation of effects of plant biostimulants on tuber quality. Interactions among Treatment, Variety and Year influenced all evaluated quality traits (Table 7; Figs. 6 and 7, Supplementary Figs. 3–7).

Regarding protein content, Fitostim® treatment increased protein content in Désirée and Kennebec only in the wetter-warmer year, whereas Fitostim® Alga treatment increased protein content in Kennebec in the colder-dryer year. In the wetter-warmer year, there was an increase in protein content in all the potato varieties with exception of Spunta, whose concentration remained stable across the two growing seasons. The highest values were observed in Kennebec treated with Fitostim® in the wetter-warmer year.

Starch content was increased by Fitostim® Alga in Désirée in the colder-dryer year and, in Spunta e Kennebec in the wetter-warmer year. Conversely Fitostim® increased the starch content in Kennebec in the wetter-warmer year. Spunta showed the lowest values compared to Désirée and Kennebec. The highest values (14.15%) were observed in Kennebec in control treatment in colder-dryer year, whereas the lowest values were registered in Spunta treated with Fitostim® Alga in colder-dryer year. The content of starch was stable in Kennebec across the two growing seasons, while in Désirée and Spunta was influenced by weather conditions.

Regarding the level of reducing sugars (glucose and fructose), the same effects of treatments observed on the content of glucose were also observed on fructose content. Fitostim® Alga decreased the content of these compounds in Désirée in both years. Conversely, a contrasting effect was observed on Spunta and Kennebec depending on weather conditions. Fitostim® decreased the content of reducing sugars in all the genotypes only in colder-dryer year while an opposite behaviour was observed in wetter-warmer year. The highest values of reducing sugars were observed for Désirée, whereas lowest levels of glucose and fructose were observed for Kennebec and Spunta, respectively. In the wetter-warmer year, there was a reduction in these compounds compared to the colder-dryer year. The lowest values of fructose content were registered for Fitostim® Alga treatment showing a 22% decrease.

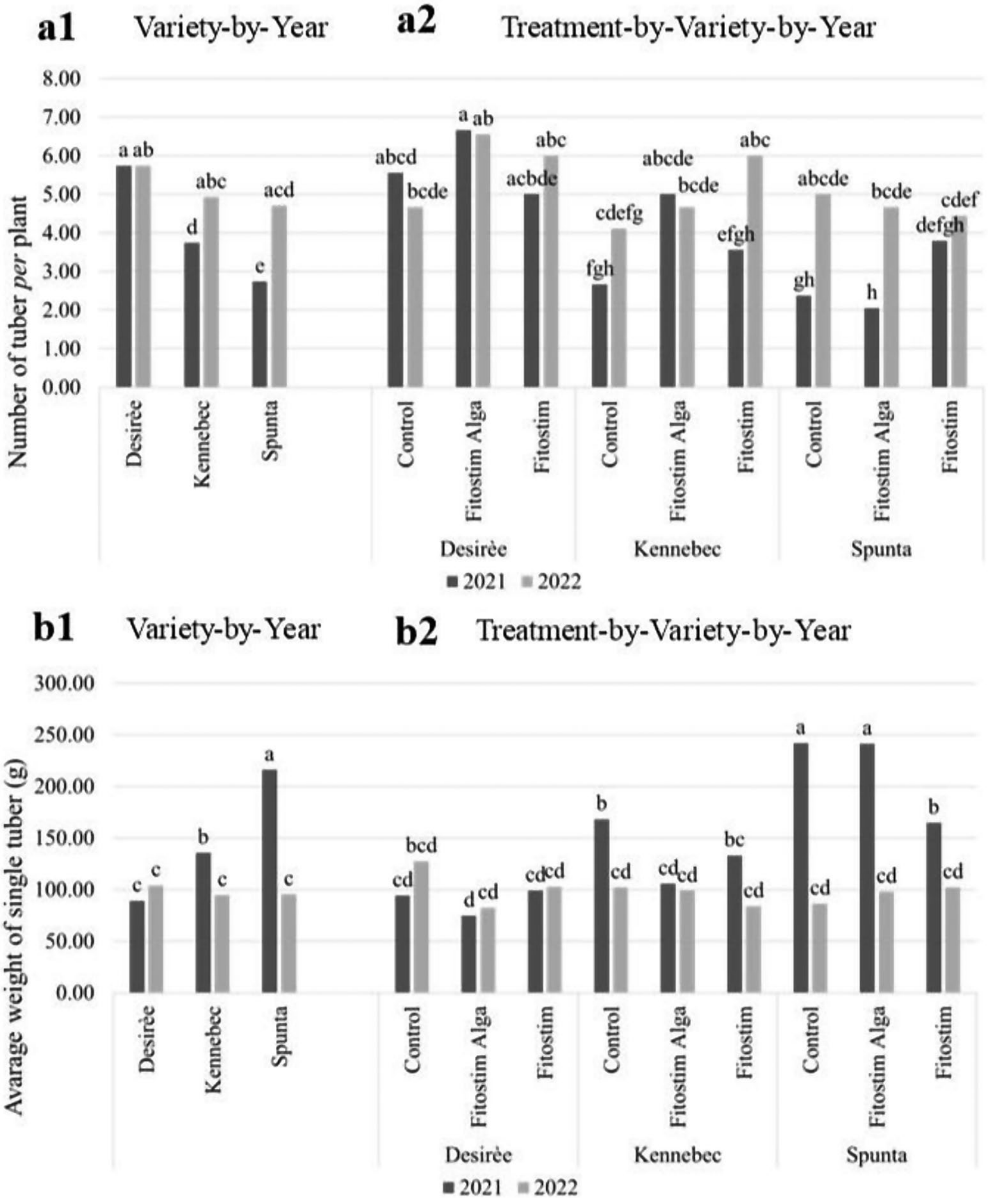

**Fig. 5** Interactions between factors for tuber size and weight traits. **a** – Variety-by-Year (* significant at 0.05) and Treatment-by-Variety-by-Year (* significant at 0.05) interactions for the number of tuber *per* plant. **b** – Variety-by-Year (***significant at 0.001) and Treatment-by-Variety-by-Year (* significant at 0.05) interactions for the average weight of single tuber (g)

Quantification of polyphenols highlighted their high levels, especially in Spunta and in the wetter-warmer year. The content of polyphenols was stable in Kennebec while depended on weather conditions in Désirée and Spunta. Treatments had opposite effects depending on weather condition. Fitostim® Alga increased the content of these compounds in Désirée in the colder-dryer year and in Kennebec and Spunta in the wetter-warmer year. Conversely, Fitostim® increased polyphenol content in Kennebec in the colder-dryer year and in Spunta in wetter warmer in comparison with the control. The highest values were observed in Spunta treated with Fitostim® Alga in the wetter-warmer year (1040.7 mg $L^{-1}$).

## Potato Fingerprinting with SSR Markers

Agarose gel electrophoresis enabled to exclude 6 primers pairs from further analysis as no amplicons were observed. The remaining 13 SSR were subjected to capillary electrophoresis using the genetic analyzer ABI PRISM 310, and generated complexed amplification patterns with one, two, three or even four peaks obtained for each marker.

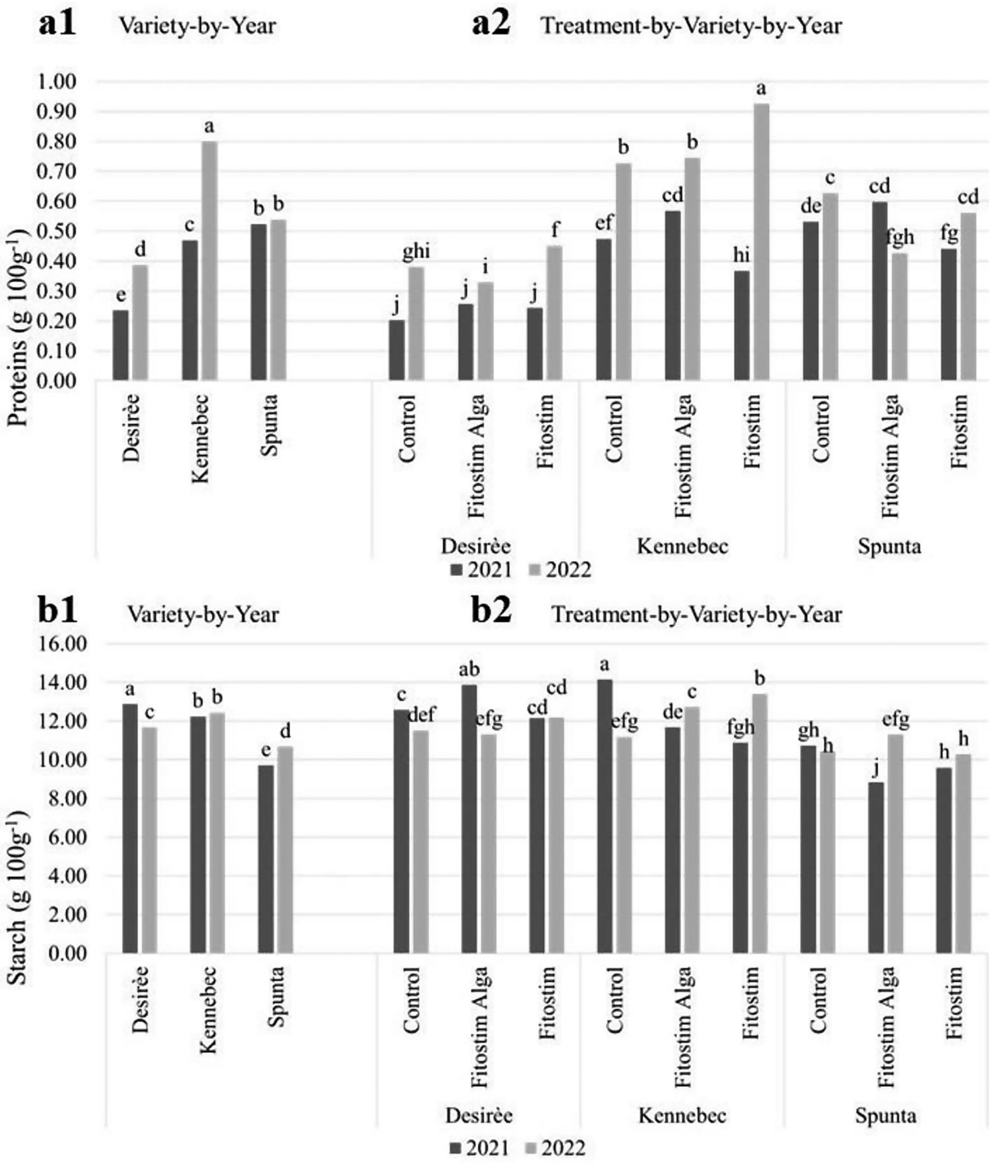

**Fig. 6** Interactions between factors for protein content (**a**) and starch contente (**b**) (g 100 g$^{-1}$). a1-b1 - Variety-by-Year have ***significant at 0.001. a2b2 Treatment-by-Variety-by-Year have ***significant at 0.001

Only for three tetra-nucleotide motif markers (16410, 4026/4027 and 12002), all retrieved from a study by Kishine et al. (2017), the amplification patterns were different for each genotype of Montese potatoes and enabled their unique identification. These three markers are thus a minimum set able to discriminate between the varieties considered in the present study (Table 8; Supplementary Figs. 8–10).

The other ten markers were either monomorphic (non-discriminative) in the present study or not able to distinguish all the Montese varieties from each other (partially discriminative). Partially discriminative markers can be however used for an integrative, double-check analysis due to good reproducibility and reliability of allele calling (Table 8).

## Discussion

Potatoes are a staple food in the European diet and are widely cultivated across diverse pedoclimatic conditions, from the Mediterranean regions to the Baltic countries (Levy & Veilleux, 2007). However, several factors can hinder potato production in mountainous areas (Pacifico, 2018). Upland ecotypes are generally less productive than the widely grown lowland varieties, making mountain potato farming less competitive. In Italy, for example, potato yields vary

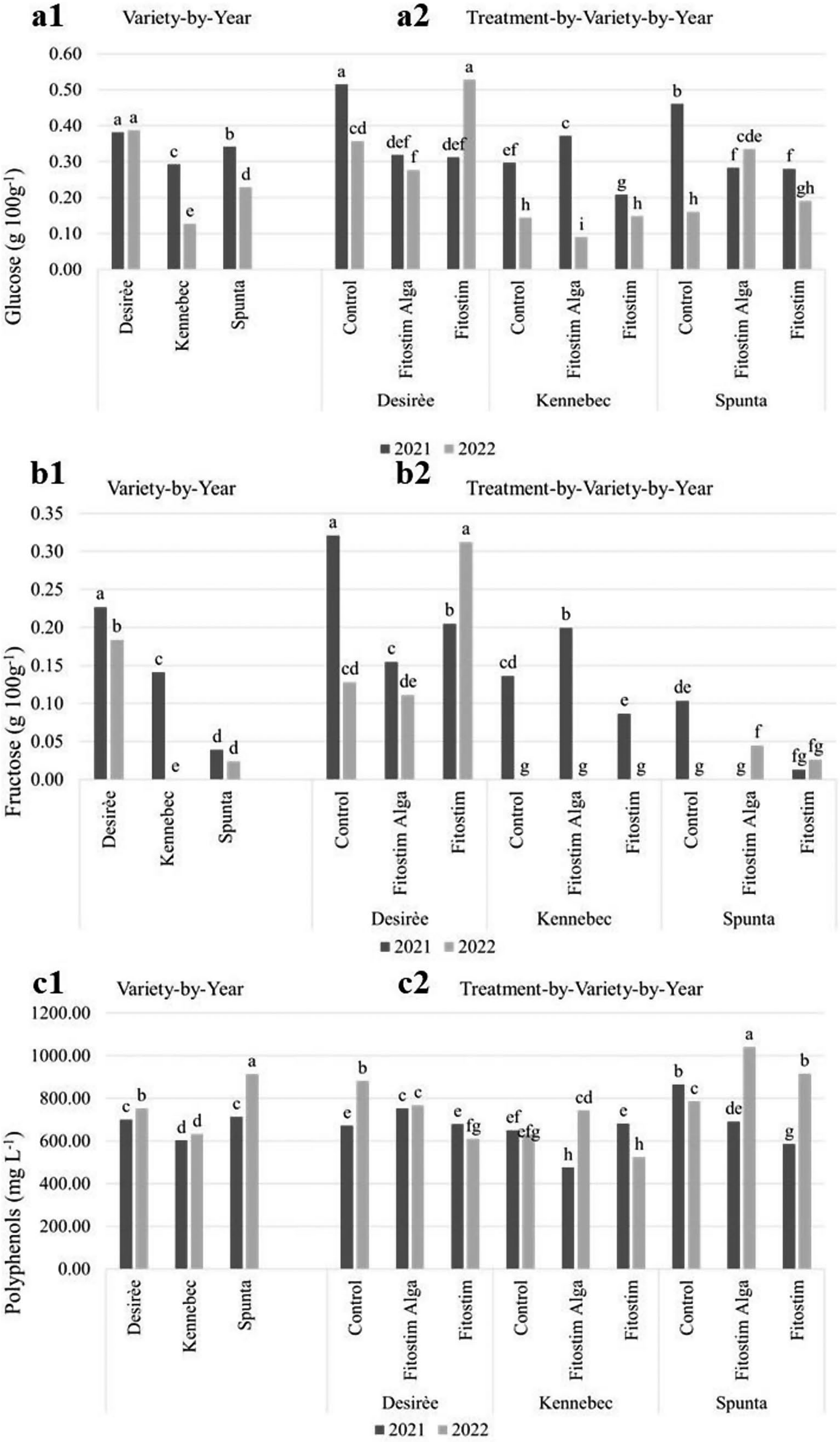

**Fig. 7** Interactions between factors for glucose (**a**) and fructose (**b**) content (g 100 g$^{-1}$) and polyphenol (**c**) content (mg L$^{-1}$). a1b1c1 Variety-by-Year have ***significant at 0.001. a2b2c2 Treatment-by-Variety-by-Year have ***significant at 0.001

**Table 7** Quality analysis of tubers

| | | Proteins (g 100 g$^{-1}$) | | Glucose (g 100 g$^{-1}$) | | Fructose (g 100 g$^{-1}$) | | Starch (g 100 g$^{-1}$) | | Polyphenols (mg L$^{-1}$) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Treatment (T) | Control | 0.49 | ns | 0.32 | a | 0.11 | a | 11.77 | a | 747.90 | a |
| | Fitostim Alga | 0.49 | ns | 0.28 | b | 0.09 | b | 11.62 | ab | 745.10 | a |
| | Fitostim | 0.50 | ns | 0.28 | b | 0.11 | a | 11.42 | b | 666.10 | b |
| | | | ns | | *** | | *** | | * | | *** |
| Variety (V) | Désirée | 0.31 | c | 0.38 | a | 0.21 | a | 12.27 | a | 727.20 | b |
| | Kennebec | 0.63 | a | 0.21 | c | 0.07 | b | 12.34 | a | 617.70 | c |
| | Spunta | 0.53 | b | 0.29 | b | 0.03 | c | 10.20 | b | 814.20 | a |
| | | | *** | | *** | | *** | | *** | | *** |
| Year (Y) | 2021 | 0.41 | b | 0.34 | a | 0.14 | a | 11.61 | ns | 672.70 | b |
| | 2022 | 0.57 | a | 0.25 | b | 0.07 | b | 11.59 | ns | 766.70 | a |
| | | | *** | | *** | | *** | | ns | | *** |
| Treatment-by-Variety | | | *** | | *** | | *** | | ** | | *** |
| Treatment-by-Year | | | *** | | *** | | *** | | *** | | *** |
| Variety-by-Year | | | *** | | *** | | *** | | *** | | *** |
| Treatment-by-Variety-by-Year | | | *** | | *** | | *** | | *** | | *** |

Data are presented as mean (Treatments = mean of 27 plants; Variety = mean of 27 plants; Year = mean of 45 plants). Different letters indicate statistically significant differences among varieties. *significant at 0.05; **significant at 0.01; ***significant at 0.001

significantly by region, with lower yields in the South and mountainous areas (21.7 t/ha) and higher yields in the north-eastern plains (44.6 t/ha) (ISMEA-ISTAT, 2008). In Emilia Romagna (North-East of Italy), two primary areas are known for high-quality potato production: the fertile Po River Valley in Bologna and Ravenna provinces, and scattered areas in the Apennine mountains, at elevations between 600 and 1,300 m above sea level. In these mountainous regions, ensuring crop productivity and quality is crucial for farmers' remuneration and consumer satisfaction. The common traits desirable in mountain potato varieties include high productivity, low irrigation needs, medium-late to late maturation, and suitability for local culinary traditions. This study evaluated three of the most cultivated potato varieties exposed to two plant biostimulants in order to improve, in a sustainable way, the yield and quality of production of potatoes cultivate in marginal areas, such as Tuscan-Emilian Apennines.

Plant biostimulants are products suitable for eco-friendly agriculture systems, easily degradable and effective at low concentration (Ambrosini et al., 2022). In particular, seaweed extracts are rich in bioactive substances such as phytohormones, amino acids, vitamins, betaine, lipids which can stimulate chlorophyll biosynthesis and plant development (Asadi et al., 2022; Sharma et al., 2014). Whereas, hydrolysed proteins contain peptides and amino acids that are involved in chlorophyll synthesis, can interfere with the phytohormone balance, help the plants cope with abiotic stresses (Gendaszewska et al., 2023; Von Wettstein et al., 1995), and act as signalling molecules modulating plant metabolism (Buffagni et al., 2021).

The variations in weather conditions between the two years of the present study, particularly the differences in temperature and rainfall, significantly influenced the growth and development of potato plants. The water demand for the entire potato production cycle is approximately 500 to 700 mm (FAO, 2013). Potatoes grown in the northern Apennines are typically rainfed and during the study, the first-year experienced severe drought, while the second year had moderate stress conditions and the potato plants were more vigorous and greener, and the tubers were larger, regardless of genotype. Also, potato plants treated with plant biostimulants exhibited increased vigour and greenness due to enhanced leaf development and accumulation of chlorophyll pigments, confirming results obtained in other crops such as maize, wheat, and lettuce (Ambrosini et al., 2022; Asadi et al., 2022; Gendaszewska et al., 2023). However, in a study on three potato varieties (Denar, Lord and Miłek) grown in Poland over three growing seasons (Wadas & Dziugieł, 2020), no statistical difference in leaf chlorophyll content were not found after the application of two commercial products based on seaweed extracts (Bio-algeen S90 and Keplak SL). These contrast results could be due both to differences in formulation and environmental conditions. In fact, in the former case, the differences in extraction solvent composition, temperatures, time, and pH may have influenced the amount and type of biologically active substances in the formulation (Michalak & Chojnacka, 2014). In latter case, they confirms the results observed in our study, in which the efficacy of both plant biostimulants in increasing leaf chlorophyll content was influenced by weather conditions showing, in our study, a higher efficacy when the temperature and rainfall were higher. This suggests that severe drought stress may have decrease the potato crop's ability to utilize seaweed extract and hydrolysed proteins, potentially

**Table 8** Chromosome location, peak position (bp) and the heights of the peaks for each microsatellite markers performed on ABI PRISM

| Marker ID* | Chromosome | Genotype | Peak position (peak height) | | | |
|---|---|---|---|---|---|---|
| 4026/4027 | 1 | Désirée | | 329 (8894) | | 362 (6029) |
| | | Kennebec | 288 (8162) | | | 362 (4237) |
| | | Spunta | 288 (1352) | | 341 (1447) | 362 (583) |
| 8242 | 2 | Désirée | 85 (247) | 215 (238) | | |
| | | Kennebec | 85 (2651) | 215 (1489) | 236 (3249) | |
| | | Spunta | 85 (531) | 215 (124) | 236 (165) | |
| 12,002 | 3 | Désirée | 237 (1032) | 238 (2060) | 239 (1113) | 251 (737) |
| | | Kennebec | 237 (597) | 238 (601) | | 256 (209) |
| | | Spunta | 237 (644) | 238 (1376) | 239 (709) | 256 (711) |
| STI0012 | 4 | Désirée | 190 (667) | | | |
| | | Kennebec | 202 (1231) | 208 (525) | | |
| | | Spunta | n/a | n/a | n/a | |
| 16,410 | 4 | Désirée | | | 303 (1702) | 359 (486) |
| | | Kennebec | 289 (420) | | 303 (617) | 369 (805) |
| | | Spunta | 289 (872) | 301 (752) | 303 (936) | 369 (812) |
| STI0032 | 5 | Désirée | 126 (2973) | 139 (1911) | 142 (495) | 146 (1239) |
| | | Kennebec | 129 (182) | 139 (213) | 142 (170) | |
| | | Spunta | 129 (100) | 139 (56) | 142 (110) | |
| STM3009 | 7 | Désirée | 174 (36) | 180 (107) | | |
| | | Kennebec | 174 (1464) | 180 (1720) | | |
| | | Spunta | 174 (3956) | 180 (5413) | | |
| STM1016 | 8 | Désirée | 261 (9613) | 265 (8046) | 277 (9725) | |
| | | Kennebec | 261 (606) | 265 (156) | 277 (1094) | |
| | | Spunta | 261 (1321) | 265 (489) | 277 (1679) | |
| STM1104 | 8 | Désirée | 56 (58) | 187 (6839) | 191 (7087) | |
| | | Kennebec | 56 (2841) | 187 (9315) | 191 (8350) | |
| | | Spunta | 56 (268) | 187 (1927) | 191 (2694) | |
| 35,584 | 9 | Désirée | 105 (1104) | 112 (4794) | 124 (2114) | |
| | | Kennebec | | 112 (4665) | | |
| | | Spunta | | 112 (5040) | | |
| STM1106 | 10 | Désirée | 176 (8998) | | | |
| | | Kennebec | | 211 (2003) | | |
| | | Spunta | 176 (809) | | | |
| STM2028 | 12 | Désirée | 62 (8308) | | | |
| | | Kennebec | 62 (8424) | | | |
| | | Spunta | 62 (8407) | | | |
| 46,514 | 12 | Désirée | 150 (7038) | 180 (2879) | | |
| | | Kennebec | 150 (1645) | 180 (1637) | | |
| | | Spunta | 150 (1310) | 180 (803) | | |

leading to closure of stoma and reduced the cuticle permeability. These data confirmed that plant biostimulants based on seaweed extracts has a higher efficacy on potato crop during warm and very wet growing seasons (Wadas & Dziugieł, 2020), suggesting a similar behaviour also for hydrolysed proteins. In fact, while plant biostimulants can promote growth under various conditions, their effectiveness is optimized under specific environmental settings (Bulgari et al., 2019).

An increase in plant height after the application of seaweed extracts on the leaves of potato plants was previously reported in other studies (Garai et al., 2021; Pramanick et al., 2017). In our study, significant effects were also found after hydrolyzed protein treatments. Hydrolyzed proteins and seaweed extracts are, in fact, rich in free amino acids such as tryptophan, which is a precursor of indole-3-acetic acid, a phytohormone involved in cell division and elongation, and thereby in plant growth (Consentino et al., 2020).

Tubers' shape, size, and lack of blemishes are crucial for consumer preference (Bahadirov et al., 2020). In the present study, no misshapen or blemished tubers in any variety were found in any variety. All varieties produced few small or very small tubers and many tubers with diameters greater than 6 cm. In general, a negative relationship between the number of tubers and their weight is commonly reported (Nouri et al., 2016). In the present study, Fitostim® Alga and Fitostim® were able to modify yield components, such as tuber size and weight, without modifying or compromising the yield. The effect of plant biostimulants in increasing tuber size and decreasing tuber weight of plant biostimulant products could be linked both to low availability of essential nutrients, such as nitrogen and phosphorus, in the soil, and to cytokinin accumulation in stolons during tuber formation and tuberization (Saidi & Hajibarat, 2021). In the Montese area of cultivation, the use of organic fertilizer does not make essential nutrient readily available to potato plants since microorganisms must first mineralize these nutrients. On the other hand, hydrolyzed animal proteins have already been observed to increase cytokinin (trans-zeatin) content in tomato leaves (Casadesús et al., 2019), and seaweed extracts to upregulate cytokinin-related genes in maize (Trivedi et al., 2021), suggesting a role in cytokinin balance also in potato tubers. Thereby, hydrolysed proteins and seaweed extracts sprayed on leaves can affect tuber formation but their direct impact on yield appear much more complex and influenced by factors such as environmental conditions, genotype characteristics and species. For instance, the same formulations applied under similar pedoclimatic conditions (Italian Northern Apennines) increased lavandin crop yield (Caccialupi et al., 2022), while recently, Fitostim® Alga application have decreased cucumber yield in greenhouse cultivation (Zamljen et al., 2024).

Considering the genotype effect on yield components, Désirée was the most productive variety with a higher number and weight of tubers suggesting that some varieties may be better suited to certain environmental conditions. Studies by Levy (1986) and Ávila-Valdés et al. (2020) also indicated that Désirée can tolerate slight drought stress, and thus a higher suitability for rainfed cultivation as in mountain agricultural system.

Beside shape and weight, the biochemical characterization of potato tubers is a key aspect of their quality. In this study, the biochemical analysis of tubers showed genotype, plant biostimulants and environmental conditions can influence the nutritional profile of potatoes. Much attention has been given to the potato carbohydrate content, as it is a primary source of energy in the human diet. In potatoes, carbohydrate content is mainly due to starch which, besides supplying metabolic energy, can influence both sensory features and the shelf life of potato products and may be used as a food ingredient or as an industrial raw material (Dupuis & Liu, 2019). Our results showed a variation of 2 g in starch content among the three varieties, highlighting a strong influence of genotype and weather conditions. These results confirmed the data obtained by Lombardo et al. (2017), which found a difference of 17.7% in starch content among seven genotypes grown in plain conditions. When the performance of the Spunta variety was compared in the two studies, contrasting results were observed, highlighting that the environment (mountain versus plain) can play an important role in affecting the quality of potato tubers in terms of starch content, and the importance of developing studies in specific pedoclimatic conditions to find tailored genotypes. Considering the plant biostimulants applications, the effects varied depending on environmental condition and variety as found also by Wadas and Dziugieł (2020).

Besides starch content, the other carbohydrates present in potatoes are simple sugars that play a key role in determining the quality of French fries and chips. When potatoes are fried at high temperatures, the Maillard reaction between sugars and free amino acids in the tubers produces a brown color, a bitter taste, and a toxic contaminant known as acrylamide (Kumar et al., 2004). For these reasons, it is very important to select genotypes with a low concentration of sugars to reduce the content of acrylamide in final products based on French fries (Amrein et al., 2003). In this study, glucose was the most abundant reducing sugar in potato tubers, with a significant difference in concentration among varieties, suggesting genotype effects on sugar content in tubers. Our data pointed out Kennebec as the best variety, regardless of the growing seasons, for the production of French fries due to a lower amount of reducing sugars and, consequently, a lower probability of generating toxic acrylamide. These data are consistent with the data Głosek-Sobieraj et al. (2022), which linked the content of total reducing sugars to flesh color and found lower levels in cream- and yellow-fleshed varieties (such as Kennebec). In the same study, the content of reducing sugars in potato tubers was increased by plant biostimulant treatments based on microorganisms, seaweed extracts, and plant natural nitrophenols. Interestingly, in our study, the effect of plant biostimulants on glucose was the same as on fructose, and the reducing sugar content was consistently decreased by seaweed extracts application in genotype with the higher content of these compounds (Désirée). On the other hands, contrasting results were observed in the genotypes with lower content of reducing sugars (Spunta and Kennebec), depending on weather conditions. Garai et al. (2021) found that the effect of plant biostimulants in decreasing the amount of reducing sugar in potato tubers depended on both the concentration of plant biostimulants and content of potassium inside potato tubers. However, these factors were not evaluated in our study.

Considering protein tuber content, results obtained in this study revealed a protein content stable in Spunta and variable in Kennebec and Désirée depending on weather conditions. In general, the protein tuber content observed was lower than values found in other papers, probably due to lower nitrogen supply, different agricultural management (Burlingame et al., 2009; Galdón et al., 2012; Lombardo et al., 2017), and pedoclimatic conditions. According to data

Głosek-Sobieraj et al. (2022), no significant differences in protein amount were found in tubers after plant biostimulant treatments. However, when the effect of plant biostimulants was considered for individual variety *per* growing season, an improvement in protein content, depending on weather conditions, was found in Désirée and Kennebec varieties treated with hydrolysed proteins.

Among human health-promoting substances, polyphenols are certainly one of the main groups of substances studied and sought in foods. Polyphenols can be involved in different biological activities; they are considered antioxidants and have an important protective role against cardiovascular diseases, cancer, and neurodegenerative disorders (Swer et al., 2019). In fresh potatoes, the content of polyphenols may range from 53.0 to 1098.0 mg kg$^{-1}$, and phenolic acids, flavonoids, and anthocyanins are the main molecules found (Mystkowska et al., 2020). A study on native Andean potato tubers (*Solanum tuberosum* L.) reported a significant variation in polyphenol content among the analyzed varieties (Andre et al., 2007). Hamouz et al. (2013) linked these differences to the color of the potato parenchyma. In our samples, a medium-high concentration of polyphenols was found in all the varieties, and Spunta tubers showed a steady high concentration of these bioactive substances, increasing the quality of tubers and the probability of attracting diet/health-conscious consumers.

Considering the plant biostimulant effects on polyphenols, these were influenced both by variety and weather conditions., according to a study by Mystkowska et al. (2020) that evaluated the application of 4 plant biostimulants (Kelpak SL®, Titanit®, Green Ok®, BrunatneBio Złoto ®) on 3 potato genotypes (Jelly, Honorata, Tajfun) in Poland in open field. Higher quality tubers are not always rewarded with higher income for farmers, and the lack of an increase in yield could discourage plant biostimulant use by farmers. Currently, the price of plant biostimulants applied in this study is about 4 € kg$^{-1}$ (data provided by the manufacturer). Following the label indication of three applications of a single plant biostimulant product per season, the total cost is around 438 € ha$^{-1}$ (24 € ha$^{-1}$ for the products and 438 € ha$^{-1}$ for three sprayer applications). However, Fitostim® Alga and Fitostim®, as reported on the label, could be applied together with normal insecticidal and fungicidal molecules without problems, except for products based on copper; in this way, it is possible to reduce the cost of application to 24 € ha$^{-1}$ and make the intervention economically feasible for farmers.

Mountain farmers, usually small-scale producers, acquire seed tubers from different suppliers. To maintain traceability and genetic identity from year to year, it is necessary to develop a suitable molecular tool. As reported in many studies, microsatellite markers have demonstrated high polymorphism and competence in characterizing potato cultivars and accessions, compared to other marker systems (Barandalla et al., 2006; Ghislain et al., 2006; Norero et al., 2002). Microsatellite markers, also known as SSR markers, are short sequence repeats distributed over the genomes that undergo frequent variation in the number of repeated units; this variability makes SSRs efficient markers to distinguish potato varieties and establish DNA fingerprinting. Although random amplified polymorphic DNA markers (RAPD) have been widely used for potato genotype studies, SSRs have proved to be more efficient for potato variety identification/discrimination. In fact, in a study by Rocha et al. (2010), where SSR and RAPD markers were used to characterize 16 potato varieties, a set composed of six RAPD primers was necessary for the discrimination of all the potato varieties evaluated in the study, while a set of only three SSR markers proved sufficient. SSR markers also proved to be good indicators of ploidy for potatoes (Ghislain et al., 2006). SSR markers can be considered attractive due to their abundance in the genome, relatively simple technical requirements, and the availability of primers and protocols in published literature.

In the present study, among all the markers tested, three SSR markers (16410, 4026/4027, and 12002) belonging to the robust and informative microsatellite-based genetic identity set presented in the Kishine et al. (2017) study were able to discriminate the three potato varieties of Montese and thus constitute a minimum microsatellite set with high discriminatory power. The application of this set of three markers generates a characteristic and unique electropherogram profile for each of the varieties under study. Microsatellite markers STM1016, STM1104, and STM1106 were already applied in many studies conducted on American, South American, and European potato varieties (Favoretto et al., 2011; Ghislain et al., 2006; Milbourne et al., 1998); however, they were not able to fully discriminate the three varieties analyzed in the present study (Table 6). At the same time, STM1106, together with 16,410, 4026/4027, and 12,002, were the only SSR markers in this study able to distinguish Kennebec and Spunta genetic profiles, even if Kennebec and Spunta do not have a common origin and pedigree, nor similar morphological traits (Table 1). Molecular characterization gained from this study supports the use of SSR markers for the molecular identification and characterization of potatoes cultivated in Montese. The specific microsatellite set found for the varieties widespread in the Montese area could be available as a tool for routine potato identification, for maintaining genetic identity and consequently phenotypic identity and stability, to guarantee specific characteristics and quality to consumers and producers and to avoid random mislabelling. At the same time, the study conducted on morpho-physiological and qualitative parameters could provide important knowledge of production and variety performance in mountain conditions, for creating a more aware and sustainable production system.

## Conclusions

This study has important implications for potato cultivation and contributes to raise awareness on the use of protein hydrolysate and seaweed extracts in managing the crop and highlighting their potential to enhance growth and quality in mountain environments. The evaluated genotypes and seasonal variability influenced the efficacy of protein hydrolysate and seaweed extracts suggesting that farmers should consider the variety and the specific environmental conditions when applying biostimulants to optimize the whole crop performance. Additionally, the positive effects of protein hydrolysate and seaweed extracts on nutritional quality traits such as the reducing sugar content suggest potential benefits for consumer health and market value of potatoes.

While this study provided valuable insights, the experiment was conducted over two growing seasons only and focused its attention to three potato varieties. Therefore, future research should include a larger number of diverse genotypes and a long-term evaluation to validate and expand upon our findings. Future research should also focus on elucidating the mechanisms underlying the observed interactions between biostimulants, genotypes, and environmental conditions.

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## Declarations

**Competing Interests** Not applicable.

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