



# **Yield and Compositional Profile of Eggplant Fruits as Affected by Phosphorus Supply, Genotype and Grafting**

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Abstract: The present experiment addressed the effects of two phosphorus regimes (30 and 90 kg ha<sup>-1</sup>, hereafter  $P_{30}$  and  $P_{90}$ ) on yield and composition of eggplant fruits in 'Birgah' and 'Dalia', whether or not these cultivars were grafted onto *Solanum torvum* 'Espina'. The  $P_{30}$  regime did not reduce yield, and promoted fruits' dry matter and total phenols content, along with their concentrations of macronutrients, mesonutrients (S and Na) and micronutrients (mostly Cu, B, Zn); however, their Fe concentrations were depressed. The rootstock 'Espina' increased fruit yield, dry matter content, epicarp chroma (in 'Birgah') and Ca content, together with their concentrations of B and Zn (especially at  $P_{30}$ ), but reduced their Fe content, mostly under  $P_{30}$ . Thus, the reduced P supply and grafting proved to be effective tools to enhance fruit yield, carpometric and almost all nutritional traits in eggplant, in a framework of more sustainable crop management. However, the reduced fruit concentration of Fe suggests that the affinity of the rootstock with specific micro minerals should be taken into account, along with the option to adopt complementary practices (e.g., targeted micronutrient fertilizations) to manage the micro mineral composition of eggplants.

Keywords: Solanum melongena L.; rootstock; total polyphenols; mineral content

# 1. Introduction

Phosphorus (P) is present in all living cells as a component of biomolecules, such as nucleic acids, phospholipids, ATP or NADPH [1]. In plants, P participates in multiple metabolic events and, in the human body, is the second most abundant mineral, being a constituent of the skeletal system. As a result of its biological importance, P fertilization is irreplaceable to ensure adequate food provision to mankind, both from a quantitative and qualitative viewpoint [2]. Despite its biological ubiquity, phosphate rocks, i.e., the only nonrenewable sources of P fertilizers, are unevenly distributed worldwide, with Morocco, China and USA accounting for 94% of the estimated 300 billion Mt of phosphate rocks worldwide [1]. Agriculture represents the main consumer of the estimated 71 billion Mt of P reserves, which, at the actual consumption rates, are expected to be significantly depleted in the next 50–100 years, consequently posing future concerns on global food security [3]. Hence, there is a compelling need to optimize P use in agriculture, especially



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**Copyright:** © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). in horticultural systems which largely contribute to the current global annual consumption of P fertilizers (~21 Mt) and related environmental impacts [4–7].

Vegetables are pivotal sources of nutraceuticals, with antioxidants and minerals being among the most prominent ones provided to the human diet. However, these plant foods do not always fit the dietary requirements, since several factors may affect their compositional profiles, including irrational fertilization practices [8,9]. Among fruit vegetables, eggplant (Solanum melongena L.) is a popular solanaceous crop, whose edible, immature berries are important sources of minerals and antioxidants in the Mediterranean diet [10]. Italy is the main producer among the European countries, with 304,690 out of the 960,227 t of eggplants (31.7% of total) produced in 2020 from a 9510-hectare surface area, mainly concentrated in the southern regions [11]. In the Mediterranean Basin, the crop is often grown in open field, on soils with high limestone content, a feature that is usually correlated to strong responses of many vegetable crops to P fertilization [12]. This leads to implications for eggplant cultivation, as high P fertilization rates are often adopted under field conditions, being a reputed primary tool to maximize fruit yield under P-limiting conditions. Currently, rational P fertilizations are desirable in horticultural systems, in order to address the concerns of agriculture-derived burden on P reserves and ecosystems, food provision to future generations, and rising consumer demand for environmentally sound plant foods [13]. In this view, vegetable grafting has been proven as a useful means to modulate yield and product composition of vegetable crops in a wide array of growth conditions [14,15]. In greenhouse-grown eggplants, grafting has been able to modify the fruit yield and quality in terms of phenolic profile or mineral distribution inside the plant [16,17]. Currently, no attempts have been focused on defining the relationships among grafting and P supply in open-field eggplants, in terms of product yield and quality traits, including its nutraceutical composition. To this end, the central role of P fertilization is well known to impact the product quality and mineral profile in many crops (e.g., grain crops) [4], given the multiple and complex interactions among P and other nutrients [18]. Thus, the abovementioned information may be highly instrumental in the double perspective to obtain adequate nutrient-dense eggplants in a more sustainable way. For these reasons, the present study addressed the effects of different P fertilization regimes and grafting on yield, quality traits and mineral composition of two field-grown eggplant genotypes largely cultivated around the Mediterranean Basin.

#### 2. Materials and Methods

#### 2.1. Experimental Site

A field experiment was conducted during 2019 at the experimental farm of the University of Catania (37°24'27" N, 15°03'35" E, 5 m a.s.l.), on a xerofluvent soil, having the characteristics reported in Table 1. The soil is characterized by an alkaline pH, a very high content of exchangeable K, total Mg and Ca, and a low content of total N and available P. The local climate is semiarid-Mediterranean (Cs climate according to Köppen classification), with warm and rainless summers. The meteorological conditions were recorded by an integrated station near the crop (~40 m). During the experiment (from May 2 to September 1), mean monthly air temperature progressively increased from May (17.7 °C) to June (22.0 °C) and July (25.8 °C), then slightly decreased in August (25.3 °C) (Figure 1). These values were similar to those of the long-term average (2009-2018), which were equal to 17.9 (May), 21.9 (June), 25.5 (July) and 25.6 °C (August). The lowest daily temperatures were recorded at 22 days after transplanting (DAT) (19.9 and 8.1 °C for maximum and minimum mean temperature, respectively) and the highest ones at 90 DAT (35.6 and 19.7 °C, respectively) (Figure 1). Mean relative humidity showed similar values in May and June, peaking at 6, 8 and 44 DAT (82%, on average), and showing the lowest values in July, at 73, 74 and 80 DAT (47%, on average) (Figure 1). No rainfall occurred during the experimental period, such that the overall difference compared to the long-term average was equal to 34.1 mm. Before transplanting, the soil was ploughed (0.40 m depth) and disk-harrowed (0.20 m depth).

| Soil Characteristic      | Unit             | Value |
|--------------------------|------------------|-------|
| Son characteristic       |                  | varue |
| Clay                     | %                | 30    |
| Silt                     | %                | 13    |
| Sand                     | %                | 57    |
| Organic matter           | %                | 2.8   |
| pH                       | $\log[H^+]$      | 7.5   |
| Cation exchange capacity | $cmol_c kg^{-1}$ | 19.1  |
| Total N                  | $mg kg^{-1}$     | 1.4   |
| Available P              | $mg kg^{-1}$     | 1.5   |
| Exchangeable K           | $mg kg^{-1}$     | 278   |
| М́g                      | $mg kg^{-1}$     | 433   |
| Ca                       | $mg kg^{-1}$     | 5245  |
| Na                       | $mg kg^{-1}$     | 125   |
| Fe                       | $mg kg^{-1}$     | 107   |
| Cu                       | $mg kg^{-1}$     | 12    |
| Zn                       | $mg kg^{-1}$     | 6     |

Table 1. Soil characteristics at the experimental site (0–40 cm depth).

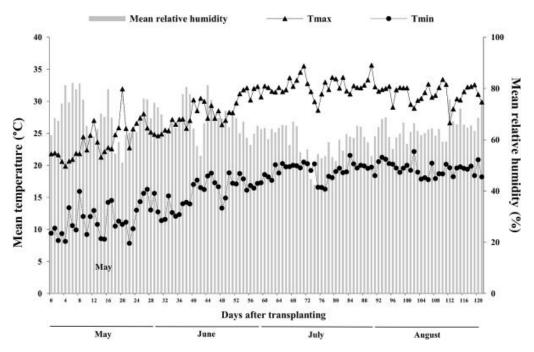


Figure 1. Meteorological conditions recorded during the trial.

#### 2.2. Experimental Design and Crop Management

The experiment was arranged as a randomized split-split-plot design with four replications based on  $3.60 \times 3.50$  m net experimental units (12 plants). A few days before transplanting by hand, the soil along the rows was mulched with black polyethylene sheets (40-centimeter width, 40-micrometer thickness), whereas plantlets at the stage of three true-leaves were transplanted on May 2 in a  $0.50 \times 1.20$  m format, then trained at two stems up to September 1. Two P regimes were arranged as main plots, corresponding to 30 and 90 kg P ha<sup>-1</sup> (hereafter P<sub>30</sub> and P<sub>90</sub>, respectively), whereas two eggplant cultivars ('Birgah' and 'Dalia'; Seminis, Milan, Italy) were imposed as sub-plots, either ungrafted or grafted onto the *Solanum torvum* Swartz rootstock 'Espina' (Esasem, Casaleone, Italy) (sub-sub-plots). Overall, we had 24 experimental units (2 P regimes × 2 eggplant cultivars × 2 graft configurations × 3 replications). The P<sub>90</sub> level was chosen since it represents a high supply commonly encountered in the reference area, whereas P<sub>30</sub> represents the dose calculated for a better balance with the other administered macronutrients. 'Birgah' and 'Dalia' are two cultivars largely grown

in south Italy, differing for their fruit typology (globose/violet and ovoidal/black, respectively). The rootstock 'Espina' was chosen because of its extensive root system and adaptation to warm/dry conditions; thus, it is among the most widespread in the reference area. The crop was drip irrigated, restituting 100% of  $ET_M$  when accumulated daily evapotranspiration (calculated using a class A-Pan evaporimeter near the crop) reached 40 mm. Fertigation was performed once a week from transplanting up to August 22 (17 interventions), overall administering the different P levels together with 120, 140, 16 and 5 kg ha<sup>-1</sup> for N, K<sub>2</sub>O, MgO and CaO, respectively. Micronutrients were provided in the following amounts (in kg ha<sup>-1</sup>): B (1), Cu (1), Fe (4), Mn (4), Mo (0.4) and Zn (2). Pests control was performed as per local custom.

#### 2.3. Fruit Collection, Carpometric Determinations and Sample Preparation

Commercially ripe fruits were harvested by hand from June 18 when they were near the maximum size, but before the onset of the epicarp color turning [19]. At each harvest, marketable fruits were counted and weighed, in order to calculate yield and number of fruits per plant. After harvesting, 12 fruits per plot were washed with distilled water, dried with paper, after which the epicarp color was measured along the equatorial region (2 readings per fruit) using a tristimulus Chroma meter CR-400 (Minolta Corporation, Ltd., Osaka, Japan), measuring L\* (lightness), a\* (green-red axis) and b\* (blue-yellow axis). The epicarp color was expressed in terms of chroma (C\*), calculated as  $(a^{*2} + b^{*2})^{1/2}$ . Fruits were then weighed to determine their fresh weight (FW) and dry matter (DM) content (after oven-desiccation at 75 °C, until constant weight). Fruits samples harvested from 20 to 22 July, i.e., when the crop attained the full yield rate, were ground after freeze-drying (-50 °C) and passed through a 1-millimeter sieve, then stored at -80 °C until further analyses.

#### 2.4. Determination of Total Phenols Content

Fruit total phenols content (TPC) was determined from lyophilized pulp powder (0.10 g), which was put into 5 mL 80% ethanol (v/v) in centrifuge tubes. The samples were extracted for 15 min using an ultrasonic bath LBS1-3 (Falc Instruments, Treviglio, Italy), maintaining water temperature below 10 °C. The tubes were then centrifuged (5000 g) at 5 °C for 20 min and the supernatant was transferred into vials. The procedure was performed 3 times. The combined extracts were diluted in measuring flasks to a 25-milliliter volume; 1.5 mL of diluted samples were filtered through a 0.45-micrometer nylon filter. Then, 200 µL of extract solution were added to 1000 µL, 1:10 diluted Folin-Ciocâlteu reagent with ultrapure water, vortexed for 1 min, after which 800 µL of 0.7 M Na<sub>2</sub>CO<sub>3</sub> were added. The liquid was vortexed and left in the dark for 60 min at room temperature. TPC was determined by reading the absorbance at 765 nm with a Jenway 7315 UV-vis spectrophotometer (Cole-Parmer, Stone, UK) and expressed as mg chlorogenic acid equivalent (CAE) kg<sup>-1</sup> FW.

#### 2.5. Determination of Total N and Mineral Profile

Two hundred mg of each sample were digested with 2 mL of 30% (m/m)  $H_2O_2$ , 0.5 mL of 37% HCl and 7.5 mL of HNO<sub>3</sub> 69% solution. The acid digestion was performed using a high-pressure laboratory microwave oven Mars plus (CEM srl, Cologno al Serio, Italy) operating at 1200 W. The temperature was linearly increased from 25 to 180 °C in 37 min, then held at 180 °C for 15 min. The digested samples were diluted to a final volume of 25 mL with ultrapure water. Blanks were prepared in each lot of samples. All determinations were performed in triplicate. For the elemental quantification, an ICP-OES (8000 DV, PerkinElmer, Shelton, CT, USA) was used, with an axially viewed configuration equipped with an ultrasonic nebulizer. All reagents used for the microwave-assisted digestions, i.e., HCl, HNO<sub>3</sub> and H<sub>2</sub>O<sub>2</sub>, were of suprapure grade (Merck, Darmstadt, Germany). High-purity water (18 MΩ cm) from a Milli-Q purification system (Millipore, Bedford, MA, USA) was used for diluting the standards.

Multi-elemental, high-purity grade was purchased from CaPurAn (CPAchem Ltd., Bogomilovo, Bulgaria). The purity of the plasma torch argon was greater than 99.99%. The external calibration solutions were prepared from standard certified elemental solutions (CaPurAn). Total N and S were determined using the combustion analysis by CHNS Elemental Analyzer (EA Flash 2000 Thermo Fisher Scientific CHNS-O determination, Rodan, Milan, Italy).

### 2.6. Statistical Procedures

All collected and calculated data were firstly subjected to Shapiro–Wilk and Levene's tests, in order to check for normal distribution and homoscedasticity, respectively. Then, a 'phosphorus level × cultivar × rootstock' analysis of variance (ANOVA) was applied according to the experimental layout adopted in the field. Percentage data were Bliss' transformed before the ANOVA (untransformed data are reported), whereas means comparisons were performed through Tukey's HSD test ( $p \le 0.05$ ).

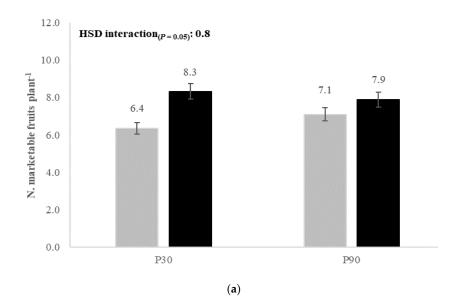
## 3. Results

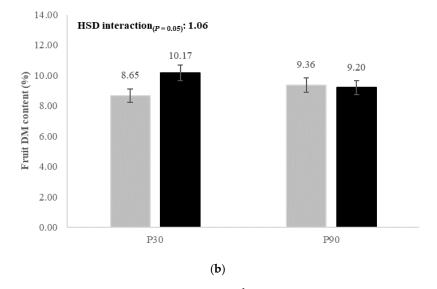
## 3.1. Yield and Carpometric Traits

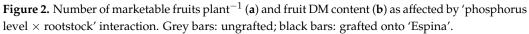
Marketable yield, number of fruits plant<sup>-1</sup> and fruit FW were not affected by the P supply in the studied cultivars (Table 2). These last showed contrasting yield traits, as 'Birgah' showed the highest marketable yield and fruit FW, whereas 'Dalia' had the highest number of fruits plant<sup>-1</sup> (Table 2). The rootstock 'Espina' promoted marketable yield (+38%), fruit FW (+16%) and the number of fruits plant<sup>-1</sup> (Table 2), with this last variable showing the strongest increase under  $P_{30}$  (+30%) (Figure 2A). Concerning fruit traits, higher firmness, chroma and TPC were noticed in  $P_{30}$  compared to  $P_{90}$  and in 'Birgah' compared to 'Dalia' (excepting TPC), whereas grafting boosted fruit firmness (+7%) and DM content (Table 2), with this last variable only in  $P_{30}$  (+1.52%) (Figure 2B). Among the tested genotypes, 'Birgah' showed a strong increase in epicarp chroma when P fertilization was reduced from  $P_{90}$  to  $P_{30}$  (+45%) (Figure 3).

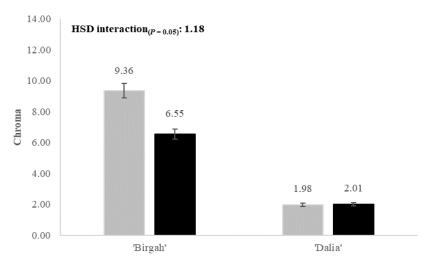
**Table 2.** Yield performances, carpometric traits and TPC of eggplants as affected by the main factors (mean  $\pm$  standard deviation). Different letters among factors' means indicate significance at Tukey's HSD test ( $p \le 0.05$ ).

| 37 1.1.  | Phospho                            | rus Level                | Cult                     | tivar                    | Root                     | Mean                     |                 |  |
|--|------------------------------------|--------------------------|--------------------------|--------------------------|--------------------------|--------------------------|-----------------|--|
| Variable                                       | P <sub>30</sub>                    | P <sub>90</sub>          | 'Birgah'                 | 'Dalia'                  | Control                  | 'Espina'                 | wiedii          |  |
| Marketable yield<br>(kg plant <sup>-1</sup> )  | $2.80\pm0.74~a$                    | $2.80\pm0.50~\text{a}$   | $2.97\pm0.68~\mathrm{a}$ | $2.64\pm0.53~\text{b}$   | $2.36\pm0.49~\text{b}$   | $3.25\pm0.57~a$          | $2.80\pm0.62$   |  |
| Marketable fruits<br>(n. plant <sup>-1</sup> ) | $7.34\pm2.10$ a                    | $7.49\pm1.97~\mathrm{a}$ | $5.71\pm1.86~\mathrm{b}$ | $9.12\pm1.70$ a          | $6.73\pm1.81~\text{b}$   | $8.11\pm2.00~\text{a}$   | $7.42 \pm 1.99$ |  |
| Fruit FW<br>(g)                                | $402\pm125~\mathrm{a}$             | $399\pm128~\mathrm{a}$   | $514\pm105~\mathrm{a}$   | $287\pm127\mathrm{b}$    | $371\pm109b$             | $430\pm142~\mathrm{a}$   | $401\pm124$     |  |
| Fruit DM content<br>(%)                        | Fruit DM content $9.70 \pm 1.06$ a |                          | $9.43\pm0.98$ a          | $9.27\pm1.13~\mathrm{a}$ | $9.01\pm0.90~b$          | $9.69\pm1.08~\mathrm{a}$ | $9.35 \pm 1.03$ |  |
| Fruit firmness<br>(N)                          | $14.6\pm2.7~\mathrm{a}$            | $13.5\pm2.2~\mathrm{b}$  | $16.0\pm1.6~\mathrm{a}$  | $12.0\pm1.2\mathrm{b}$   | $13.5\pm2.4~\mathrm{b}$  | $14.5\pm2.6$ a           | $14.0\pm2.5$    |  |
| Chroma<br>(adimensional)                       | $5.67\pm1.06~\mathrm{a}$           | $4.28\pm1.40~\text{b}$   | $7.95\pm2.07~a$          | $2.00\pm1.09~b$          | $4.90\pm1.70~\mathrm{a}$ | $5.05\pm1.81~\mathrm{a}$ | $4.97 \pm 2.05$ |  |
| TPC<br>(mg CAE kg <sup>-1</sup> FW)            | $2012\pm380~\text{a}$              | $1721\pm373~\mathrm{b}$  | $1538\pm268~\mathrm{b}$  | $2196\pm377~\mathrm{a}$  | $1878\pm415~\mathrm{a}$  | $1795\pm398~\mathrm{a}$  | $1837\pm456$    |  |









**Figure 3.** Fruit chroma as affected by 'phosphorus level  $\times$  cultivar' interaction. Light grey bars: P<sub>30</sub>; black bars: P<sub>90</sub>.

## 3.2. Fruit N and Mineral Profile

## 3.2.1. Macronutrients

The  $P_{30}$  regime promoted N, P, and K accumulation in eggplants (by 18, 11 and 9%, respectively), whereas the heterograft configuration boosted K content (+7%) and depressed N (-15%) (Table 3). The genotypes differed for fruit N and K content (both higher in 'Dalia') (Table 3); however, regarding K, 'Birgah' showed a significant increase under  $P_{30}$  supply (+13%) (Table 4) whereas 'Dalia' showed the highest K rise passing from control plants to those grafted onto 'Espina' (+9%) (Table 6).

**Table 3.** Total N and minerals content of eggplants as affected by the main factors (mean  $\pm$  standard deviation). Different letters among factors' means indicate significance at Tukey's HSD test ( $p \le 0.05$ ).

|                       | Phospho                        | rus Level                                     | Cul                                | tivar                                    | Root   | stock                                       |                |  |
|-----------------------|--------------------------------|---|------------------------------------|--|--|---|----------------|--|
| Variable              | P <sub>30</sub>                | P <sub>90</sub>                               | 'Birgah'                           | 'Dalia'                                  | Control  | 'Espina'                                    | Mean           |  |
| Macronutrients        |                                |   |                                    |  |  |   |                |  |
| $(mg kg^{-1} FW)$     |                                |   |                                    |  |  |   |                |  |
| Ň                     | $140\pm14~\mathrm{a}$          | $140 \pm 14 \text{ a}$ $119 \pm 19 \text{ b}$ |                                    | $137\pm13$ a                             | $139\pm19$ a                                     | $118\pm18\mathrm{b}$                        | $130\pm19$     |  |
| Р                     | $165\pm16$ a                   | $148\pm12b$                                   | $150 \pm 21$ a                     | $162\pm 8$ a                             | $157\pm16$ a                                     | $155\pm18~\mathrm{a}$                       | $156\pm17$     |  |
| Κ                     | $625\pm45$ a                   | $575\pm75\mathrm{b}$                          | $561\pm54~\mathrm{b}$              | $640\pm53~\mathrm{a}$                    | $580\pm79~\mathrm{b}$                            | $620\pm53~\mathrm{a}$                       | $600\pm66$     |  |
| Mesonutrients         |                                |   |                                    |  |  |   |                |  |
| $(mg kg^{-1} FW)$     |                                |   |                                    |  |  |   |                |  |
| Mg                    | $102\pm 8~\mathrm{a}$          | $98\pm9~\mathrm{a}$                           | $97\pm9~\mathrm{a}$                | $103\pm8$ a                              | $100\pm11~\mathrm{a}$                            | $100\pm 6~\mathrm{a}$                       | $100\pm9$      |  |
| Ca                    | $69\pm20~\mathrm{a}$           | $78\pm36~\mathrm{a}$                          | $56\pm10~{ m b}$                   | $56 \pm 10$ b $91 \pm 31$ a              |  | $62 \pm 10 \text{ b}$ $85 \pm 37 \text{ a}$ |                |  |
| S                     | $9.5\pm1.5$ a                  | $8.4\pm1.8~{ m b}$                            | $9.1 \pm 1.9$ a $8.8 \pm 1.6$ a    |  | $10.3 \pm 0.9 \text{ a}$ $7.5 \pm 1.0 \text{ b}$ |   | $8.9\pm1.7$    |  |
| Na                    | $15.8\pm8.8$ a                 | $11.5\pm6.7~\mathrm{b}$                       | $9.4\pm3.1\mathrm{b}$              | $9.4 \pm 3.1 \text{ b}$ 17.9 $\pm$ 9.1 a |  | $9.2\pm2.2\mathrm{b}$                       | $13.6 \pm 7.9$ |  |
| Micronutrients        |                                |   |                                    |  |  |   |                |  |
| $(\mu g k g^{-1} FW)$ |                                |   |                                    |  |  |   |                |  |
| Fe                    | $2900\pm857\mathrm{b}$         | $3339\pm871$ a                                | $3702\pm757~\mathrm{a}$            | $2538\pm535~\mathrm{b}$                  | $3139\pm930~\mathrm{a}$                          | $3101\pm856$ a                              | $3120\pm874$   |  |
| Mn                    | $521\pm63$ a                   | $512\pm76$ a                                  | $512\pm62$ a                       | $512\pm78~\mathrm{a}$                    | $466\pm64\mathrm{b}$                             | $558\pm35$ a                                | $512\pm69$     |  |
| Cu                    | $495 \pm 42$ a $376 \pm 105$ b |   | $423 \pm 125$ a $448 \pm 67$ a     |  | $463 \pm 63 \text{ a}$ $408 \pm 121 \text{ b}$   |   | $436\pm99$     |  |
| В                     | $1809\pm972~\mathrm{a}$        | $1374\pm661~\mathrm{b}$                       | $1246 \pm 416$ b $1937 \pm 1025$ a |  | $1053\pm294\mathrm{b}$                           | $2131\pm\!874$ a                            | $1592\pm84$    |  |
| Zn                    | $3106 \pm 1832$ a              | $2172\pm1224\mathrm{b}$                       | $2198\pm564b$                      | $3080 \pm 2124$ a                        | $1657\pm604\mathrm{b}$                           | 3621 ±1692 a                                | $2639 \pm 159$ |  |

**Table 4.** Potassium, magnesium, manganese and copper concentrations in eggplants (mean  $\pm$  standard deviation) as affected by 'phosphorus level  $\times$  cultivar' interaction.

| Variable —                               | P          | 30          | P           | $HSD_{interaction (p = 0.05)}$ |    |
|--|------------|-------------|-------------|--------------------------------|----|
| variable                                 | 'Birgah'   | 'Dalia'     | 'Birgah'    | 'Dalia'                        |    |
| K (mg kg <sup><math>-1</math></sup> FW)  | $596\pm14$ | $636\pm59$  | $527\pm38$  | $643\pm51$                     | 54 |
| Mg (mg kg <sup><math>-1</math></sup> FW) | $106\pm7$  | $106 \pm 9$ | $88\pm7$    | $100\pm 6$                     | 14 |
| Mn ( $\mu$ g kg <sup>-1</sup> FW)        | $543\pm31$ | $499\pm80$  | $480\pm69$  | $525\pm81$                     | 57 |
| Cu ( $\mu$ g kg <sup>-1</sup> FW)        | $521\pm34$ | $469\pm32$  | $325\pm101$ | $427\pm88$                     | 67 |

3.2.2. Mesonutrients

Sulphur and Na concentrations were higher in  $P_{30}$  than in  $P_{90}$  (by 13 and 42%, respectively) (Table 3), without interactive effects. Regarding Mg concentration, an analogous response was noticeable only in 'Birgah' (+20%), whereas no significant differences were recorded for 'Dalia' (Table 4). These genotypes differed in terms of fruit Ca and Na concentrations, as both minerals attained higher levels in 'Dalia' (Table 3), i.e., the cultivar that also showed strongest Ca and Na variations in response to grafting (+62 and -60%, respectively) (Table 6). On the other hand, the heterograft configuration depressed the S accumulation (-27%), irrespective of the P level and genotype (Table 3).

#### 3.2.3. Micronutrients

Increased concentrations in Cu, B and Zn were found in response to the reduced P supply (Table 3), whereas 'Birgah' showed a stronger response to P<sub>30</sub> for fruit Mn ( $\pm$ 13%) and Cu ( $\pm$ 62%) concentrations (Table 4). A different trend was recorded for Fe, whose fruit content was strongly decreased, passing from P<sub>90</sub> to P<sub>30</sub> (Table 3), with the rootstock 'Espina' depressing fruit Fe under P<sub>30</sub> supply (-17%) and increasing it at P<sub>90</sub> ( $\pm$ 15%) (Table 5). Such response was opposite to that of B and Zn, whose contents were promoted by 'Espina' mostly in P<sub>30</sub> (by 125 and 143%, respectively) (Table 5). Excluding Fe and Cu, grafting promoted the accumulation of micronutrients in eggplants (Table 3), although in some cases such response was genotype-dependent. Indeed, while for Mn concentration the graft-induced increase was consistent among P levels and cultivars ( $\pm$ 20%) (Table 3), for B and Zn concentrations 'Dalia' showed the highest rise passing from control to grafted onto 'Espina' ( $\pm$ 169 and  $\pm$ 320%, respectively). 'Birgah' displayed a strong reduction in Cu concentration in the heterograft configuration (-23%) (Table 6).

**Table 5.** Iron, boron and zinc concentrations in eggplants (mean  $\pm$  standard deviation) as affected by 'phosphorus level  $\times$  grafting' interaction.

| Variable                          | P               | 30              | P            | 90              | $HSD_{interaction (p = 0.05)}$ |
|-----------------------------------|-----------------|-----------------|--------------|-----------------|--------------------------------|
| variable                          | Control         | 'Espina'        | Control      | 'Espina'        |                                |
| Fe ( $\mu$ g kg <sup>-1</sup> FW) | $3176 \pm 1161$ | $2625\pm290$    | $3102\pm741$ | $3577\pm993$    | 550                            |
| B ( $\mu g k g^{-1} FW$ )         | $1113\pm304$    | $2505\pm907$    | $993\pm298$  | $1756\pm722$    | 319                            |
| Zn ( $\mu g kg^{-1} FW$ )         | $1813\pm600$    | $4399 \pm 1736$ | $1501\pm621$ | $2843 \pm 1353$ | 441                            |

**Table 6.** Potassium, calcium, sodium, copper, boron and zinc concentrations in eggplants (mean  $\pm$  standard deviation) as affected by 'cultivar  $\times$  grafting' interaction.

| Variable –                               | 'Bir         | gah′          | 'Da          | $HSD_{interaction (p = 0.05)}$ |     |
|--|--------------|---------------|--------------|--------------------------------|-----|
| variable –                               | Control      | 'Espina'      | Control      | 'Espina'                       |     |
| K (mg kg <sup><math>-1</math></sup> FW)  | 546 ±59      | $577\pm49$    | $612\pm42$   | $667\pm53$                     | 52  |
| Ca (mg kg <sup><math>-1</math></sup> FW) | $53\pm8$     | $59\pm12$     | $69\pm3$     | $112\pm32$                     | 20  |
| Na (mg kg <sup><math>-1</math></sup> FW) | $10.5\pm3.3$ | $8.3\pm2.6$   | $25.5\pm6.4$ | $10.2\pm1.4$                   | 3.7 |
| Cu ( $\mu g kg^{-1}$ FW)                 | $477\pm76$   | $369 \pm 147$ | $450\pm52$   | $447\pm85$                     | 67  |
| $B (\mu g kg^{-1} FW)$                   | $1055\pm365$ | $1437\pm400$  | $1051\pm237$ | $2824\pm607$                   | 319 |
| Zn ( $\mu$ g kg <sup>-1</sup> FW)        | $2130\pm344$ | $2266\pm755$  | $1184\pm384$ | $4976 \pm 1150$                | 441 |

## 3.2.4. Correlation among Variables

The results of the correlation analysis are reported in Table 7. Overall, 171 correlations were analyzed, of which 67 (39% of total) showed significance, highlighting 44 positive and 23 negative relationships. In the case of yield and carpometric traits, 18 out of 21 correlations (86%) were significant, whereas they were 53 out of 150 (35%) in the case of N and mineral concentrations (Table 7). In the case of positive correlations, the highest correlation coefficients were recorded among B and Zn (0.901 \*\*\*), fruit FW and chroma (0.879 \*\*\*), fruit FW and firmness (0.868 \*\*\*), and among fruit firmness and chroma (0.864 \*\*\*). The strongest relationships in the dataset of positive correlations were recorded among marketable fruits plant<sup>-1</sup> and chroma (-0.799 \*\*\*), marketable fruits plant<sup>-1</sup> and fruit FW (-0.722 \*\*\*), fruit FW and TPC (-0.691 \*\*\*), and among marketable fruits plant<sup>-1</sup> and Fe concentration (-0.598 \*\*).

|                      | Marketable<br>Yield | Marketable<br>Fruits | Fruit FW  | Fruit DM<br>Content | Fruit<br>Firmness | Chroma    | TPC       | Ν         | Р        | К        | Mg      | Ca        | s         | Na       | Fe      | Mn        | Cu   | В         |
|----------------------|---------------------|----------------------|-----------|---------------------|-------------------|-----------|-----------|-----------|----------|----------|---------|-----------|-----------|----------|---------|-----------|------|-----------|
| Marketable<br>fruits | NS                  | -                    |           |                     |                   |           |           |           |          |          |         |           |           |          |         |           |      |           |
| Fruit FW             | 0.543 **            | NS                   | -         |                     |                   |           |           |           |          |          |         |           |           |          |         |           |      |           |
| Fruit DM content     | 0.539 **            | NS                   | NS        | -                   |                   |           |           |           |          |          |         |           |           |          |         |           |      |           |
| Fruit<br>firmness    | 0.464 *             | -0.535 **            | 0.868 *** | 0.602 **            | -                 |           |           |           |          |          |         |           |           |          |         |           |      |           |
| Chroma               | NS                  | NS                   | 0.879 *** | NS                  | 0.864 ***         | -         |           |           |          |          |         |           |           |          |         |           |      |           |
| TPC                  | NS                  | 0.574 **             | NS        | NS                  | -0.583 **         | -0.550 ** | -         |           |          |          |         |           |           |          |         |           |      |           |
| Ν                    | NS                  | NS                   | -0.434 *  | NS                  | NS                | NS        | 0.474 *   | -         |          |          |         |           |           |          |         |           |      |           |
| Р                    | NS                  | NS                   | NS        | NS                  | NS                | NS        | 0.502 *   | 0.658 *** | -        |          |         |           |           |          |         |           |      |           |
| K                    | NS                  | 0.455 *              | -0.546 ** | NS                  | NS                | NS        | 0.713 *** | 0.450 *   | 0.516 ** | -        |         |           |           |          |         |           |      |           |
| Mg                   | NS                  | NS                   | NS        | NS                  | NS                | NS        | 0.497 *   | 0.613 **  | 0.503 *  | 0.497 *  | -       |           |           |          |         |           |      |           |
| Ca                   | NS                  | 0.692 ***            | -0.571 ** | NS                  | -0.499 *          | -0.597 ** | NS        | NS        | NS       | NS       | NS      | -         |           |          |         |           |      |           |
| S                    | -0.553 **           | NS                   | NS        | NS                  | NS                | NS        | NS        | 0.644 *** | NS       | NS       | NS      | -0.462*   | -         |          |         |           |      |           |
| Na                   | -0.566 **           | NS                   | -0.595 ** | NS                  | -0.423 *          | NS        | 0.588 **  | 0.551 **  | NS       | 0.594 ** | NS      | NS        | 0.439 *   | -        |         |           |      |           |
| Fe                   | NS                  | -0.598 **            | 0.669 *** | NS                  | 0.537 **          | 0.535 **  | -0.531 ** | NS        | NS       | NS       | NS      | NS        | NS        | -0.497 * | -       |           |      |           |
| Mn                   | 0.534 **            | NS                   | NS        | 0.459 *             | NS                | NS        | NS        | NS        | NS       | NS       | NS      | 0.437 *   | -0.516 ** | -0.466 * | NS      | -         |      |           |
| Cu                   | NS                  | NS                   | NS        | NS                  | NS                | NS        | NS        | 0.479 *   | 0.615 ** | 0.442 *  | NS      | NS        | 0.424 *   | NS       | NS      |           | -    |           |
| В                    | NS                  | 0.665 ***            | NS        | 0.452 *             | NS                | NS        | NS        | NS        | NS       | NS       | 0.452 * | 0.670 *** | -0.411 *  | NS       | NS      | 0.637 *** | NS   | -         |
| Zn                   | NS                  | 0.528 **             | NS        | NS                  | NS                | NS        | NS        | NS        | NS       | NS       | NS      | 0.546 **  | NS        | NS       | -0.460* | 0.579 **  | NS ( | 0.901 *** |

**Table 7.** Pearson's product-moment correlation coefficients (r) among variables. \*, \*\* and \*\*\* indicate significance at  $p \le 0.05$ , 0.01 and 0.001, respectively. NS: not significant.

## 4. Discussion

In the present experiment, none of the studied eggplant cultivars showed negative yield responses under reduced P fertilization, demonstrating the agronomic inefficacy to exceed the  $P_{30}$  threshold. Consequently, the  $P_{90}$  level depressed the efficiency of P fertilization; for every kg ha<sup>-1</sup> of supplied P, the crop yielded 1.56 t ha<sup>-1</sup> of marketable fruits in  $P_{30}$ , against 0.52 t ha<sup>-1</sup> in  $P_{90}$  (data not shown). It is possible that the high fractionation of P supply we adopted contributed to these differences too, by reducing the P immobilization into the soil and subsequently yielding more efficient absorption by the plants [4]. Accordingly, the absence of any significant variation of yield components passing from  $P_{90}$  to  $P_{30}$  (i.e., number of marketable fruits plant<sup>-1</sup> and fruit FW) indicates that the plants had no need for extra P supplied beyond 30 kg ha<sup>-1</sup>. On the other hand, the rootstock 'Espina' promoted yield, number of marketable fruits plant<sup>-1</sup> (mainly at P<sub>30</sub>) and fruit FW, confirming previous results obtained by grafting eggplant onto S. torvum [20,21]. The highest yields of grafted vegetable crops have been often attributed to the enhanced water and nutrient uptakes, flowing in turn from a more expanded root system [22]. Both P supply and grafting are able to modify the nutritional flows between vegetative and reproductive organs [22,23]. In this view, the higher fruit yield recorded in  $P_{30}$ /grafted eggplants suggests a synergistic effect among  $P_{30}$  and 'Espina' in generating more favorable source:sink relationships, promoting fruit yield.

Fruit DM content and firmness results were clear examples of fruit quality improvement under a more rational P supply, with the former variable being maximized by combining P<sub>30</sub> and grafting, likely as a consequence of an enhanced carbon flow toward the fruits. Indeed, it has been reported that the optimal tuning among macronutrient supplies through fertilization (notably among N and P) acts to improve the photosynthates translocation toward the reproductive organs [12]. Accordingly, we found that marketable yield was positively correlated to fruit FW (0.543 \*\*) and DM content (0.539 \*\*). This finding suggests implications for postharvest behavior of eggplants, as higher DM content and firmness values are often associated with improved vegetable shelf-life and tolerance to mechanical injuries along the distribution chain [24].

The  $P_{30}$  supply also improved the skin chroma (in 'Birgah') and TPC of eggplants, thus highlighting positive effects on both commercial (more vivid external colors) and nutraceutical traits. Indeed, the health-promoting properties of eggplants largely rely on their phenolic acids composition (mostly chlorogenic acid derivatives), whereas the typical hue of eggplant skin is primarily defined by the high accumulation of anthocyanins, such as delphinidin-3-rutinoside, delphinidin-3-rutinoside-5-glucoside or delphinidin-3-coumaroylrutinoside-5-glucoside (nasunin), which show antioxidant and antiagiogenic activities [25,26]. These outcomes could be explained by a more sustained growth rate of the fruits, allowing them an earlier achievement of perceived suitable size for harvest. In this sense, higher anthocyanin and phenol concentrations have been reported in younger eggplants, with the former variable being highly correlated to the external fruit color appearance [27,28]. For TPC, the  $P_{30}$  supply apparently acted as an eustressor, promoting their accumulation in eggplants. Accordingly, higher phenolic concentrations have been reported under reduced P supply, e.g., in lavender, strawberry or Chinese kale [29–31].

Potassium was the prevailing macronutrient in eggplants, confirming the important role of this vegetable as a K provider in the human diet. The reduced P supply promoted the fruit accumulation of K only in 'Birgah', probably as a consequence of its different K partitioning inside the plant. Differently, P<sub>30</sub> boosted N and P concentrations in eggplant fruits, with differences (+17.6 and +11.5%, respectively) largely overcoming that recorded for fruit DM (+7.8%). To this end, an adequate N:P ratio into the soil has been reported to optimize root architecture and the ability of the plant to absorb both macronutrients [32]. This hypothesis seems to be confirmed by the correlation analysis, which revealed a strong relationship between both macronutrient concentrations (0.658 \*\*\*). On the other hand, fruit P and K concentrations positively responded to grafting, confirming the ability of this propagation technique to promote the accumulation of these macronutrients in fruit

l among these last macronutrients

vegetables [10,33]. Despite the positive correlation found among these last macronutrients (0.516 \*\*), the highest increase in K content recorded in 'Dalia' suggests a strong influence of the genotype in remodeling the fruit macromineral profile of grafted eggplants. This finding is consistent with the known importance of the "scion  $\times$  rootstock" interactions reported in this crop [34]. Taken together, our results show the possibility to enhance the fruit macronutrient profile in 'Birgah' and 'Dalia' by combining a more rational P supply and grafting. Beyond its environmental importance, this outcome is nutritionally relevant mainly for P and K because of their role in the human body, regulating e.g., bone formation and cardiovascular functions (P), or blood pressure and transmission of signals in neuromuscular tissues (K) [35,36].

Where mesonutrients were concerned, S and Na showed similar responses to P fertilization and grafting, as their fruit concentrations were promoted by  $P_{30}$  and restricted by the heterograft configuration. However, no differences were found for S by comparing  $P_{90}$ /ungrafted vs.  $P_{30}$ /grafted treatments of both eggplant cultivars. Differently, the rootstock effect was largely prevalent for Na, so that the  $P_{30}$ /grafted eggplants showed the poorest Na concentration, consistent with the reduced Na uptake observed in grafted eggplant plants [16]. On the other hand, a positive response to grafting was noticed for Ca concentration (up to  $\pm 62\%$  in 'Dalia'), confirming the positive effects of the rootstock 'Espina' on Ca accumulation in eggplants [21].

Regarding the micromineral composition, we observed strong, positive effects of P<sub>30</sub> mainly on B and Zn concentrations, consistent with their improved root uptake observed under more proper P soil content [37–40]. On the other hand, beyond the differences among eggplant cultivars, grafting always promoted the accumulation of Mn, B and Zn, probably as a consequence of more expanded roots exploring deeper soil layers, where more convenient oxidative and thermal conditions foster the absorption of these minerals [41–43]. Accordingly, highly significant correlations were found between Mn and B (0.637 \*\*\*), Mn and Zn (0.579 \*\*), and between B and Zn (0.901 \*\*\*). Consequently, in both cultivars, the  $P_{30}$ /grafted combination produced fruits with the best micronutrient compositions in terms of Zn ( $\pm 193\%$ ), B ( $\pm 152\%$ ), Mn ( $\pm 24\%$ ) and Cu ( $\pm 15\%$ ), despite grafting per se acting to reduce the Cu concentration in 'Birgah'. Many rootstock species, including S. torvum, are well known to restrict the translocation of some potentially toxic metals toward the shoot, a feature that, together with the peculiar scion traits, would explain the reduced fruit Cu concentration we recorded in 'Birgah' [44,45]. In any case, the overall increase in these four minerals through rational P supply and grafting is relevant from a nutritional viewpoint due to the primary role of these elements in human metabolism, including cardiovascular integrity (Cu), brain (Mn) and bones (B) development, or synthesis of nucleic acids and proteins (Zn) [8,41,42]. Differently, fruit Fe concentration was reduced under P<sub>30</sub>, mostly in the grafted eggplants, thus showing an opposite trend when compared to Zn and B. The contrasting trends of Fe and Zn ( $r = -0.460^{*}$ ) is one of the main outcomes we recorded, as these are among the most important trace elements for the human body. Indeed, their dietary deficiencies lead to serious metabolic disorders, including growth retardation, weakened immunological defense and anemia; their suboptimal levels in human diet are perceived as a growing concern both in developed and developing nations [8,46,47]. By comparing the P<sub>30</sub>/grafted and P<sub>90</sub>/ungrafted combinations, our results show a dramatic reduction of the fruit Fe:Zn ratio (from 1.26 to 0.13); this suggests an alteration of their homeostasis in fruits, despite no visual symptoms of Fe chlorosis being recorded during the experiment (data not shown). Overall, the correlation analysis supports the hypothesis that the reduced Fe concentration in fruits flows from an enhanced  $P_{30}$ -driven absorption of Zn, probably exacerbated by the rootstock, given the high capability of *S. torvum* for the uptake of Zn from the growth substrate [48]. Accordingly, it has been noted that Fe and Zn interact because of the chemical similarity of their divalent cations and basic transporter proteins, leading in many plants to reciprocal interference in their uptake and translocation, as part of the complex tripartite interaction among P, Fe and Zn homeostasis [49,50].

## 5. Conclusions

The present experiment provides information about the positive effects obtainable by combining a more targeted P supply and grafting in terms of fruit yield and quality in open field eggplants. Such information appears highly instrumental from the perspective of improving the sustainability and nutritional quality of this pivotal Mediterranean vegetable crop. Although the fruit constituents reported in this study (total phenols and inorganic constituents) provide no energy, their sufficient intake represents a necessary component of adequate human growth and metabolism; their suboptimal dietary concentrations can lead to metabolic disorders and increased incidence in chronic diseases. Beyond the differences among eggplant cultivars, our results show that the reduction in P fertilization did not affect fruit yield, but instead was promoted by grafting. The  $P_{30}$ /grafted eggplants showed enhanced marketable yields, along with increased concentrations in total phenols, macronutrients (mostly P and K), Ca and micronutrients (Zn, B, Mn and Cu), hence revealing an overall better nutraceutical composition. The depressed Fe concentration represented an exception having nutritional relevance, probably consequent to both complex P-Fe-Zn interactions in the soil and intrinsic rootstock characteristics. This outcome would suggest that under similar conditions, the affinity of the rootstock with specific micro minerals should be taken in due account, along with the option to adopt complementary practices (e.g., more targeted micro mineral fertilizations) to optimize the overall accumulation of these constituents in eggplant fruits.

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