



Bumblebees sense rootstock-mediated nutrition and fertilization regime in tomato

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Abstract

Aims Since producing more with less is required for increasing agricultural sustainability and reducing its environmental impact, breeding varieties with increased yield stability under reduced fertilizer application is an important goal, particularly in high valued horticultural crops such as tomato (*Solanum lycopersicum* L.). However, because of the difficulties to conciliate yield and fertilizer use efficiency through breeding, the graft-compatible genetic biodiversity existing in horticultural species offers the possibility to directly approach this objective in high-yielding elite varieties through improving nutrient capture and promoting ecosystem services such as insect pollination. We hypothesized that rootstocks affect pollinator

foraging decisions through the nutritional status that impacts yield.

Methods Fifteen genetically diverse experimental rootstocks were grafted to a scion tomato variety and cultivated under optimal and reduced (25% of optimal) P and NPK fertilization in the presence of managed bumblebee pollinators (*Bombus terrestris*).

Results Up to twofold yield variability between rootstocks was associated with leaf nutrition and photosynthesis of the scion. Interestingly, fertilization regime and the rootstock genotype influenced the pollinator foraging decisions since bumblebees showed feeding preference for plants cultivated under low P, and for the most yielding and nutritious graft combinations under reduced but not under optimal fertilization. Bumblebees can sense plant nutritional status through source-sink relations, as supported by the consistent relationship between pollinator preferences and leaf carbon concentration.

Conclusions This study opens new perspectives for using pollinators as “phenotypers” to select the most resilient plants under suboptimal conditions and/or genotypes that synergistically increase crop productivity by promoting the ecosystem service provided by the insects.

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Introduction

Intensive horticulture aims at maximizing both crop yields and the efficiency in the use of natural and artificial inputs such as land, water, fertilizers and energy, often under suboptimal growing conditions imposed by extreme temperatures, salinized water and soil and the incidence of pests and diseases. To achieve this goal, farmers use different technological and biological tools such as climatized greenhouses, artificial substrates, intelligent fertirrigation systems, highly yielding elite varieties, vigorous rootstocks, biostimulants, and beneficial soil microorganisms such as mycorrhizal fungi and plant growth-promoting rhizobacteria to improve plant growth and reproduction. The mutualistic interactions with other organisms include direct (managed) or indirect (ecosystem-based) use of pollinator insects that ensure flower fertilization and fruit set, thus optimizing yields and the efficiency of the input resources. The use of fertilizers is of particular interest, since the application of insufficient or excessive amounts leads to physiological disorders and yield penalties, with negative economic and environmental impacts.

The mineral macronutrients nitrogen (N), potassium (K) and phosphorus (P) are the most important fertilizers in horticulture, where most studies are focused due to their economic and environmental associated costs, particularly in the case of N and P. Deficiencies in those macronutrients lead to physiological, biochemical, and metabolic disorders resulting in reduced photosynthesis, stunted growth and yield reduction (Tewari et al. 2007; Chen et al. 2018; Maia et al. 2019). Beside precise fertilization, reducing fertilizer application without penalizing yield is possible through developing varieties with increased efficiency in the uptake and use of single or combined nutrients. Tomato (*Solanum lycopersicum* L.) is a big consumer of water and resources and in general, commercial horticulture uses rootstocks that essentially provide generic vigor and some resistances to soilborne pathogens such as bacteria, fungi and nematodes. However, while optimizing yield is prioritized, little attention has been paid by seed companies to the breeding of varieties or rootstocks with improved capacity to maintain yields with reduced amounts of water and fertilizers. Indeed, most studies dealing with grafting and fertilizers use commercial F1 rootstocks with similar properties

and similar genetic backgrounds, and focus on the capacity to accumulate particular nutrients in the leaves, but rarely pursued the agronomic evaluation of genetically diverse experimental rootstocks under limited fertilizer supply (Martínez-Andújar et al. 2020; Pérez-Alfocea 2021).

Mineral fertilization can also alter mutualistic interactions such as pollinating services. Since P-fertilization can modify flower attributes such as colour, scent, nectar and pollen quantity and quality, it can also impact flower attractiveness for pollinating insects (Borghi and Fernie 2018) and therefore flower fertilization and fruit set. Indeed, a recent pioneering study has reported that high P improves fruit yield in almond through mobilizing starch reserves for nectar secretion and improving bee pollinator visitation and fruit set (Karunakaran et al. 2021). The authors suggest that plant-pollinator mutualism could be used to optimize P fertilization in almond orchards, as has also been reported in canola (Adamidis et al. 2019). The question is whether mutualism can also serve to optimize the input of other resources in other pollinator-dependent species and how to select the most efficient or resilient individuals under suboptimal (e.g. low fertilizer application) conditions.

Animal pollinators are required for production in 75% of crop species used by humans (Klein et al. 2007) while there is a global decline in pollinator populations (Potts et al. 2010), that is increasing the interest in crop-pollinator interdependence because of the key impact on food production and stability (Garibaldi et al. 2011; Adamidis et al. 2019). Although tomato is an autogamous self-pollinated species that does not strictly rely on pollinators, its pollination is significantly improved by the wind and insects (Picken 2015). Indeed, the importance of insects for tomato pollination has been long time recognised in both open field and greenhouse conditions by either natural or managed pollinators (Toni et al. 2021). In recent years, the use of pollinating insects, commonly buzzy bumblebees (e.g. *Bombus terrestris*) has been developed and applied at the commercial level worldwide to ensure flower fertilization, fruit set, yield and quality (Velthuis and van Doorn 2006; Yoon and Park 2019).

Bees require nectar and pollen floral resources that provide necessary carbohydrates, proteins, lipids, and micronutrients for survival, reproduction, and resilience to stress (Vaudo et al. 2015). However,

nectar and pollen nutritional quality varies widely among host-plant species and environments, which in turn influences how bees forage to obtain their nutritionally appropriate diets. Although the preference of pollinators for some varieties is well known by farmers, it has been little explored (Klatt et al. 2013), and no study has assessed the impact of whole plant or rootstocks genotypes in interaction with the environment on the foraging decisions of pollinators, and whether that preference is related to the resistance of the phenotype, beyond the influence on the pollinating efficiency.

Plant nutritional status, mediated by the own root or by the rootstock, will influence the source-sink relations and, therefore, plant yield. The optimization of such relations will be reflected in the amount of assimilates that the sink structures receive for growth, including flower development and fertilization. Besides, since tomato does not produce nectar, pollinating insects will feed on pollen produced by flowers and will take successive foraging decisions based on the quantity and quality of food that they receive during their visits (Chittka 2017; Borghi and Fernie 2018). Therefore, the pollinator's preference determined by foraging decisions could be used as an indicator of the physiological status of the plant and, ultimately, of yield under any environmental conditions.

In the case of grafted plants, the use of foraging decisions as indicator of plant physiological status is particularly interesting since the differences between different rootstocks on the same variety often appear when the physiology of the plant is becoming exhausted at the end of the growing cycle, when the adverse environmental conditions (e.g. high temperature) and stress-induced physiological disorders are intensified. According to our hypothesis the pollinators can sense those particularities before the agronomic differences become evident, thus selecting the most promising rootstocks. Additionally, although not addressed in this study, those rootstocks could synergistically increase yield by stimulating pollination, which is key under some environmental constraints.

In this study, we addressed for the first time the impact of a set of experimental rootstocks on crop agronomic components under P and NPK deficiencies, and on the pollinator foraging activity in an important crop species as the tomato. We hypothesize that the rootstock influences the nutritional and physiological status of the variety (optimising

source-sink relations) and those changes can be sensed by the insect pollinator thus influencing its foraging decisions. Ultimately, the pollinator preference could be used (i) as a tool for searching synergistic rootstock x pollination effects on crop yield under suboptimal growing conditions, and (ii) as a phenotyping parameter for plant selection and breeding based on ecological decisions.

Material and methods

Plant material, grafting, and growth conditions

Scions of the indeterminate normal-fruited tomato F1 hybrid cv. Julita (Unigenia Semillas SLU, Los Alcázares, Spain) were grafted onto fifteen experimental rootstocks. Non-grafted plants of the scion variety (denoted as rootstock #1 in the figures), self-grafted (rootstock #2) and grafted onto the widely-used commercial F1 rootstock Maxifort (rootstock #9; Bayer-De Ruiter, Netherlands) were utilized as controls. The experimental rootstocks include five introgression lines (ILs, #3, 4, 5, 6) derived from crosses between *Solanum lycopersicum* cv M82 with *Solanum pennellii* LA716 (Eshed and Zamir 1995), and with *S. habrochaites* LA1777 (rootstock #8), five domestic (rootstocks #7, 10, 13, 16, 18) and two wild (rootstocks #15, 17) accessions and three mutant lines affected in abscisic acid (rootstock #14) and ethylene (rootstocks #11, 12) production. Those hormones have been involved in rootstock-mediated responses to salinity and nutrient stress in tomato (Martínez-Andújar et al. 2016, 2017, 2021)

The sowing of scion and rootstock seeds was synchronized to obtain the appropriate stem diameter to ensure grafting viability and homogenous grafted plants. The graft was performed using the splicing method at the 2–3 true leaf stage (3–4 weeks after sowing), and the scion was attached at the first node of the rootstock. Four weeks later, eighteen plants per graft combination were transferred to a commercial greenhouse, randomly distributed in three blocks (6 plants per genotype), and cultivated in sandy soil until completing the growing cycle. All plants were initially irrigated with complete Hoagland's solution (Hoagland and Arnon 1950) for 25 days. Then, one block received (in mM) reduced (0.5) phosphorus fertilization (low-P), another block received reduced

nitrogen (3), phosphorus (0.5) and potassium (1.5) supply (low-NPK), while the remaining plants received optimal N (12), P (2) and K (6) fertilization (control), for a period of 140 days. Low-P and low-NPK treatments were applied in two steps: first the nutrients were reduced by 50% compared to Hoagland solution for one month, and then by 75% until the end of the experiment. The other macronutrient concentrations (in mM) were as follows: Control (Ca, 3.5; Mg, 1; S, 1), Low-P (N, 12; K, 6.6; Ca, 3.5; Mg 1; S, 1.6) and low-NPK (Ca, 2.9; Mg, 1; S, 2.8). The micronutrient concentrations (in μM) were: Fe, 100; B, 22.64; Mn, 3.14; Zn, 2.09; Cu, 0.60; Mo, 0.06. Fruit yield (kg/plant) was weekly recorded until the end of the experiment 171 days after transplanting. Plants received conventional phytosanitary treatments compatible with the commercial use of managed bumblebees. Pollinator activity, gas-exchange and nutritional status were assessed as described below.

Pollinator preference quantification

Managed hives (Agrobio SL, Almeria, Spain) of pollinating insects (*Bombus terrestris*) were placed to ensure pollination of all plants following commercial guidelines. Briefly, a hive containing 50 workers ensures pollination of 300–500 plants for 8–12 weeks. Pollinator preferences were evaluated weekly throughout the complete reproductive stage of the plant, for which the flower trusses already assessed were labelled. The pollinator's preference was quantified by scoring the brown marks that the bites of the insects leave on the stamens during their visits to the flowers, so that Level 0 (L0), Level 1 (L1), and Level 2 (L2) correspond to absence, low intensity and high intensity of bites (as a percentage with respect to the total flowers assessed across the whole period), respectively (see Fig. 4a). Seven trusses (between 30 and 50 flowers) per plant and three plants per graft combination and treatment (around 1100 flowers) were assessed by three different observers and used for quantification of pollinator preference.

Gas exchange parameters

To assess the rootstock-mediated leaf physiological status at the end of experiment, photosynthesis (A_N) and stomatal conductance (g_s) were measured after 130 days of starting the treatments in the youngest

fully expanded leaves (one leaf per plant) using a CIRAS-2 system (PP Systems, Massachusetts, USA) between 09:00 h and 12:00 h. CO_2 was set at ambient level (400 ppm) and radiation matched the chamber conditions ($1300 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPF). Three plants per graft combination and treatment were used for photosynthesis and stomatal conductance measurement.

Foliar nutritional analysis

After 140 days of starting the low-P and low-NPK treatments, leaf nutritional status was determined in the same leaves used for gas-exchange measurements. To determine the elemental composition, leaves were dried for 48 h at 80 °C, milled to a powder and 200 mg of dry tissue was digested with a HNO_3 : HClO_4 (2:1, v/v) solution. Samples were analyzed by using inductively coupled plasma spectrometry (ICP-OES IRIS INTREPID II XDL, Thermo Fisher Scientific Inc., Loughborough, UK). Total C and N concentrations were determined in 200 mg of dry leaf material by the combustion method using an elemental analyzer (LECO TRUSPEC, Netherlands).

Statistical analysis

The objective of the study was to analyze the variability generated by the rootstocks on agronomic and physiological parameters of the grafted commercial variety and the preference of pollinators rather than any specific comparison between rootstocks. Therefore, data were subjected to the analysis of variance (two-way ANOVA) to test the main effects of rootstock genotype, fertilization regimes and their interaction. Means were compared using Tukey's test at 0.05 confidence level. Scatter, violin and correlation plots were built using all data since outliers removal did not significantly affected ANOVA results. Scatter plots allow to visualize the distribution of the data across treatments. Mean and error bars are shown inside of the scatter plot. Violin plots illustrate the distribution shape of the data across treatments and its probability density. Heatmaps were made using the Pearson correlation coefficient values in order to illustrate the type and strength of relationship between variables.

Fig. 1 Box plot showing the yield of *Solanum lycopersicum* cv. Julita F1' grafted onto different rootstocks growing under control (a), low-P (b) and low-NPK (c) conditions during 140 days (n=4–6). Circled numbers denote the control rootstock genotypes. (d) Scatter plot with individual data points showing the yield by the treatment (n=94–104). Different letters indicate significant differences between treatments. Δ indicates fold change in yield between the highest and lowest rootstock. Results of two-way ANOVA (p values reported) for genotype (G), treatment (T) and their interaction (G x T) are indicated in the top right of the panel. ** and *** indicate statistically significant difference at p<0.01 and p<0.001, respectively

All statistical analyses were performed using SPSS for Windows (Version 22.0, SPSS Inc., Chicago, IL, USA).

Results

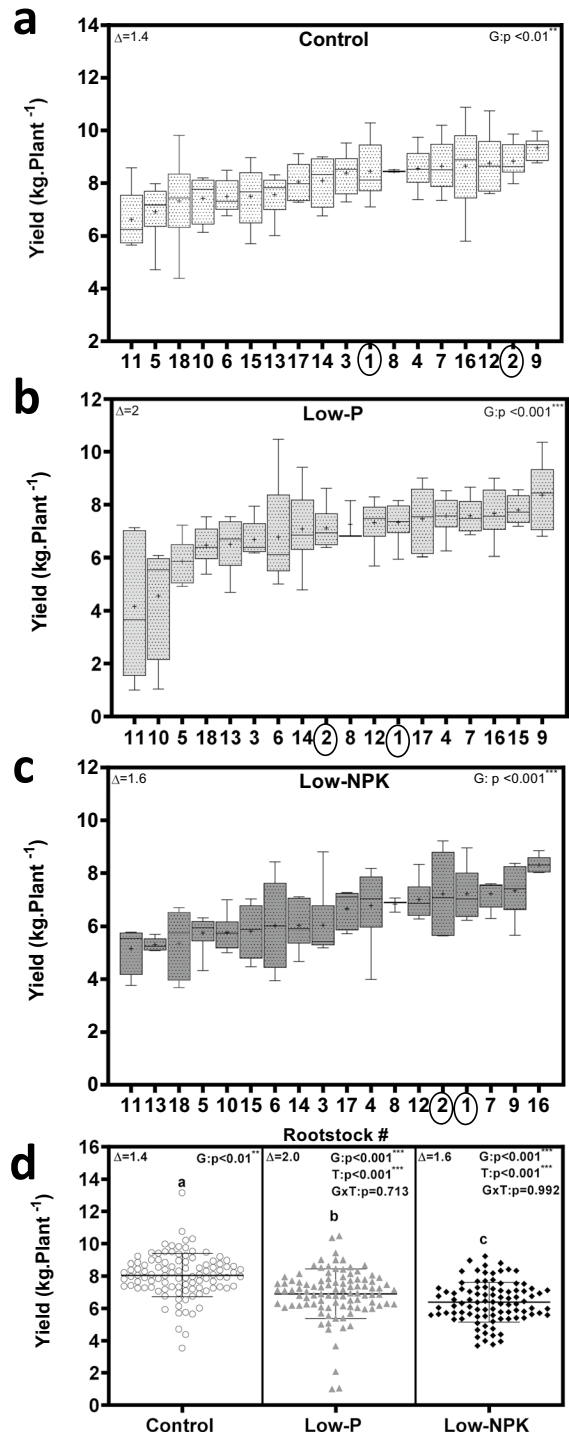
Rootstock-mediated variation in shoot agronomical and physiological parameters

Fruit yield and gas exchange

Experimental rootstocks provoked 1.4, 2 and 1.6-fold variation in fruit yield of the commercial variety under optimal and limited P and NPK fertilization, respectively (Fig. 1a-c). On average, reduced P and NPK fertilization (25% of optimal) decreased fruit yield by 15 and 20% compared to control conditions, respectively (Fig. 1d). Importantly, the photosynthetic rate and stomatal conductance were also reduced under nutrient deficiency, more significantly under low-NPK (Fig. 2a, b).

Leaf nutritional status

Irrespective of the deficitary fertilization regime, the total concentrations of macro (N, P, K, Mg, S, and Ca) and micro (Fe, Mn, B, and Zn) nutrients in the leaves were reduced by 19 and 33% compared to optimal fertilization, respectively (Fig. 2c, d). Leaf N concentration was significantly reduced (10%) under low-NPK conditions, but not under low-P, although the rootstocks provoked a similar 1.2 (low-P) to 1.3-fold (control and low-NPK) variation in this parameter (Fig. 3a). Leaf P was reduced by 14% under low nutrient supply compared to optimal nutrition, and the rootstocks generated a similar 1.6 (optimal) to 1.8-fold (low-P) variation



(Fig. 3b). Leaf K was reduced under limited nutrition, particularly under low-NPK (20%), and the rootstocks generated 1.4 (optimal) to 1.6-fold (low nutrition) variation (Fig. 3c). Interestingly,

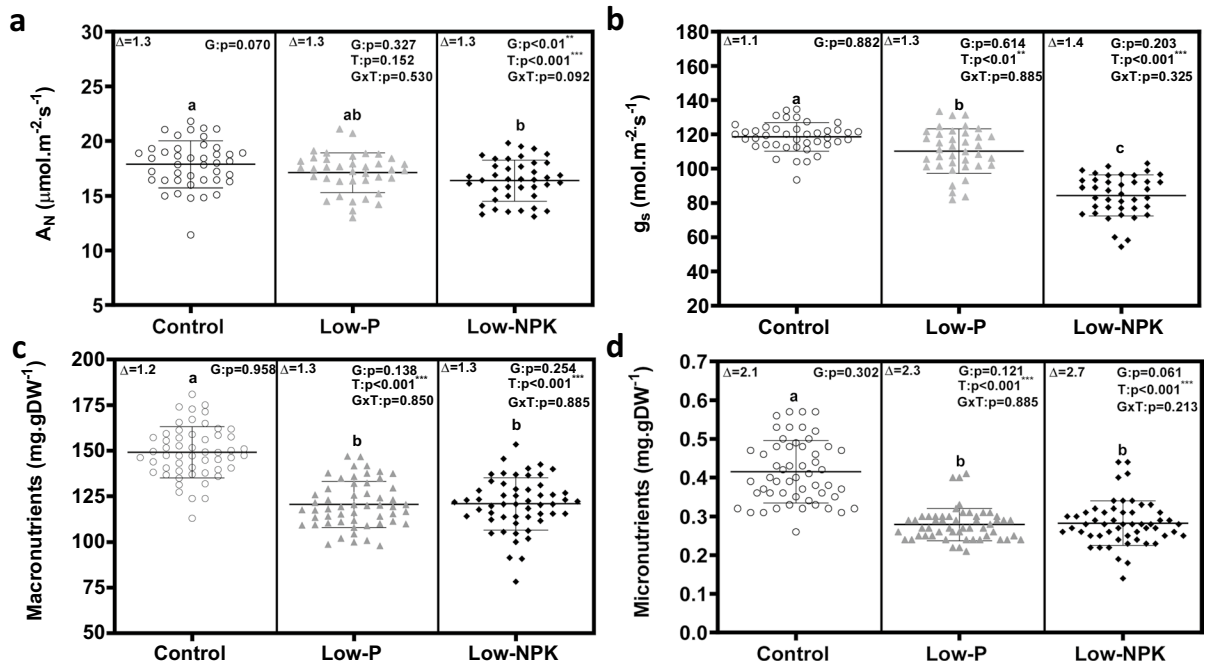


Fig. 2 Scatter plot with individual data points showing the photosynthesis rate (A_N) (a), stomatal conductance (g_s) (b), macronutrients (c) and micronutrients (d) concentrations in leaves of *Solanum lycopersicum* cv. ‘Julita F1’ grafted onto different rootstocks growing under control, low-P and low-NPK conditions during 140 days ($n=42\text{--}54$). Different letters indicate significant differences between treatments. Δ indicates

fold change in each parameter between the highest and lowest rootstock. Results of two-way ANOVA (p values reported) for genotype (G), treatment (T) and their interaction (G x T) are indicated in the top right of the panel. ** and *** indicate statistically significant difference at $p<0.01$ and $p<0.001$, respectively

the highest rootstock-mediated change was found for leaf Na concentration (2.4 to threefold; Fig. 3d), while the minimal variation was found for leaf C (1.1-fold, irrespective of the treatment), although it was the only element that increased under low-P (10%) and low-NPK (8%) conditions, compared to optimal nutrition (Fig. 3e). In general, the leaf concentrations of other macro (Mg, S, Ca) and micro (Fe, Mn, B, Zn) nutrients were reduced under low-P and low-NPK with a rootstock-mediated variability ranging between 1.5 and 2.4-fold (Fig. 3f-l).

Pollinator preference

Based on stamen marks scoring levels observed over the flowering period, where L0 (percentage of not visited flowers, no marks) is the lowest and L2 (percentage of visited flowers with most intense marks) is the highest (feeding

interest) score (Fig. 4a), plants cultivated under optimal fertilization had the highest L0 value, while those growing under low-NPK scored the highest in intermediate interest (L1) (Fig. 4b). Indeed, according to L1+L2 (percentage of flowers visited), the plants grown under limited fertilization, particularly under low-P, were more visited than those grown under optimal nutrient supply (Fig. 4b). However, flowers on plants grown under low-P were preferred by the pollinators in their foraging activity, since they registered the highest L2 score, while flowers under low-NPK were the less preferred according to this criterion (Fig. 4b, c). The set of experimental rootstocks also altered the variation in pollinator preference depending on the fertilization regime, inducing 1.6 (control), 1.9 (low-NPK) and twofold (low-P) variation in the highest pollinator preference criterion L2 (Fig. 4d-f).

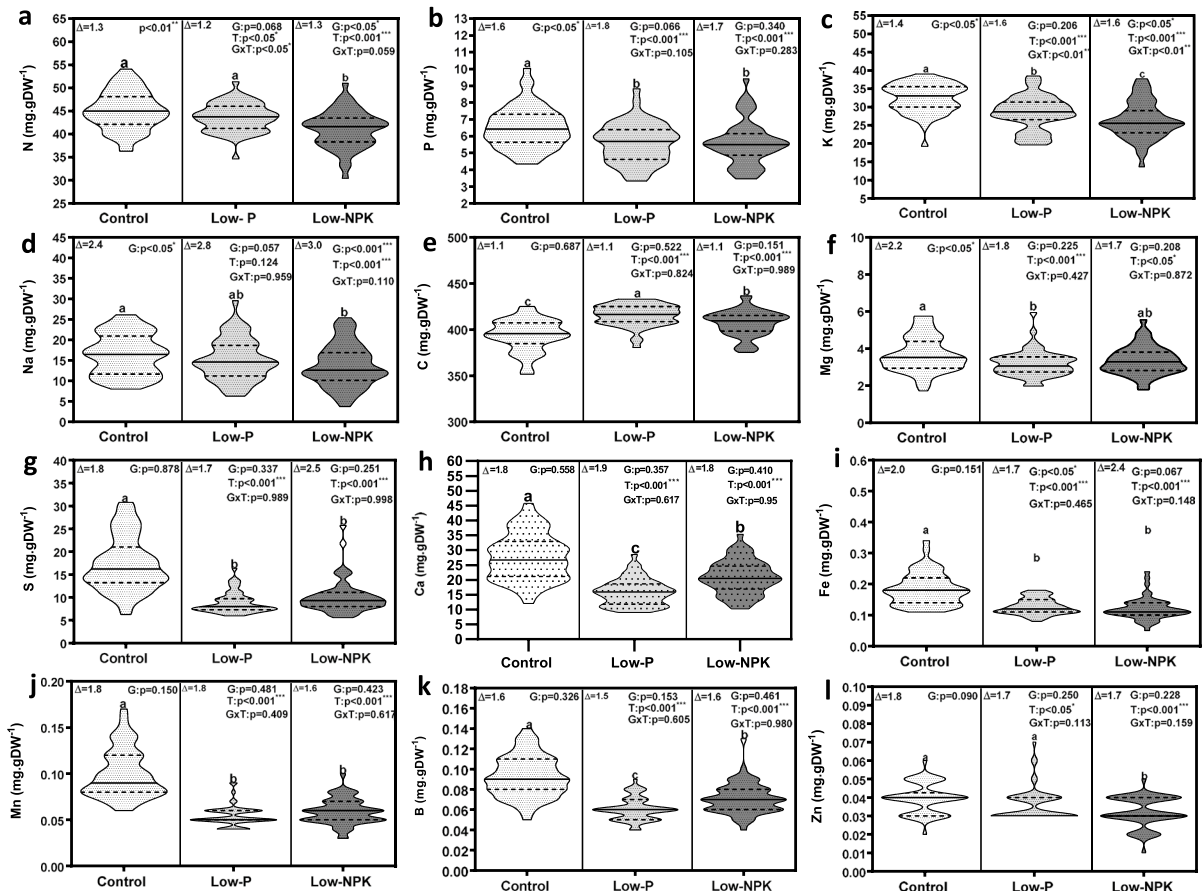


Fig. 3 Violin plot of nitrogen (N)(a), phosphorus (P)(b), potassium (K)(c), sodium (Na)(d), carbon (C)(e), magnesium (Mg) (f), sulphur (S)(g), calcium (Ca)(h), iron (Fe)(i), manganese (Mn)(j), Boron (B)(k) and zinc (Zn)(l) leaf concentrations of *Solanum lycopersicum* cv. 'Julita F1' grafted onto different rootstocks growing under control, low-P and low-NPK conditions during 140 days ($n=54$). The violin plot shows the median (marked as black line) and the first and third quartile

(marked as dashed black line). Different letters indicate significant differences between treatments. Δ indicates fold change in each parameter between the highest and lowest rootstock. Results of two-way ANOVA (p values reported) for genotype (G), treatment (T) and their interaction (G x T) are indicated in the top right of the panel. *, ** and *** indicate statistically significant difference at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively

Correlations between agronomical and physiological parameters and pollinator preferences

To check whether the level of pollinator preference can be an indicator of the plant performance, the intensity of visits during the overall flowering period was compared with the final fruit yield and the physiological and nutritional status in all graft combinations, expecting that the choice of the pollinator reflects the benefits of the rootstock on scion performance. Interestingly, the fruit yield of grafted plants correlated positively with pollinator preference L2 under low-NPK ($r=0.48$, $P \leq 0.01$) and

low-P ($r=0.35$ $P \leq 0.05$) deficit, while this correlation was negative under optimal fertilization ($r=-0.32$, $P \leq 0.05$) (Fig. 5a-c). These weak but significant correlations despite the low variability existing between rootstocks suggest the pollinator preference as a promising indicator to discern between plant genotypes under stress conditions, probably by capturing nutritional and metabolic cues.

Fruit yield correlated positively with A_N , g_s , and leaf P, S, Mg, Ca, Fe, Mn and total macronutrients, while the correlation was negative with leaf C concentration, irrespective of the fertilization regime (Fig. 6). Leaf N only correlated with yield under

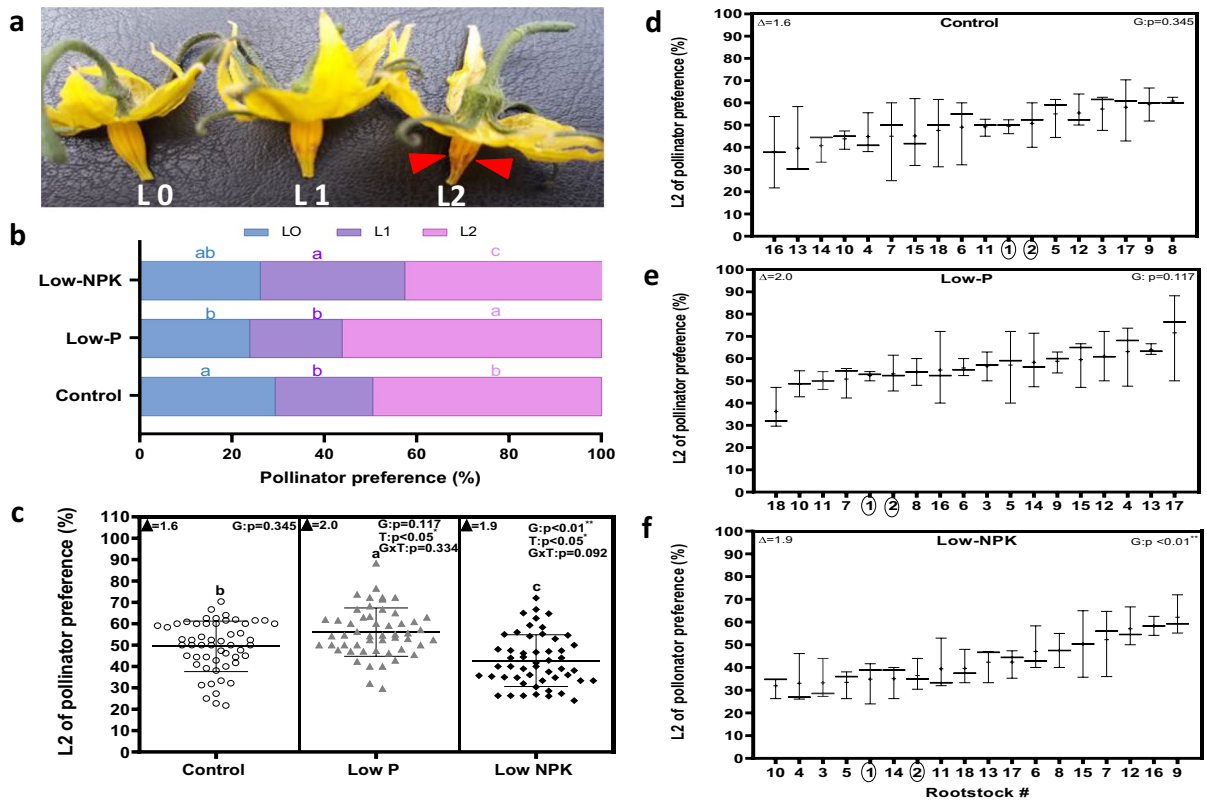


Fig. 4 Picture showing the levels of intensity of brown mark of pollinator bites on tomato flowers stamens indicating pollinator preference (a). Percentage of each of the three levels of pollinator preference depending of the treatment. Level 0 (L0), Level 1 (L1) and Level 2 (L2) indicate none, low and high pollinator preference, respectively. In the bar chart data are means of 54 values. Different letters indicate significant differences ($p < 0.05$) of preference level (L0, L1 or L2) between treatments (b). Scatter plot with individual data points showing the Level 2 of pollinator preference by the treatment ($n = 54$). Different letters indicate significant differences between treat-

optimal and low-NPK fertilization, but not under low-P, while leaf K consistently positively correlated with yield under low-P and low-NPK. Leaf Zn and B correlated positively with yield but more significantly under limited fertilization, while leaf micronutrients strongly correlated with yield under low-P but not under low-NPK.

Under optimal fertilization, photosynthesis positively correlated with leaf N, C and K, and negatively with the rest of the nutrients (Fig. 6a). However, A_N positively correlated with most of the leaf nutrients under limited fertilization, but negatively with leaf C and Na, although with some differences:

ments (c). Box plot showing the highest level of pollinator's preference (L2) of *Solanum lycopersicum* cv. 'Julita F1' grafted onto different rootstocks growing under control (d), low-P (e) and low-NPK (f) conditions during 140 days ($n = 3$). Circled numbers denote the control rootstock genotypes. Δ indicates fold change in L2 between the highest and lowest rootstock. Results of two-way ANOVA (p values reported) for genotype (G), treatment (T) and their interaction (G x T) are indicated in the top right of the panel. * and ** indicate statistically significant difference at $p < 0.05$ and $p < 0.01$, respectively

while leaf Ca, Mg, K, S, Mn and Zn are common positive factors under both limiting conditions (Fig. 6b, c), leaf N and P positively influenced A_N under low-NPK (Fig. 6c), but not under low-P (Fig. 6b).

Pollinator preference L2 was positively correlated with leaf N, C, K, and Zn under limited fertilization, particularly leaf N (both), K and Zn (-NPK), while leaf S, Na, B, Ca, and Fe correlated negatively (Fig. 6b, c). However, the opposite was found under control conditions, since L2 positively correlated with most nutrients, including Na, Ca, B and Fe, but negatively with major nutrients N, C and P (Fig. 6a).

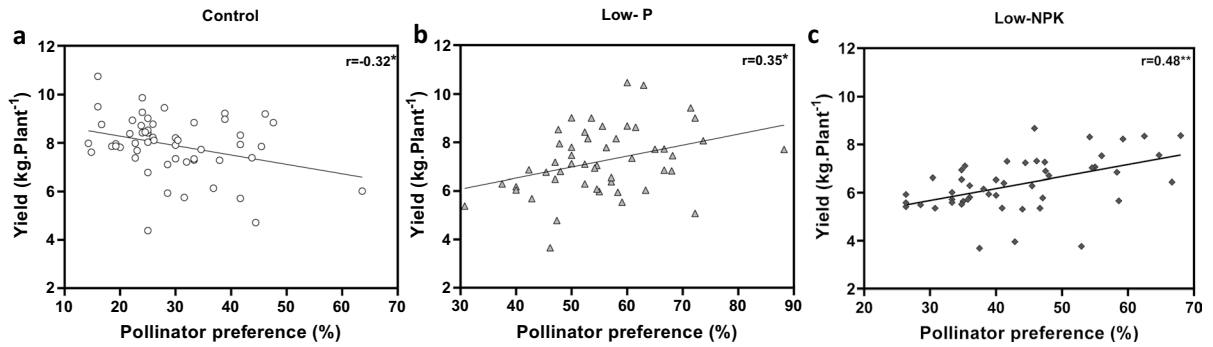


Fig. 5 Correlation between pollinator’s preference (Level 2) and total yield of *Solanum lycopersicum* cv. ‘Julita F1’ grafted onto different rootstocks growing under control (a), low-P (b) and low-NPK (c) conditions during 140 days ($n=54$). The

values of Pearson correlation coefficient (r) are given. Significance values for correlations are indicated by asterisk, where $*=p \leq 0.05$ and $**=p \leq 0.01$

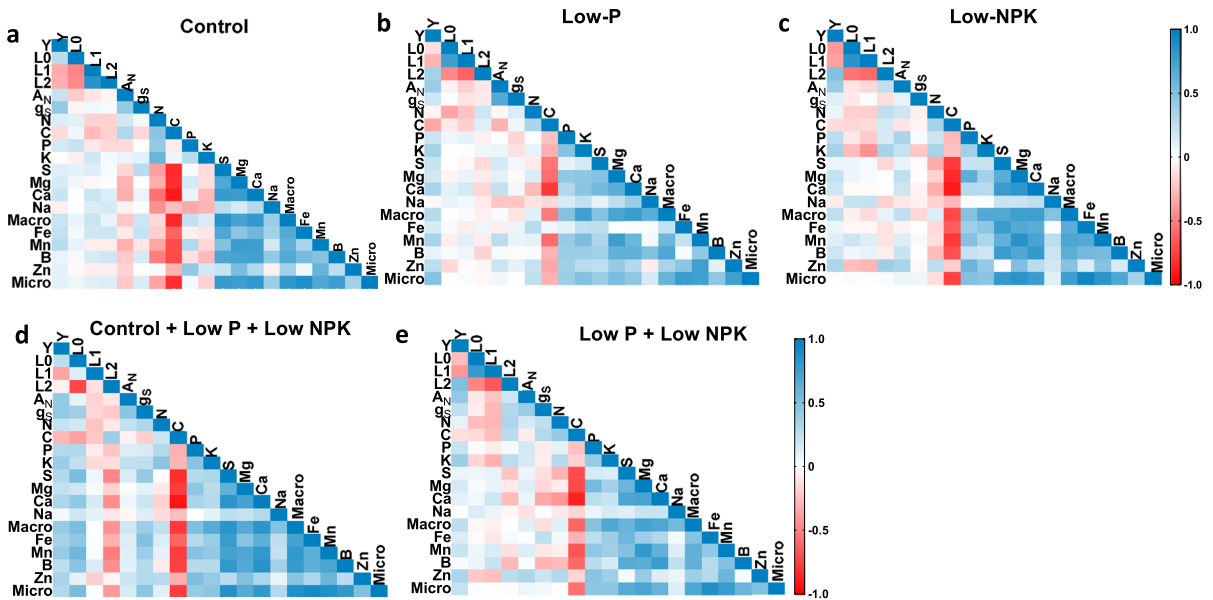


Fig. 6 Pearson correlation coefficient heatmaps for yield (Y), pollinator’s preference levels (L0, L1 and L2), photosynthesis (A_N), stomatal conductance (g_s), nitrogen (N), carbon (C), phosphorous (P), potassium (K), sulfur (S), magnesium (Mg), calcium (Ca) sodium (Na), macronutrients (Macro), iron (Fe), manganese (Mn), boron (B), zinc (Zn) and micronutrients

(Micro) concentrations in leaves of *Solanum lycopersicum* cv. ‘Julita F1’ grafted onto different rootstocks and grown under control (a), low-P (b), low-NPK (c), control+low-P+low-NPK (d) and low-P+low-NPK (e) conditions. The colour-code denotes a gradient from positive (blue) to negative (red) r values

Leaf N positively correlated with leaf C, P and K under optimal, and more weakly under low-NPK and low-P (only C and K). However, leaf N correlated negatively with the rest of nutrients under optimal fertilization, but positively with Fe and Zn under limited supply (Fig. 6a-c). Except for N, leaf C negatively correlated with most of nutrients irrespective

of the fertilization regime (Fig. 6a-c). Leaf P correlated positively with the rest of nutrients (except Na, Fe and B under control), more significantly under low fertilization, particularly with Zn.

By pooling the three treatments together (Fig. 6d), yield is positively influenced by A_N and leaf mineral status, negatively affecting leaf C concentration, but

uncoupled from the pollinator preference (L2), which in turn is mainly influenced by the leaf C status. However, under limited fertilization (-P and -NPK), yield is strongly linked to pollinator preference, which is positively related to the leaf C and mineral status, particularly N, K, and Zn, which positively affect A_N (Fig. 6e).

Discussion

The effects of the rootstocks on the yield are related to different processes affecting the vigour of the plant and the uptake and transport of water and nutrients to the scion. Those effects are expected to be more evident under suboptimal conditions, as reflected in the 0.2 (low-NPK) to 0.6-fold (low-P) higher yield variability generated under limited compared to optimal fertilization (Fig. 1a-c). Given the diversity and cumulative rootstock-mediated traits and the complex relationship between nutrient availability and plant growth, it is not necessarily expected to find a correlation between the fruit yield and the leaf concentration of a particular nutrient deficiently available to the plant. However, the positive effects of those rootstock-mediated traits are integrated with the physiological status of the scion, as supported by the positive correlation between A_N and yield (Fig. 6). Indeed, this study reveals that both the rootstock-mediated yield and A_N under limited fertilization are related to the leaf concentration of K, P, and, to a lesser extent, N, depending on the limiting nutrient, besides other macro (S, Mg, and Ca) and micro (Mn and Zn) nutrients (Fig. 6b, c, e).

Although insect-mediated pollination is one of those non-studied rootstock-mediated traits that could influence yield, this study investigated if the pollinator preferences during their foraging decisions can reflect the physiological status of the plant growing under suboptimal conditions. Based on visual tracking of bumblebees' activity on flower stamens and the positive relationship between yield and pollinator preference (L2), the results support such a hypothesis (Figs. 5b, c; 6b, c, e). Even if L2 was not necessarily related to the leaf status of the deficient nutrient, as it occurs with P under low-P (Fig. 6b), pollinator preference was associated with the nutritional status for N, C (low-P) and N, C, P K, and Zn (low-NPK), supporting that pollinator preference is a good indicator of

the plant nutritional status under limited fertilization. However, L2 did not correlate with yield across different treatments (Fig. 6d) and even correlated negatively under optimal fertilization (Figs. 5a and 6a), suggesting that the relationship between pollinator preference and yield can be specific to the environmental conditions, coupling this ecological decision to the plant phenotype.

On average, pollinator preference (L2) increased under low-P, but decreased under NPK deficit, while the percentage of flowers visited by bumblebees (L1 + L2) was higher under limited fertilization, compared to control conditions (Fig. 4b, c). Interestingly, the only chemical species analyzed in the leaves that followed a similar pattern was the carbon concentration (Fig. 3e). While leaf C concentration increased under nutrient limitation, it was consistently and negatively correlated to yield across treatments (Fig. 6d). Furthermore, despite leaf C correlated positively with photosynthesis and negatively with pollinator preference L2 under control conditions (Fig. 6a), the opposite was found under both nutrient deficit conditions (Fig. 6b, c). Those results couple C concentration, assimilate metabolism and partitioning and plant growth. Indeed, high growth capacity is related to high respiration and higher nutrient uptake rates, while reduced growth under stress may be related to increased concentration of cell wall material and metabolites (Lambers and Poorter 1992), supporting the negative correlation between leaf C concentration with yield and the leaf mineral nutrient concentration (Fig. 6). Moreover, in plants with slow growth, the concentration of non-structural carbohydrates (NSC, i.e. starch and low molecular weight sugars) is commonly high, and vice versa (Körner 2003, 2015). Therefore, pollinators could be sensing the homeostasis of assimilate metabolism and source-sink relations in the plants, as reflected in their foraging decisions.

Besides the differences in structural carbohydrates such as cellulose, hemicellulose and lignin, the increases in C concentration under reduced fertilization may be rather due to the accumulation of NSC, such as sucrose, hexoses and starch (Ma et al. 2018), which could negatively impact on photosynthesis through non-stomatic feedback inhibition (Dewar et al. 2022), but positively on pollinator foraging decisions if assimilates accumulate in the flowers. This situation is likely to occur under low-P because of the strong induction of leaf C accumulation (Fig. 3e) and

its negative correlation with A_N and yield (Fig. 6b). Adequate P levels are necessary for key processes such as photosynthesis, respiration, signalling (Vance et al. 2003), cell division, enzyme activation, membrane structure (Hoehenwarter et al. 2016), through the synthesis, transport, and activation of high-energy biomolecules (Marschner et al. 2011). However, P deficiency induces starch and soluble sugars accumulation in the leaves, reducing Calvin Cycle activity and CO_2 fixation by limiting RuBP regeneration (Fredeen et al. 1990), although limited export to sink tissues may also occur due to reduced ATP availability and sucrose loading in the phloem (Rao et al. 1990). However, a better rootstock-mediated P nutritional status in the leaves can mobilize starch reserves through phosphorylation (Jacobsen et al. 1998) and promotes source-sink relations and sugar transport to the flowers (Lemoine 2000) by inducing sink strength in the reproductive structures (Erel et al. 2016). Interestingly, reduced P fertilization also increased the pollinator preference compared to optimal and low-NPK (Fig. 4c), which could be due to the metabolic changes provoked, non-visually observed but detected by the insects. Recently, it has been reported that P fertilization can improve almond yield by promoting pollinator (honeybees) visitation through mobilizing starch reserves and producing extra nectar (Karunakaran et al. 2021). The results suggest that pollinator visitation can be used as an indicator for precise P application to maximize yield and P use efficiency in a pollinator-dependent species such as almonds. Hence, P fertilization affects sink activity and quality attributes of flowers affecting fruit set as has been observed in olive (Erel et al. 2016) and almond (Karunakaran et al. 2021) trees, as it also seems to occur in tomato.

Therefore, if we assume that changes observed in total C concentration are mainly due to NSCs, it seems reasonable to find a consistent negative relationship between leaf C concentration and yield. The mobilization of carbohydrates for growth and other sink activities in those plants with a better nutritional and physiological status under suboptimal conditions could explain the negative relationship between leaf C concentration and A_N , while the pollinators will benefit from the quantity and/or quality of pollen thanks to the carbohydrates and assimilates transported from the leaves to the flowers in the healthiest genotypes. A higher rootstock-induced growth/yield would also

result in lower mineral nutrient concentrations in the leaves, thus supporting the negative covariation with total C concentration. Hence, the capacity to attract photoassimilates of the flower or sink strength can influence not only the pollinator visitation and the fruit set (Nepi et al. 2018), but also the preference of insects for some plants over others, reflecting the physiological homeostasis, particularly under suboptimal conditions. Leaf K and Zn concentrations also seem to be key factors in rootstock-mediated benefits on photosynthesis, yield and pollinator preference under reduced fertilization, particularly under low-NPK (Fig. 6c, e). Both nutrients are essential in carbohydrate and photosynthetic metabolism, energy production, assimilate mobilization to the sinks and in pollen tube formation (Gondal 2021; Luo et al. 2021). Moreover, leaf N was the only mineral nutrient positively correlated with leaf C in all treatments, supporting a major role for both highly coordinated elements not only in plant productivity, but also in ecosystemic pollinator foraging decisions.

The results indicate that while the rootstocks can essentially improve the nutritional status of the plants, this improvement is mainly translated into yield under nutrient-deficient conditions, which undoubtedly contributes to increase nutrient use efficiency. Interestingly, it seems that pollinators can sense the leaf nutritional status of the plants provided by different rootstocks, and could additionally influence yield through promoting pollination under P and NPK limited supply (not assessed in this study). Under those limiting conditions, total and particular nutrients were related to the photosynthetic activity that could in turn influence the quality of pollen feeding the insects and influence the number and intensity of visits during their foraging activity. Curiously, this relationship between pollinator activity, nutritional status and plant yield seems to be uncoupled under control (optimal) nutrient conditions, which suggests that increased nutritional status does not additionally influence photosynthesis and yield and can negatively influence the energetic/nutritional rewarding of the flowers/pollen and pollinators activity. This idea is similar to the general negative relationship existing between fruit yield and fruit quality (e.g. concentration of soluble solids in tomato), thus explaining the negative correlation observed between yield and pollinator visitation under optimal conditions in the grafted

population under evaluation. Overall, although the visiting decisions to flowers could initially depend on scent signals, the results suggest that pollinators can naturally select the most ‘nutritious’ and yielding rootstock genotypes under a selection pressure provided by nutrient limitations through sensing metabolic cues, which is being investigated.

Although the yield dependence on pollinators at crop level is well-known, a high varietal dependence can also exist, as reported in rapeseed (Hudewenz et al. 2014) and canola (Adamidis et al. 2019). In this study, the yield trait has been uncoupled from any putative varietal dependence, since the variety is always the same and all the plants have been cultivated with managed bumblebees. Therefore, the differences in pollinator visitation should be provided by the rootstock genotype and the environment. However, the influence of specific unknown interactions with other environmental factors (e.g. phytosanitary treatments) and/or a putative specific yield-dependence of some graft combinations on insect pollination cannot be ruled out, particularly under resource-limiting conditions. Understanding to what extent the pollinators decisions affect crop yield and resource use efficiency through selecting the most resilient and pollinator-dependent genotypes is of the utmost interest in horticulture. The development of methods for the automated sequential analysis of genotype x environment x pollinator interactions and the underlying multi-omics components are being investigated.

Conclusion

Our study indicates that rootstock breeding using wide genetic variability existing in *Solanum* sp. is a promissory strategy to enhance fruit yield in elite tomato varieties under reduced fertilization through improving its nutritional and physiological status. Furthermore, we have shown for the first time that pollinators preferences during their foraging decisions covary with the nutritional and physiological status of the plants as influenced by the rootstocks and the fertilization regime. Overall, rootstock-mediated yield and A_N are intimately related through the leaf mineral status, influencing leaf C and pollinator foraging decisions, probably through source-sink relations, which is agronomically more relevant under limited fertilizer supply. Such findings suggest that

pollinators can be used as natural plant phenotypers under environmental pressure, which opens new perspectives in phenotyping, selection and breeding based on ecological decisions.

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Author contribution F.P.-A. conceived the Project and designed the research, C.M.-A and R.B grew the plants and performed all the stress experiments. C.M.-A, A.S.P., M.O., J.A.-M. executed the agronomical agronomic analyses, C.M.-A, A.A. and P.A.-M. performed the physiological analysis. C.M.-A. and R.B carried out the pollinator preference quantification. C.M.-A performed the data analysis, C.M.-A. and F.P.-A wrote the original draft preparation. F.P.A reviewed and edited the final manuscript. All the authors provided critical suggestions for the realization of the manuscript.

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Data availability All data generated or analyzed during this study are included in this article and are available from the corresponding author on reasonable request.

Declarations

Competing interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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