ITALUS HORTUS
Vol. 32 (2025), Pages 1-15
doi: 10.26353/j.itahort/2025.2.115
Original Research Paper

Biostimulant effect of apple and potato by-product extracts on viability and germinability of fresh and stored pollen in several fruit species

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Received: 25 February 2025; Accepted: 13 July 2025; Available on line: 28 November 2025

Abstract: The recovery of waste from agri-food industry, representing a valuable source of bioactive compounds, intends to promote a circular economy, fostering more sustainable development and reducing environmental impact. In this context, application of biostimulants based on natural materials has proved to have a positive impact on plant growth parameters. This study aimed to evaluate the potential biostimulant impact of aqueous extracts derived from apple and potato by-products on the *in vitro* germination of fresh and stored pollen from six fruit species (almond, apricot, hazelnut, olive, pear, and pomegranate). Thus, potato and apple extracts (PE and AE), firstly characterized for mineral composition, total antioxidant capacity and total phenols, were added to the basic germination medium at 1 mgL^{-1} and 10 mgL^{-1} concentrations. Results showed that, while the AE did not give positive effect in most genotypes, the PE at the lowest concentration improved the pollen performance. In particular, the PE was able to restore pollen germination ability in cultivars that did not retain a good viability after storage. This booster effectiveness could represent a promising tool to develop new biostimulants for improving the fertilization process, offering an opportunity to enhance the utilization of byproducts, leading to a reduction in the environmental impact of potato processing industries.

Keywords: Almond; Apricot; Hazelnut; Olive; Pear; Pomegranate.

1. Introduction

The processing of agricultural raw materials results in a significant volume of organic waste, which entails additional costs for food industries. These wastes must be managed, stored, and disposed of in accordance with prevailing regulations, both at the European and national levels (L.166/2016 and Directive 2008/98/EC).

Processing waste from fruits and vegetables agri-food industry mainly contain leaves, peels, seeds, and pulp which represent a valuable source of bioactive compounds, including phenols, proteins, alkaloids, sugars, and lipids (Cassani and Gomez-Zavaglia, 2022). Their recovery aims to promote a circular economy, transitioning from 'waste to wealth', fostering more sustainable development and reducing environmental impact. In the context of a green-agriculture, promising products, originated from several organic matrices, have been proposed as an alternative to chemical treatments (du Jardin, 2015). These new products, called biostimulants, have heterogeneous compositions including humic acids, protein hydrolysates, and several plant extracts (Povero et al., 2016; Bulgari et al., 2017; Rouphael et al., 2018). To obtain the natural products from raw materials, conventional methods, including unsafe solvents extraction, are the most widely used (Zhang et al., 2018). In alternative, new green technologies, such as cryoextraction, have been successfully tested on different plant matrices (Bartolini et.al., 2022).

Recently, the extraction based on water-maceration method of raw plant material was exploited as a simple, cheap, and sustainable innovative methodology (Bulgari et al., 2017).

Among vegetable and fruit processing industries, those of potatoes and apples generate different types of waste. Concerning potatoes, the largest portion of discarded materials is represented by the peels, which are a source of very interesting substances that could be employed as antioxidants, precursors of steroid hormones and dietary fibres (Schieber and Aranda Saldaña, 2009). Similarly, the apple sector produces wastes resulting from apple processing into juice, puree, canned, dried, or frozen products (Shalini et al., 2010). In particular, from apple juice manufacturing the so-called pomace is obtained, which is a solid residue consisting of a complex mixture of peel, core, seed, calyx, stem, and soft tissue (Vendruscolo et al., 2008). Similarly to potato waste, apple residues represent a valuable source of compounds used for nutritional, pharmacological, or cosmetic purposes, being rich in dietary fiber and polyphenols (Asma et al., 2023). Taking into account the characteristics of potato and apple waste, they have been evaluated as appropriate matrices for producing biostimulants for agricultural use demonstrating a positive impact on the growth parameters of lettuce plants in hydroponic cultivation (Orlando et al., 2021).

The application of biostimulants based on natural materials has proved to be efficient on pollen biology in promoting the germination process (Sabir, 2015; Hine-Gómez et al., 2020). In this respect, biostimulants have the potential to improve the viability of pollen grains, thereby enhancing stress tolerance under unfavorable conditions. Many natural extracts, such as flower organs and seaweed derivatives, have been effective in improving the germination process, also accelerating the rate of pollen tube growth (Jayaprakash, 2018) and the observed positive effects are attributed to the presence of substances such as hormones, vitamins, proteins, lipids, and enzymes.

Throughout the flowering period, plants are subjected to various stresses, such as fluctuations in temperature, which can substantially diminish pollen quality, with negative impact on the reproductive phase and consequently, adversely affecting crop yields (Sotomayor et al., 2012). Even, the maintenance of viability and germination capability is a crucial aspect for a long-term use of pollen. Although time and conservation conditions are genotype-specific, pollen samples are usually stored under chill or freezing temperatures, mainly for breeding purposes (Martínez-Gómez et al., 2002; Beltran et al., 2019; Calic´ et al., 2021). In this context, ensuring an optimal germination level is a key factor for a successful pollination in controlled crosses and in artificial or supplementary pollination too. Thus, bioactive substances of plant origin might be usefully employed on stored pollen, which may experience a loss of viability and a reduction in germination capacity due to genetic variations, storage duration, and conditions.

On these premises, the current study aimed to evaluate the potential biostimulant impact of extracts derived from apple and potato by-products on the *in vitro* germination of fresh and stored pollen from various fruit species.

2. Materials and Methods

2.1. Preparation and characterization of potato and apple extracts

Potato and apple wastes, peels and pomace respectively, were used to obtain aqueous extracts. Potato peels, with a thin periderm layer (about 0.5–1.0 mm), were manually collected by means of a knife from organic red potatoes (*Solanum tuberosum* L., cv. 'Blue Salad'). The solid residual pomace (mixture of skin, pulp, seeds) of organic apples (*Malus domestica* Borkh, cv. 'Fuji') were saved from a juice extractor. Both products, at ready-to use stage, were purchased from a local large-scale retail. The waste materials were minced, macerated in deionized water (500 g L^{-1}) over 14 days, in the dark, at room temperature (20 °C ± 2). Three separate potato and apple waste extractions of 5 L each were performed. The aqueous extracts were centrifuged (10.000 rpm x 5 min.), filtered with cellulose filter, properly diluted in water to 1 or 10 mL L^{-1} (Orlando et al., 2021) and stored at -20 °C before being utilized for treatments. The pH values of all aqueous extracts were measured. Chemical analysis for the

extract characterization were performed in samples taken from each extraction (three biological replicates in total) after keeping the extracts under agitation for 5 minutes.

The following mineral elements were analyzed: Mg, Zn and K contents by atomic absorption spectroscopy (Varian Model Spectra AA240 FS, Agilent Technologies Australia Pty Ltd., Mulgrave, Australia), and P-PO_4 content by UV/VIS spectrometry according to Olsen and Sommers (1982). The N-NO_3 content was also measured spectrophotometrically, using the salicylic-sulphuric method (Cataldo et al., 1975). The amount of total phenols was determined by the Folin- Ciocalteu phenol reagent, according to the method reported by Kang and Saltveit (2002). An aliquot of 100 µL methanol extract was mixed with 2.0 mL of distilled water and 300 µL of Folin-Ciocalteu phenol reagent. The mixture was incubated for 4 min at room temperature (22–24 °C) and then 7.5% sodium carbonate (1.6 mL) was added. The mixture was stored in the dark at room temperature for 2 h. and the absorbance was then measured at 765 nm. For calibration, standard solutions of gallic acid (0–500 mg L^{-1}) were prepared. The concentration of total phenols was expressed in gallic acid equivalent as milligrams of gallic acid (GAE) per gram of fresh weight (mg GAE g^{-1} FW).

Total antioxidant capacity was determined by the test of ferric reducing antioxidant power (FRAP) according to Benzie and Strain (1996). Ferric to ferrous ion reduction at low pH causes a colored ferrous-tripyridyltriazine complex. FRAP values were obtained by comparing the absorbance change at 593 nm in test reaction mixtures. For this assay, 2.0 mL of acetate buffer (0.25 M, pH 3.6), 900 µL of freshly prepared FRAP reagent (2 mM ferric chloride and 1 mM 2,4,6-tris(2-pyridyl)-s-triazine in acetate buffer), and 100 µL plant methanolic extract were used. Calibration was performed using a standard curve prepared with ammonium ferrous sulfate standard solutions. The results were expressed as micromole of Fe^{2+} equivalents per gram of fresh weight (µmoL $Fe^{2+}g^{-1}$ FW).

2.2. Plant material

The study was performed in 2019 using healthy and productive trees of cultivars belonging to six fruit species (Table 1). Two trees per cultivar were selected within a germplasm collection grown at the experimental orchards of the Department of Agriculture, Food and Environment (University of Pisa) located in Pisa province (Tuscany, Italy, 43°43′32.02’ N, 10°27′37.66’ E; altitude 3 m a.s.l.). All trees, 6-7 years old, 5 x 5 m spaced and trained to a free vase, were subjected to standard agronomic practices (dip-irrigation, fertilization, pests and diseases treatments).

Table 1. Fruit species, cultivars, rootstocks, pollen collection date (JD, Julian Days) and storage time (months).

Species	Cultivar	Rootstock	Collection (JD)	Storage (months)
Almond (*Prunus dulcis* L.)	Pizzuta d’Avola	GF 677	58	12
Apricot (*Prunus armeniaca L.)*	Sillari	Myrabolan 29C	79	11
Hazelnut (*Corylus avellana* L.)	San Giovanni	*Corylus colurna* L.	42	12
Olive (*Olea europaea* L.*)*	Leccino	Own-rooted	141	9
Pear (*Pyrus communis* L.)	Abate Fetel	BA/29	100	10
Pomegranate (*Punica granatum* L.)	Wonderful	Own-rooted	165	8

2.3. Pollen collection

Before the beginning of flowering, an adequate number of shoots with hermaphrodite flowers, and, for the hazelnut, male inflorescences (namely catkins) were isolated by paper bags. In order to have a low humidity percentage, flowers at full blooming stage were collected in the central hours of sunny days. Anthers were directly brushed in sterile Petri dishes using a soft brush. Samples were divided into two aliquots to carry out evaluations on both fresh and cold-stored pollen. In this latter case, pollen was

dehydrated over silica gel at room temperature (20 °C), stocked in Falcon tubes and stored at +4°C over 8-12 months, depending on the genotype (Table 1).

2.4. Pollen viability assessment

The pollen viability was determined by a colorimetric reaction induced by the TTC (2,3,5 triphenyl tetrazolium chloride), a redox indicator of cellular respiration (Shivanna and Rangaswamy, 1992). By the TTC test is possible to identify a different staining of pollen grains in relation to their capacity to reduce the tetrazolium salts by dehydrogenases, producing an insoluble red formazan complex (Norton, 1966).

The pollen grains were uniformly distributed with a brush on microscope slides containing drops (120 µL) of 1% TTC diluted in a solution of 15% sucrose. The slides were covered with coverslips and then incubated for 24 h under dark conditions at 37 °C. After incubation, samples were observed under an optical microscope (Fluophot, Nikon Inc., Minato City, Japan) for estimating the intensity of pollen staining. A total number of at least 200 pollen grains, in four different areas of the same slide, were visually scored as follows (Eti,1991): i) dark-red: viable; ii) light-red: semi-viable; iii) yellowish (unstained): non-viable. Based on this staining score, the pollen quality was defined as good, uncertain, or null, respectively. Results were expressed as a percentage by the ratio between the number of stained pollen grains and the total number of grains.

For each genotype, the TTC test was performed on fresh pollen and repeated after the chill preservation time (Table 1). Before performing the test, the stored pollen was first rehydrated by placing samples in a humid chamber for one hour.

2.5. Pollen germination ability

Germination of pollen grains was performed through *in vitro* assays. On fresh pollens, preliminary tests were performed to assess the best germination medium for each considered genotype. Two different basic germination substrates were tested: i) a liquid culture medium, and ii) a solid one (0.65% w/v agar), both characterized by a minimal composition of 15% (w/v) sucrose in water solution at pH 6.9. Pollen grains were dusted on microscopic slides and plastic Petri dishes (5 cm diameter) for liquid and solid medium, respectively. They were then incubated in a growth chamber, in the dark, for 24-48 hours at 24 °C. Pollen was considered as germinated when the tube length was equal to or greater than the grain diameter (Stanley and Linskens, 1974) by observations using a light microscope (ZEISS Axio Imager 2, Jena, Germany) and an inverted microscope (Wilovert S, Helmut Hund GmbH, Wetzlar, Germany) for slides and Petri dishes, respectively.

As a result, specific germination substrates were selected for each genotype: the liquid medium was the most suitable for pomegranate and hazelnut, whereas for almond, apricot, pear and olive was the solid one. Thus, the experiments consisted of comparing the basic germination medium (C) with those where the potato (PE) and apple (AE) extracts were added, at 1 mgL^{-1} and 10 mgL^{-1} concentrations. The pH of the media supplemented with PE and AE were 5.0 and 3.95, respectively. Two microscopic slides and Petri dishes were used for each treatment. After incubation, pollen germination was measured on three randomly selected visual areas for each support. The germination rate was determined as the percentage of germinated pollen in a total of at least 200 grains per treatment.

2.6. Data analysis

The statistical analysis was performed using the package GraphPad Prism (version 10.00 for Windows, GraphPad Software, La Jolla, San Diego, CA, USA). Data are showed as means ± standard errors (SE). Prior to the statistical analysis, the percentage values were subjected to arcsine square root transformation. Student's *t*-test was used to compare the differences between two experimental groups. ANOVA was used to investigate the differences among treatments, and means were compared by the

Tukey's multiple comparison test. A regression analysis was performed to determine the relationships between pollen viability and pollen germination. The confidence level at $p \leq 0.05$ was considered as statistically significant.

3. Results

3.1. Characterization of potato and apple extracts

The chemical analysis on potato and apple extracts are shown in Table 2. The recorded pH levels fell within an acidic range, between 4.10 (AE) and 5.00 (PE); the antioxidant activity was similar in both extracts with FRAP values between 3.57 (PE) and 2.27 (AE) µmol Fe^{2+} mL, while the total phenols content significantly differed being 0.78 and 0.46 mg GAE/mL for PE and AE, respectively.

As concerns the mineral elements composition (Table 3), while no differences between the two types of extracts were observed for nitrate and Zn, the PE showed higher contents for the other elements. In particular, values were about two - and three-fold higher for potassium, magnesium and phosphorus, respectively.

Table 2. Values of pH, total antioxidant capacity (TAC) and total phenols (TP) of potato (PE) and apple (AE) extracts at 10 mL L^{-1}. Means ± SE (n = 3). The asterisk denotes significant differences between extracts (Student's *t*-test; $p \leq 0.05$).

Extracts	pH	TAC (µmol Fe2+mL^{-1})	TP (mg GAE mL^{-1})
PE	5.00	3.57 ± 0.38	0.78 ± 0.13 *
AE	4.10	2.27 ± 0.43	0.46 ± 0.07

Table 3. Minerals composition of potato (PE) and apple (AE) extracts. Means ± SE (n = 3). Asterisks denote significant differences between extracts (Student's *t*-test; $p \leq 0.05$).

Extracts	$N\text{-}NO_3$ (mg L^{-1})	$P\text{-}PO_4$ (mg L^{-1})	Zn (mg $L^{-1)}$	K (mg L^{-1})
PE	14.58 ± 0.09	4.92 ± 0.16 *	0.09 ± 0.01	40.73 ± 1.09 *
AE	14.22 ± 0.06	2.94 ± 0.11	0.09 ± 0.02	12.41 ± 0.30

3.2. Viability of fresh and stored pollen grains

The TTC staining test provided a clear identification of viable (dark-red), semi-viable (light- red) and non-viable (unstained) pollen grains, both in fresh and stored samples, as shown in Figure 1.

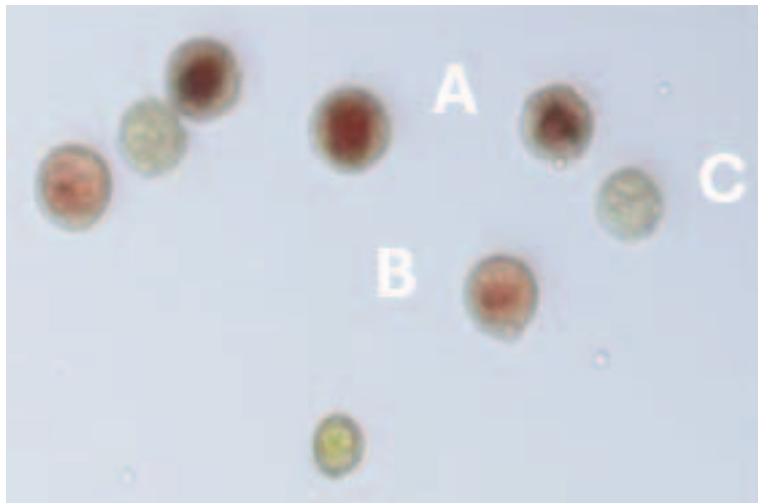

Figure 1. Viability of pollen grains by the TTC test: dark-red (A, viable), light-red (B, semi-viable), unstained (C, non-viable). Olive pollen grains (x 200).

Most of dark-red pollens were detected on fresh samples (Figure 2A), ranging from 60.1% (pomegranate) to 85.0% (almond). Light-red colored pollen grains, with uncertain germination aptitude, were from 5 to 25%, similarly to the unstained ones (5-20%) which are representative of non-viable pollens.

The long-term chill storage determined a strong viability loss in comparison with fresh pollens (Figure 2B). The most striking case was observed for hazelnut in which all the grains appeared unstained and, thus, non-viable. In pomegranate, apricot and olive, a two- to four-fold increase of light-red and unstained pollen grains was recorded, in a range of 65-85%. On the other hand, in almond and pear the percentages of light-red and unstained pollen grains did not change after storage: indeed, values for viable dark-red fresh pollen grains were maintained at about 85% (almond) and 70% (pear).

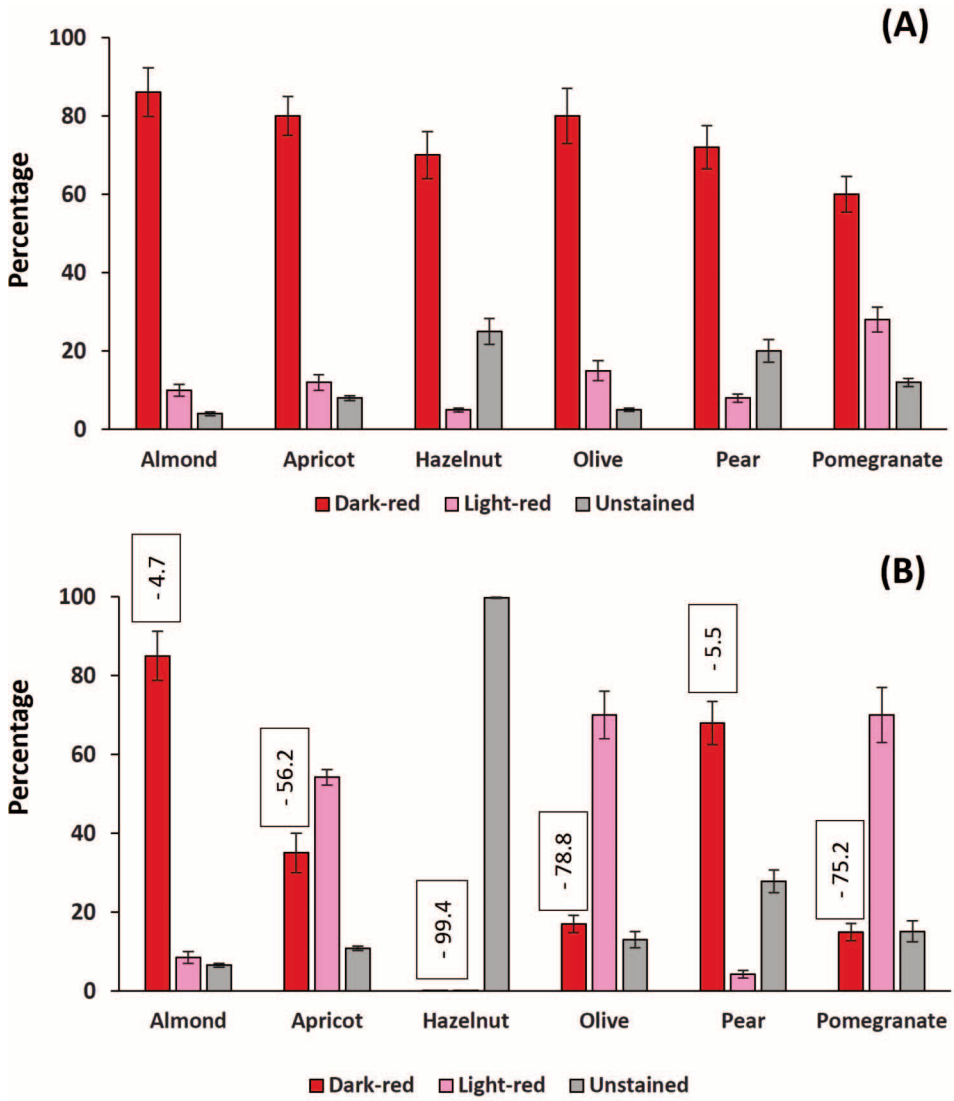

Figure 2. Average of the viability percentage (± SE) scored as dark-red, light-red and unstained grains in fresh (A) and stored (B) pollen samples. In plot B, the boxes above the dark-red bars show the decrease of viability percentage between fresh and stored pollen.

3.3. Germination ability of fresh and stored pollen grains

The mean germination values of fresh pollen, after 48 h of culture incubation, are shown in Figure 3. On the control medium, the germination rate differed widely, ranging from the lowest value 25.6% and the highest one 97.8% recorded for apricot and pear, respectively. Values recorded for the other species were 33.9% (pomegranate), 58.4% (olive), 60.5 (hazelnut), and 81.9% (almond).

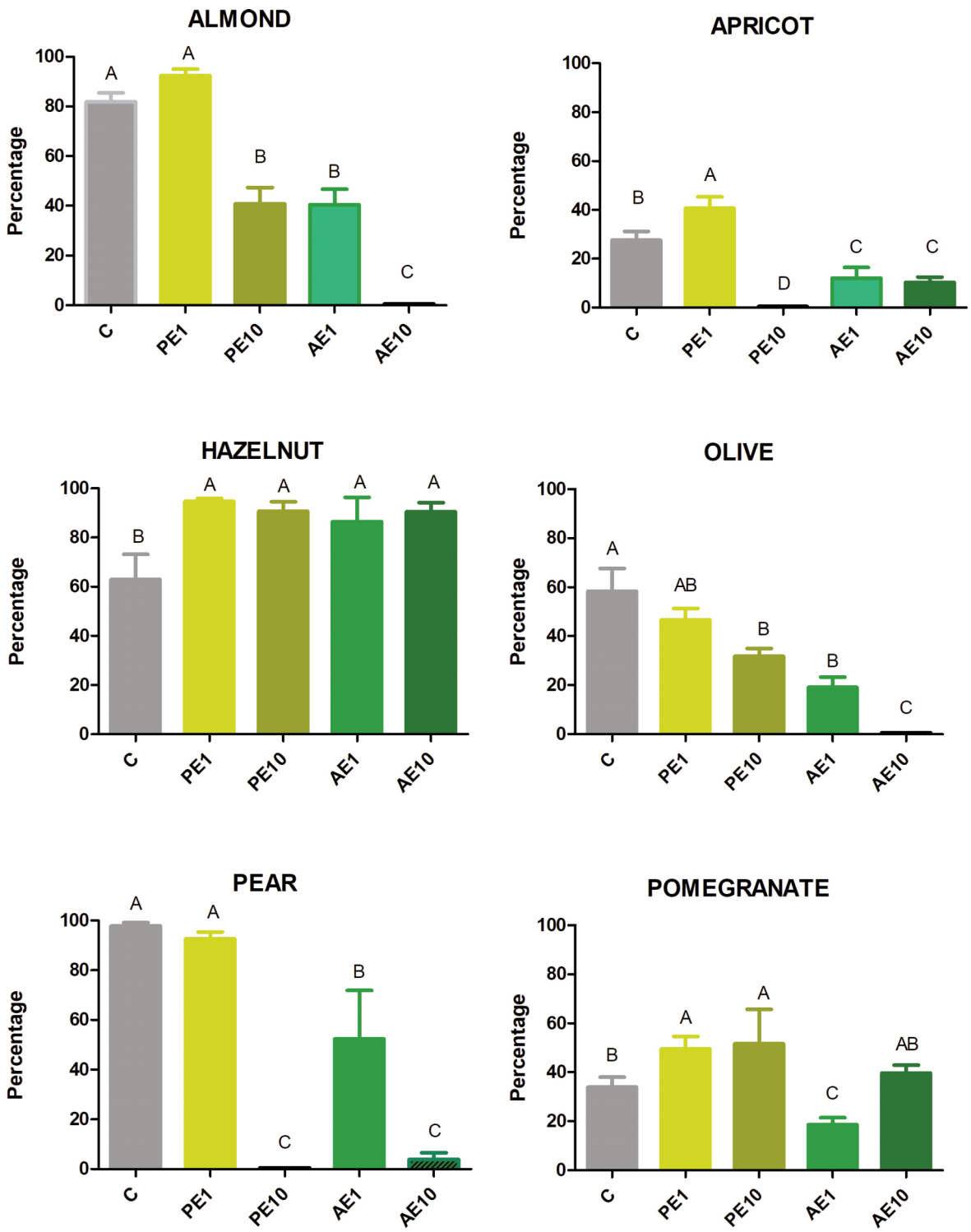

Figure 3. Fresh pollen grains: germination percentage recorded for almond, apricot, hazelnut, olive, pear and pomegranate assessed in control medium (C) and in media supplemented with potato (PE1 = 1mL L^{-1}; PE10 = 10 mL L^{-1}) and apple (AE1 = 1mL L^{-1}; AE10 = 10 mL L^{-1}) extracts. Means ± SE. Different letters indicate differences at $p \leq 0.05$.

To establish a possible relationship between germination ability and viability of fresh pollen (dark-red stained grains) recorded in control media, a regression analysis was performed for each genotype tested. As a result, positive correlations were found in all cultivars (Figure 4): the highest significance was recorded for almond, olive and pomegranate ($R^2 = 0.818 – 0.9327$).

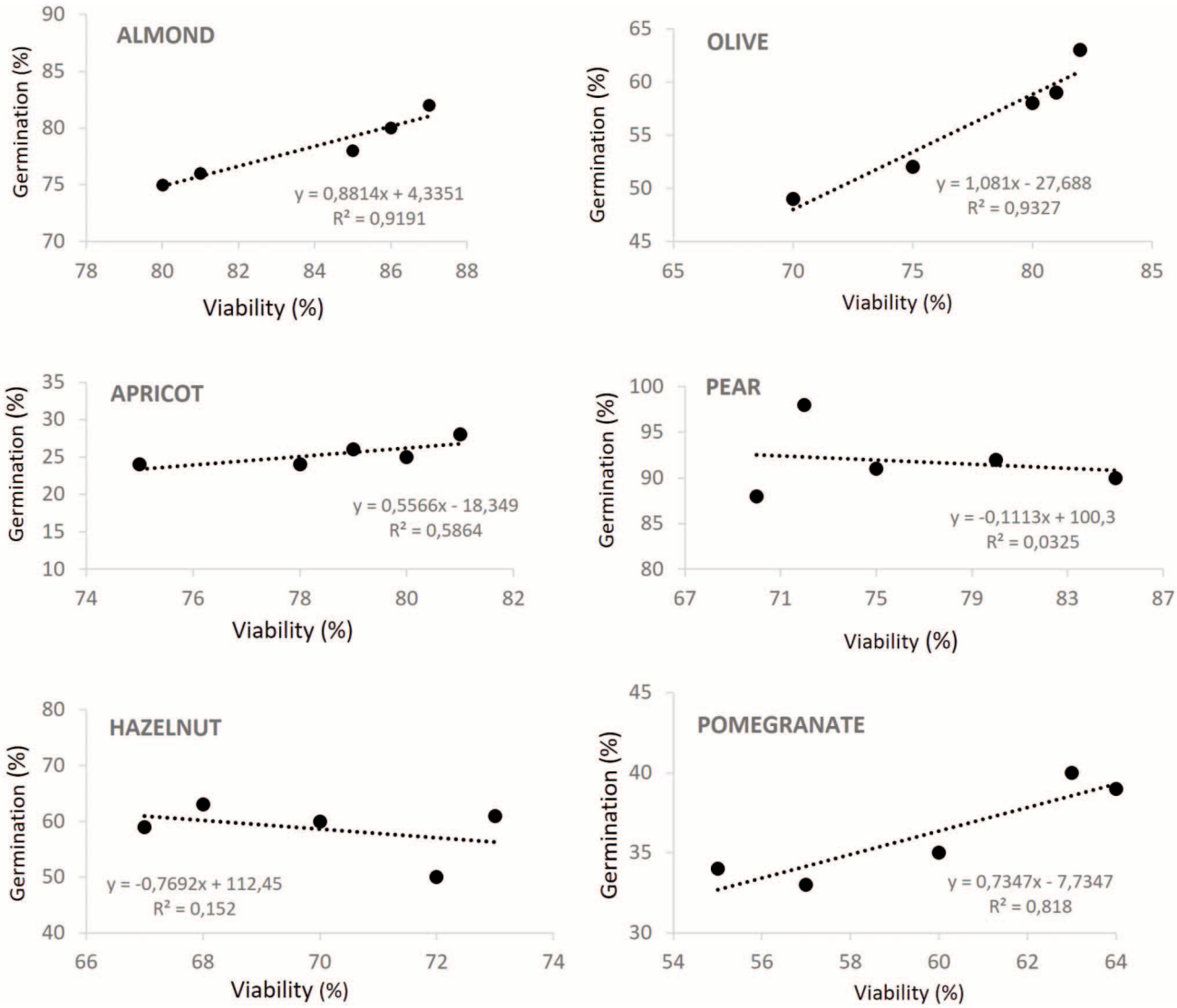

Figure 4. Relationship between percentages of viability (dark-red grains) and germination ability of fresh pollen grains recorded in control media for each species.

Concerning the pollen germination in media supplemented with PE and AE, different results emerged in relation to genotypes, extracts, and concentrations (Figure 3). Hazelnut showed the greatest benefit from both PE and AE, irrespective of the concentration. Indeed, the germination values obtained with the addition of extracts significantly exceeded those of the control medium. However, as a general trend, the AE had a detrimental effect on germination compared to the control substrate in the other genotypes. The PE at the lowest concentration (PE1) gave generally stimulating effects. On the contrary, as regards the PE10 concentration, the pollen germination was lower than control or null as in apricot and pear, whilst in pomegranate and hazelnut a similar positive effect to PE1 was observed.

In Figure 5, results are shown on the pollen germination ability recorded after a long-term preservation period (8-12 months at +4 °C). On the control medium, the chill storage determined a decrease of germination rates, ranging from less than 2% (hazelnut) to 60% (almond and pear). Comparing the mean values between fresh (Figure 3) and stored pollen (Figure 5), a wide decay range occurred as follows: 28.6% (almond), 29.5% (pomegranate), 38.4% (pear), 51.9% (apricot), 72.9% (olive), 98.6% (hazelnut).

The addition of PE and AE to the germination substrate gave different responses in relation to supplement and genotype. The supplement to the medium of AE at both concentrations resulted ineffective to restore the germination ability after pollen storage, leading to a significant reduction in pollen tube

emission. This was primarily evident at AE10, where, in most genotypes, the process was completely inhibited. The lowest concentration of PE (PE1) exhibited a positive impact on the germination ability of stored pollen across all genotypes. The strongest effect was recorded for hazelnut which was characterized by the lowest germination ability. Similarly, significant results were observed for apricot, with approximately three-fold increases in germination compared to the control. The PE10 was effective only for hazelnut. On the contrary, it had an inhibitory effect on almond, olive, and pear.

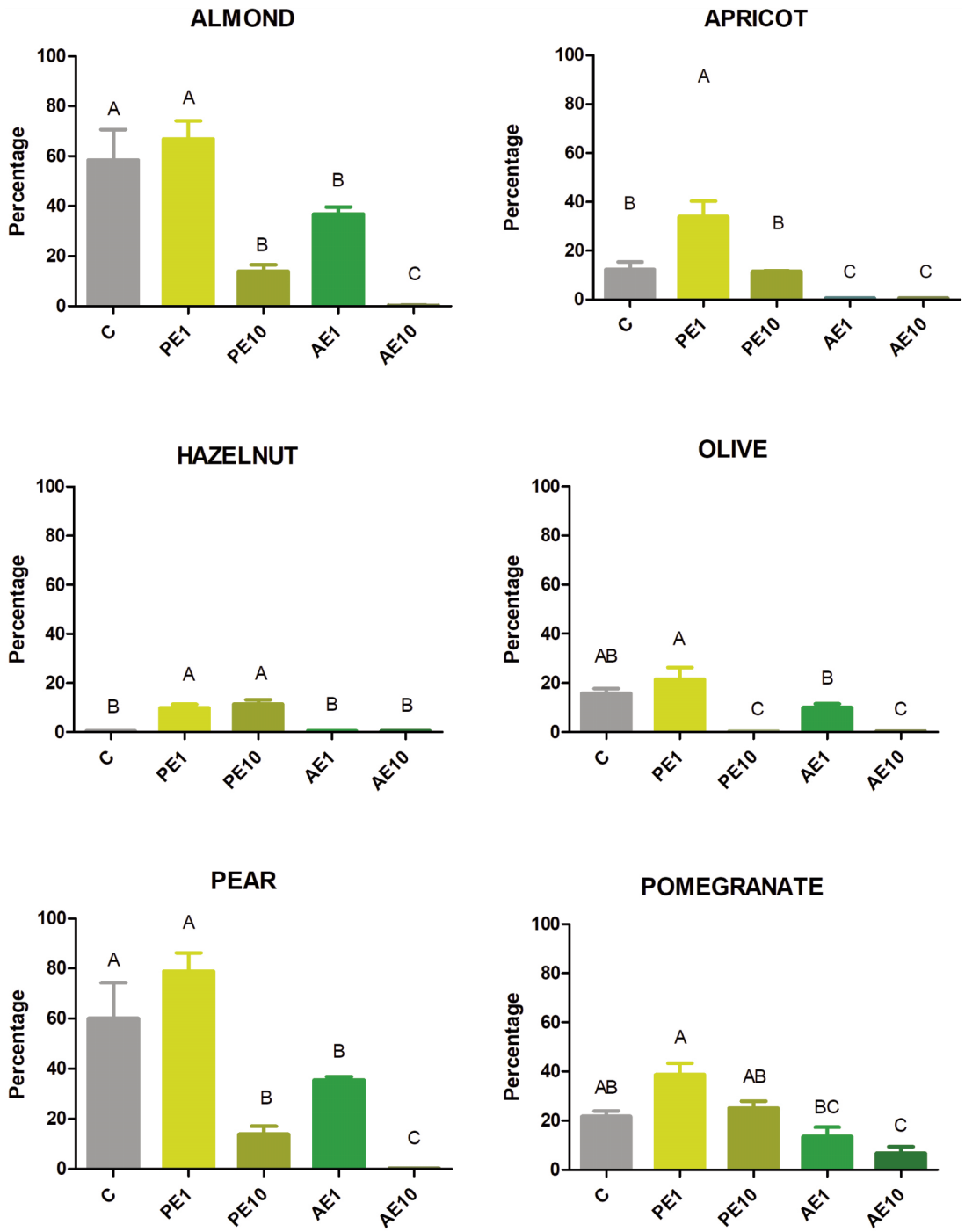

Figure 5. Stored pollen grains: germination percentage recorded for almond, apricot, hazelnut, olive, pear and pomegranate assessed in control medium (C) and in media supplemented with potato (PE1 = 1mL L^{-1}; PE10 = 10 mL L^{-1}) and apple (AE1 = 1mL L^{-1}; AE10 = 10 mL L^{-1}) extracts. Means ± SE. Different letters indicate differences at $p \leq 0.05$.

4. Discussion

The viability and germination ability of pollen depend on various factors, firstly species and cultivar (Nikkanen et al., 2000; Sharafi, 2011). The interaction with environmental conditions and nutritional status of trees can also strongly influence the pollen responses (Hedhly et al., 2005). A reduction in pollen functionality has been found under abiotic (i.e., heat, cold, drought) and biotic stresses, particularly during early stages of pollen development (Moreno-Alias and Rapoport, 2012). Moreover, lower levels of intercepted photosynthetically active radiation (PAR) within the canopy have been reported to negatively affect pollen viability and germination (Bartolini et al., 2022).

In vitro viability and germination tests are reliable controlled methods to evaluate the pollen quality, since they usually reflect the real fertilization ability (Dafni and Firmage, 2000). The TTC viability test conducted in this study on fresh pollen indicated that all examined genotypes exhibited promising viability rates, with dark-red grains ranging from 60% to 85%, consistent with findings reported by previous study (Bolat and Pirlak, 1999). Positive correlations between dark-red pollen grains and germination rates in control media were found for each genotype. Although the level of statistical significance varied across species, these results support the use of TTC staining as a reliable method to estimate the potential of pollen germination. This observation aligns with previous researches which have reported significant correlations between pollen stainability and *in vitro* germination or fruit set (Ferrara et al., 2007; Gallotta et al., 2014; Novara et al., 2017).

This study was designed to evaluate pollen germination responses in a minimal culture medium without the addition of micro- and macroelements to avoid interferences when potato and apple extracts were supplemented. Nevertheless, for the considered fruit species, the germination ability fell within ranges reported in previous studies for *in vitro* tests: almond (Maita and Sotomayor, 2015), apricot (Garcia et al., 1988), hazelnut (Okay and Ayfer, 1994), olive (Reale et al., 2006; Bartolini et al., 2022), pear (Kuroki et al., 2017), pomegranate (Gadže et al., 2011). So far, germination assessment through the *in vitro* method has proven to be a reliable tool, characterized by rapid and easy execution, high repeatability, and the ability to yield results consistent with actual pollen viability levels (Shivanna et al., 1991; Brandoli et al., 2024).

Germination and viability values were very similar in almond, pear and hazelnut. However, in apricot, olive, and pomegranate, the pollen germination rate was lower than the viability rate. This observation is consistent with the findings of Bolat and Pirlak (1999) and Khatun and Flowers (1995), who suggested that the discrepancy may arise because pollen grains that have lost their ability to germinate can still reduce tetrazolium salts.

The addition of apple and potato extracts to the standard germination medium elicited varying effects on both fresh and stored pollen. In general, the AE reduced the germination ability while the supplement of PE favored the germination process. The lowest concentration of PE was the most efficient inducing a greater and significant stimulus of fresh pollen in genotypes with the lowest germination ability such as the apricot (plus 38.0%) and pomegranate (plus 32.2%). A similar positive effect of PE was also observed in the germination of stored grains, which had lost viability to varying extents, ranging from about 10% (pear) to over 90% (hazelnut). In this latter case, the storage conditions (+4 °C; 12 months) resulted not appropriate, in agreement with Novara et al. (2017) which have evidenced that, for an efficient pollen viability conservation of several Piedmont hazelnut cultivars, the best temperature condition, for 5 months' term storage, was -30 °C. Intriguingly, the PE were able to restore the germination ability of this species. A similar beneficial effect was observed in apricot and pomegranate too, but only at the lowest concentration (PE1). About this, several reports have evidenced that a supply of certain bioactive substances were efficient on *in vitro* pollen germination but at extremely low concentrations (Hine-Gómez et al., 2020) while high doses strongly and significantly inhibited this process (Matsubara, 1981; Sabir, 2015; Aloisi et al., 2016; Çetinbaş-Gença et al., 2020).

The chemical analysis of the aqueous fraction of PE and AE evidenced substantial differences between them. About mineral ions, the PE were characterized by the highest content of potassium and

magnesium, inorganic elements that are structural components of many enzyme systems in plants (Millaleo et al., 2010) and are known to have stimulatory effects on pollen growth (Jayaprakash, 2018). A key mineral element for pollen function is also represented by zinc (Pandey et al., 2006) that, however, did not differ between the two types of extracts. Other biological active compounds as phenols were present in higher quantity in PE than AE which can contribute to maintain the quality of pollen grains and sustain the germination process. The addition of antioxidant compounds (such as flavonols and ascorbic acid) to the germination medium, even at very low concentrations, can enhance pollen germination by suppressing reactive oxygen species (ROS) generated during both chill preservation (You and Chan, 2015) and pollen germination (Ylstra et al., 1992; Smirnova et al., 2009).

In addition to organic and inorganic substances, the pH of *in vitro* culture media can influence pollen germination and pollen tube development: it has been demonstrated that an increase in the pH of the culture media stimulates pollen germination, whereas a progressively acidic medium, with a pH below 5.0, has been shown to have a detrimental effect (Van Ryn et al., 1986; Fragallah et al., 2019; Munzuroglu et al., 2003; Acar et al., 2010). About this aspect, with PE the pH values did not drop below 5.00, whilst the supplement with AE caused a stronger acidification of the germination media (pH 3.95). Thus, such a condition may have resulted in an ineffectiveness of AE for the pollen germination in most genotypes. Further, a better pollen response observed with PE could be due to the presence of key compounds, such as hormones, which are involved in stimulating the germination process. Given that metabolomic analysis on peels of yellow potatoes have shown the presence of auxins (Orlando, 2022), it is possible that this important phytohormone was present also in our extracts obtained by the peel maceration of red potato. In particular, the indole-3-acetic acid (IAA) has proven to be active in stimulating pollen tubes grow under *in vitro* culture systems in several species (Wu et al., 2008; Radović et al., 2016).

The overall results suggest a potential practical relevance of these extracts which were tested as biostimulants for the first time on different fruit species. An enhancement of pollen performance would be desirable for breeders, as it could make a significant contribution to the success of specific breeding plans. At the same time, the availability of 'green' compounds capable of enhancing the fertilization process, especially under unfavorable conditions, could meet farmers' needs for sustainable crop improvement.

5. Conclusions

The analysis, conducted to assess the potential biostimulant effect of apple and potato by-product extracts on *in vitro* pollen germination, revealed significant differences based on products and species tested. As a general trend, the AE did not have positive effects in most genotypes. On the other hand, the PE, at the lowest concentration (PE1), improved the pollen performance when reduced fresh grain germination occurred. Moreover, the PE was able to restore pollen germination in cultivars that did not retain a good viability after storage. This was particularly evident in hazelnut, which experienced a significant loss of stored pollen viability.

In conclusion, the findings of this study suggest:

- The effectiveness of potato peel extracts as pollen germination booster could be taken into account as a promising tool to develop new biostimulants for improving the fertilization process. In particular, the water maceration of waste materials can represent a sustainable alternative to obtain biostimulants avoiding unsafe chemical technologies;
- The use of potato peel extract as a biostimulant could offer an opportunity to enhance the utilization of byproducts, leading to a reduction in the environmental impact of potato processing industries;
- The preliminary results obtained *in vitro,* under a controlled experimental system, must be confirmed *in vivo,* under field conditions where complex genotype-environmental interactions occurred.

Moreover, in the frame of climate change, further investigation is needed to determine the biostimulant effect on fruit setting process, mainly under stress conditions.

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