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Early transcriptomic changes in cucumber and maize roots in response to $FePO_4$ nanoparticles as a source of P and Fe

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The use of nanoparticles as an alternative to traditional fertilizers, aiming at a more efficient use of nutrients, is a recently developed concept that requires a thorough understanding of the processes occurring in the soil-plant system. A crucial aspect in this framework is to decipher the plant responses to the unique characteristics of these materials. In this work, we aim at decoding the transcriptional responses of cucumber and maize roots to $FePO_4$ nanoparticles applied as P and Fe sources, respectively. The results demonstrate that P and Fe supplied as nanoscale salts support plant nutrition with an efficiency comparable to that of ionic forms of the nutrients. This supposition is confirmed by transcriptomic profiles that show no significant upregulation of transcripts typically induced by deficiencies in P and Fe in cucumber and maize plants in which these nutrients were provided by $FePO_4$ nanoparticles. The analysis further revealed that nanoparticles alter the expression of genes involved in root development and stress responses, effect that appeared to be independent on the nutritional status of the plants. Our data further underline the challenge to identify generalizable elements of the impact of nanomaterials on plant species, as responses are intimately linked to the type of nanomaterials and differ among plant species.

Keywords Nanoparticles, P nutrition, Fe nutrition, Cucumber, Maize, Transcriptomics

Driven by global population growth and changing dietary patterns, global food demand is projected to increase by 70% by 2050[1]. Additionally, the intensive use of pesticides and fertilizers had significant environmental impacts, including pollution, soil salinization, and the deterioration of soil biological health[2]. Therefore, a challenge of modern agriculture is to develop new technologies that enhance food supply while mitigating adverse environmental effects and addressing climate change[3]. Nano-scaled particles, ranging from 1 to 100 nm in at least one dimension[4], possess unique physical and chemical properties that are being rapidly exploited across various scientific fields including agriculture[5]. When used to support plant nutrition, these particles are known as nanofertilizers[6]. The peculiar properties of nanomaterials enable them to release nutrients in a controlled manner under specific environmental conditions, significantly enhancing nutrient use efficiency[7–9]. A critical aspect of crop fertilization is to minimize nutrient losses and to synchronize nutrient release with their interception by plant roots, thereby reducing chemical inputs and environmental impact[4,6,10].

Phosphorus (P), an element playing crucial structural, energetic, and post-translational regulatory roles in plants[11], is one of the most limiting nutrients in soils and the one with the lowest nutrient use efficiency, accounting for only 10–15%[12]. In response to P deficiency, plants activate a wide range of morpho-physiological acclimations including a modified root architecture and reduced shoot growth, resulting in an enhanced root/shoot biomass ratio, reduced leaf expansion, and, consequently, an increase in chlorophyll content[13,14]. Furthermore, P-deficient roots induce the expression of high-affinity Pi transporters and enhance the extrusion of organic acids, protons, and phosphatases to solubilize immobile P forms, thereby increasing their acquisition by crops[15].

Among the micronutrients, iron (Fe) is required in the largest amount by plants[16]. However, its solubility in soil is extremely low, particularly in calcareous soils, which account for about 30% of the world's cultivated soils[17]. Iron plays a pivotal role in essential metabolic processes such as respiration, photosynthesis, and chlorophyll biosynthesis[16,18]. Leaf chlorosis is the most evident symptom of Fe deficiency[16]. Plants have evolved two

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strategies to face Fe shortage in the environment: Strategy I, employed by non-grasses, is based on acidification of the rhizosphere and reduction of insoluble ferric Fe species[18] and Strategy II, adopted by grasses, which is based on the chelation of such forms of Fe[18].

Most transcriptional investigations on the effects of nanoparticle (NP) application in plants have primarily focused on the phytotoxicity of metal-based and carbon-based nanomaterials[19]. The mechanisms of phytotoxicity were found to be highly dependent on the type of nanomaterials, although some studies highlight a common response of genes related to both abiotic and biotic stress in plants exposed to various types of NPs[20–24]. A much smaller number of studies have evaluated gene expression changes when NPs are applied as a source of mineral nutrients[25–27]. The rationale for the present study derived from previous findings of our research group. Specifically, we developed a method for synthesizing citrate-capped $FePO_4$ NPs[28], which were shown to be an efficient source of both P and Fe, particularly when compared to their bulk, non-nano counterparts in hydroponic systems. It has been suggested that, due to their sub-micron size, NPs can reach the root surface in greater amounts[29]. Moreover, their high surface-to-volume ratio, combined with root activity, is expected to enhance their dissolution rate compared to bulk $FePO_4$, leading to species-specific plant responses[29]. Moreover, the NPs demonstrated the ability to sustain P nutrition in pot-grown cucumber plants as triple superphosphate[7] without significantly impacting soil biology[29].

Here, we attempted to catalogue the early transcriptomic responses (after 24 h) of hydroponically-grown cucumber and maize roots when $FePO_4$ nanoparticles were applied as sources of P and Fe, respectively. We compared the transcriptional profiles of roots which acquired P or Fe from NPs with those of plants treated with the corresponding ionic forms of the nutrients. We hypothesized that, in addition to their nutritional effects, NPs might induce additional root responses due to their nano-sized solid nature.

Results and discussion

Plant growth and physiological analyses

Previous research by Sega et al.[29] demonstrated that $FePO_4$ NPs are an efficient source of P and Fe for cucumber and maize plants, respectively. The experiments presented in the current manuscript corroborate these findings as evidenced by the plant growth parameters and SPAD index (Figs. 1 and 2, Figs. S1, S2, S3 and S4). Specifically, the application of $FePO_4$ NPs as a P source (PNP) to cucumber plants resulted in shoot weights that were similar to control plants, no significant differences were observed in root weights between the two conditions (Figs. 1 and 3). Furthermore, the application of $FePO_4$ NPs as an Fe source allowed maize plants to attain shoot and root dry weights comparable to those of control plants (Fig. 2). These observations suggest that $FePO_4$ NPs can effectively supply essential nutrients, promoting healthy plant growth under nutrient-deficient conditions. However, we observed significant differences in root morphology between FeNP-treated and control maize plants (Fig. 4), with FeNP-treated plants showing lower total and lateral root lengths. This indicates that $FePO_4$ NPs can adequately supply Fe but may negatively impact root morphology in maize.

Root transcriptional changes in response to NP treatment

To understand the action of $FePO_4$ NPs on nutritional processes related to the acquisition of P (in cucumber) and Fe (in maize), we studied the early transcriptomic responses in roots of these two species to short-term exposure to $FePO_4$ NPs. Specifically, we identified transcripts that were differentially expressed in roots among plants treated for 24 h with $FePO_4$ NPs, plants grown in complete nutrient solution (C), and plants grown in the absence of P (-P, for cucumber) or Fe (-Fe, for maize). The number of differentially expressed transcripts (DETs) identified in the two root transcriptome comparisons carried out for each species is reported in Table 1. The

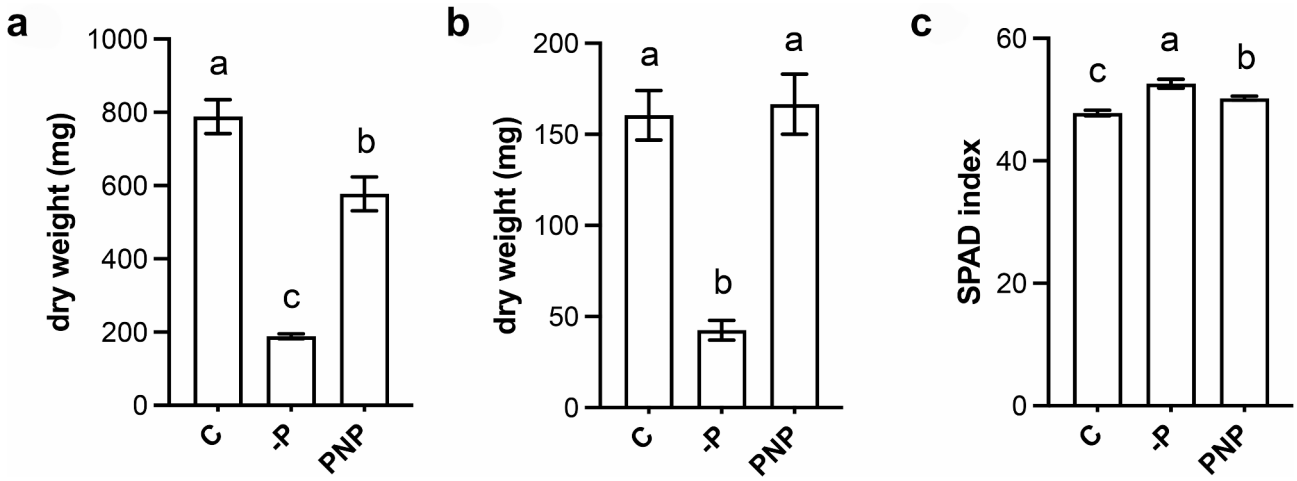

Fig. 1. Phenotypic parameters of FeNP-treated cucumber plants. **a** Shoot dry weight; **b** Root dry weight; **c** Leaf SPAD index after 13 d of growth in hydroponics. Control plants (C); plants grown without P (-P); plants grown with NPs as a source of P (PNP). Data are means ± S.E. of three independent experiments with three plants each (one-way ANOVA with Tukey's test, $n = 9$, three plants for each independent growth experiment, $p < 0.05$).

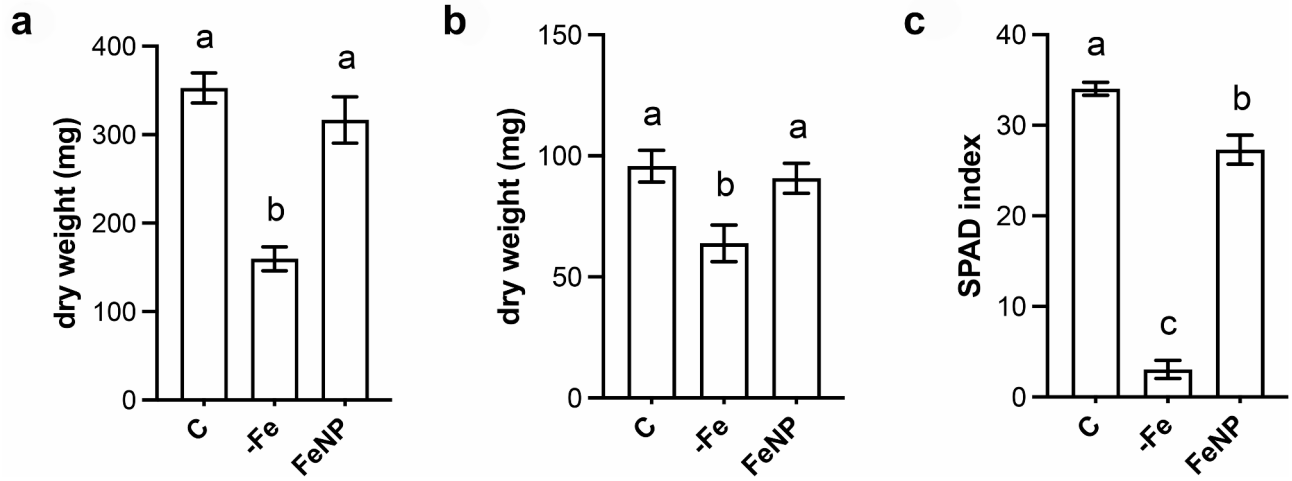

Fig. 2. Phenotypic parameters of FeNP-treated maize plants. **a** Shoot dry weight; **b** Root dry weight; **c** Leaf SPAD index of maize plants after 10 d of growth in hydroponics. Control plants (C); plants grown without Fe (-Fe); plants grown with NPs as a source of Fe (FeNP). Data are means ± S.E. of three independent experiments with three plants each (one-way ANOVA with Tukey's test, $n = 9$, three plants for each independent growth experiment, $p < 0.05$).

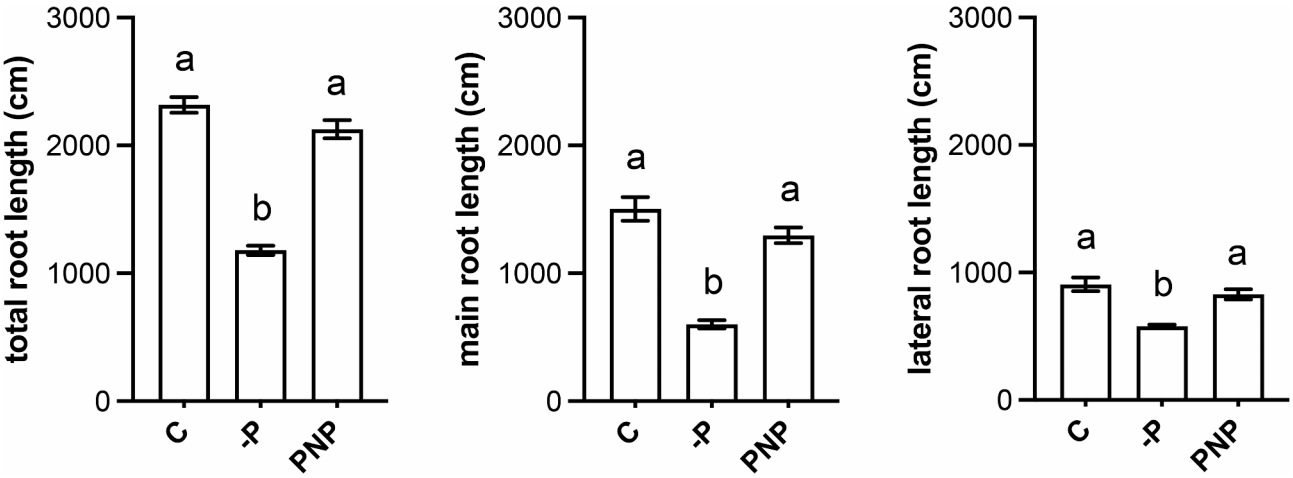

Fig. 3. Total, main, and lateral root length cucumber plants after 13 days of growth in hydroponics. Control plants (C); plants grown without P (-P); plants grown with NPs as a source of P (PNP). Data are means ± S.E. of three independent experiments with three plants each (one-way ANOVA with Tukey's test, $n = 9$, three plants for each independent growth experiment, $p < 0.05$).

differential expression of a selected set of transcripts was also analysed by RT-qPCR experiments (Table S1). The highest number of DETs was observed in maize roots when the $FePO_4$ NP treatment was compared to control plants (Table 1). The results of a GO enrichment analysis of the DETs are reported in Figs. S5 and S6.

Transcriptional differences between NP-treated and P-sufficient cucumber roots

To determine whether the role of $FePO_4$ NPs was solely nutritional (i.e., by providing P intercepted by the roots) without affecting other physiological or developmental processes, we first compared transcripts that were differentially expressed between NP-treated and P-sufficient plants (PNP vs. C, Additional file 1). In this comparison, no transcripts related to P uptake, P metabolism, or cell wall metabolism linked to root developmental processes were identified after 24 h, highlighting the role of $FePO_4$ NPs as an efficient source of the macronutrient for cucumber[29]. Interestingly, $FePO_4$ NPs seem to enhance the stress responses in the roots of cucumber plants. We recorded upregulation of two transcripts (*Cucsa.197540.10* and *Cucsa.241740.1*, Table 2), homologous to *AtNHL10* and *AtICS2*, respectively, that are putatively involved in biotic stress responses. *AtNHL10* is a pattern-triggered immunity-responsive gene[30], while *AtICS2* encodes isochorismate synthase 2, which plays a role in salicylic acid synthesis and is involved in the enhancement of plant immunity at low temperatures[31]. Another transcript induced by $FePO_4$ NPs (*Cucsa.326110.1*, Table 2) shows homology to

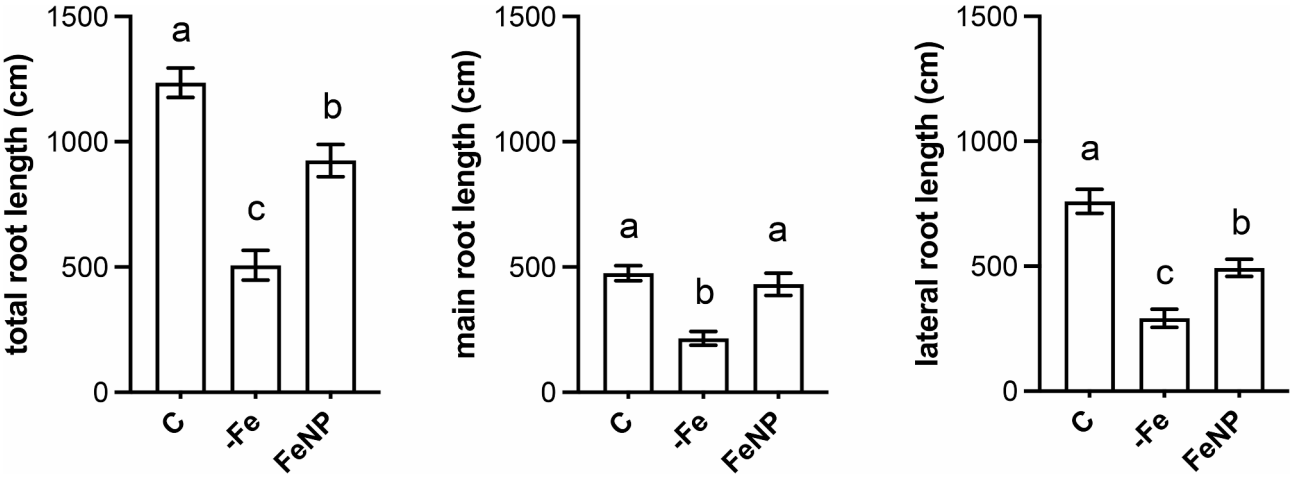

Fig. 4. Total, main, and lateral root length maize plants after 10 days of growth in hydroponics. Control plants (C); plants grown without Fe (-Fe); plants grown with NPs as a source of Fe (FeNP). Data are means ± S.E. of three independent experiments with three plants each (one-way ANOVA with Tukey's test, $n = 9$, three plants for each independent growth experiment, $p < 0.05$).

Comparison	DET	Downregulated	Upregulated
Cucumber			
PNP vs. -P	81	60	21
PNP vs. C	40	11	29
Maize			
FeNP vs. -Fe	81	14	67
FeNP vs. C	147	12	135

Table 1. Number of DETs identified in the various comparisons of root transcriptional profiles from cucumber and maize 24 h after the onset of the treatments (t-test, adjusted p-value < 0.05; FC≥ |2|).

AtPRX52, which is responsive to the fungal toxin fumonisin B1 and may play a role in modulating H_2O_2 levels during the oxidative burst in response to microbial pathogen attack[32]. Only one transcript linked to biotic stress response was found to be downregulated in $FePO_4$ NP-treated plants (*Cucsa.254210.1*, Table 2). This gene is homologous to *AtSTP13*, encoding a sugar transporter involved in resistance to *Botrytis cinerea* in *Arabidopsis*[33]. Our data on biotic stress responses contrast with previous findings in Arabidopsis, where NPs composed of different materials (TiO_2-NPs, Ag-NPs, and multi-walled carbon nanotubes) suppressed pathogen-responsive genes and those involved in salicylic acid-mediated pathways after 12 h. This response was linked to increased bacterial colonization in infection experiments[19]. It thus appears that data obtained with different experimental systems (e.g., a certain combination of NPs and plants) can lead to different results, rendering a prediction of the outcome of such experimental difficult. $FePO_4$ NPs may be perceived on the root surface as xenobiotic compounds, as indicated by the upregulation of transcripts involved in detoxifying these exogenous substances (*Cucsa.256030.1* and *Cucsa.058370.1*, Table 2). These two transcripts encode proteins homologous to AtGSTU7 and AtHYR1, respectively. AtGSTU7 is putatively involved in herbicide detoxification in roots[34], while AtHYR1 is an UDP glucosyl transferase that mediates resistance to hypostatin, an inhibitor of cell expansion[35].

Transcriptional differences between NP-treated and P-deficient cucumber roots

To investigate early effects of $FePO_4$ NPs on the nutritional status of cucumber plants, we analysed the transcriptomic profiles of plants supplemented with NPs and plants grown without P (-P) (Additional file 1). The identified DETs indicate that $FePO_4$ NPs may influence root development and metal homeostasis of the plants. A putative effect of NPs on root development is evidenced by the downregulation of an expansin transcript (*Cucsa.355990.1*, Table 2) and increased abundance of a transcript encoding a pectin lyase (*Cucsa.249050.1*). Expansins are known to facilitate cell wall loosening, a process that is crucial for cell growth and the emergence of lateral roots[36]. However, lateral root development also hinges on the activities of pectate lyases and the de-esterification of pectins by pectin methyl esterases[36]. Notably, $FePO_4$ NPs caused downregulation of a transcript encoding a plant invertase/pectin methylesterase inhibitor (*Cucsa.286280.1*, Table 2). This protein family was shown to promote lateral root development and overall root growth[37]. The supposition that $FePO_4$ NPs promote root growth is further supported by their ability to downregulate the expression of glucan synthase-like 4 (*Cucsa.058230.1*, Table 2), a homolog of *AtGSL4* involved in callose deposition in plasmodesmata. Callose deposition can inhibit root growth, particularly under stress conditions[38]. Conspicuously, these findings appear

Transcript ID	Description	TAIR10 ID	*p*-value, adj	Fold change
DETs (PNP vs. C) in cucumber roots				
Cucsa.058370.1	UDP-Glycosyltransferase superfamily protein	AT3G21760.1	0.022	2.991
Cucsa.197540.10	Isochorismate synthase 2	AT1G18870.1	0.049	2.543
Cucsa.241740.1	Late embryogenesis abundant (LEA) hydroxyproline-rich glycoprotein family	AT2G35980.1	0.017	2.068
Cucsa.254210.1	Major facilitator superfamily protein	AT5G26340.1	0.010	-2.088
Cucsa.256030.1	Glutathione S-transferase tau 7	AT2G29420.1	0.034	2.126
Cucsa.326110.1	Peroxidase superfamily protein	AT5G05340.1	0.007	2.695
DETs (PNP vs. -P) in cucumber roots				
Cucsa.058230.1	Glucan synthase-like 4	AT3G14570.1	0.004	-2.002
Cucsa.090660.1	Nicotianamine synthase 4	AT1G56430.1	0.024	-2.833
Cucsa.092390.1	Nicotianamine synthase 4	AT1G56430.1	0.007	-2.215
Cucsa.249050.1	Pectin lyase-like superfamily protein	AT3G07970.1	0.035	3.625
Cucsa.257230.1	Heavy metal transport/detoxification superfamily protein	AT1G29000.1	0.007	2.024
Cucsa.286280.1	Plant invertase/pectin methylesterase inhibitor superfamily protein	AT1G23350.1	0.028	-2.188
Cucsa.352750.1	Root hair defective 6-like 2	AT4G33880.1	0.037	-2.365
Cucsa.355990.1	Expansin-like A1	AT3G45970.1	0.023	-2.038
DETs (FeNP vs. C) in maize roots				
AC217910.3_FGT001	Heavy metal transport/detoxification superfamily protein	AT4G39700.1	0.014	2.609
GRMZM2G004276_T01	Patatin-like phospholipase family protein	AT5G04040.1	0.048	3.151
GRMZM2G015280_T01	Peroxidase superfamily protein	AT5G05340.1	0.005	-2.594
GRMZM2G074761_T01	Alternative oxidase 1 A	AT3G22370.1	0.046	-2.813
GRMZM2G090381_T01	Cyclic nucleotide gated channel 1	AT5G53130.1	0.010	2.323
GRMZM2G125669_T01	Alternative oxidase 1 A	AT3G22370.1	0.003	-3.814
GRMZM2G138450_T01	Peroxidase superfamily protein	AT5G15180.1	0.001	-21.968
GRMZM2G346095_T01	Chalcone and stilbene synthase family protein	AT5G13930.1	0.038	3.078
GRMZM2G417229_T01	Homeobox protein 22	AT4G24660.1	0.044	2.089
GRMZM2G480439_T01	Glutathione S-transferase TAU 18	AT1G10360.1	0.019	6.986
DETs (FeNP vs. -Fe) in maize roots				
GRMZM2G061776_T01	Peroxidase family protein	AT4G30170.1	0.003	2.261
GRMZM2G148374_T01	H^+-ATPase 8	AT3G42640.1	0.010	4.197
GRMZM2G177940_T01	Pectin lyase-like superfamily protein	AT5G19730.1	0.008	2.582
GRMZM2G361475_T01	Peroxidase superfamily protein	AT1G71695.1	0.038	2.005
GRMZM5G888797_T01	Pollen Ole e 1 allergen and extensin family protein	AT2G27385.2	0.033	4.326

Table 2. DETs cited in the results and discussion section.

to be congruent with our phenological analyses, revealing that plants treated with $FePO_4$ NPs develop a root system akin to control plants supplied with ionic forms of P (Fig. 3). Further evidence supporting the efficiency of these NPs as a P source is provided by the regulation of transcripts involved in root hair elongation. Generally, root hair development is responsive to the P nutritional status of the plant[39]. In *Arabidopsis*, P starvation stimulates root hair elongation through induction of the bHLH proteins *AtRSL2* and *AtRSL4*[40]. Adequate P supply suppresses root hair elongation[41]. NP-treated plants showed reduced transcript abundance of the *AtRSL2* homolog *Cucsa.352750.1* relative to P-starved plants (Table 2), indicative of an adequate P supply.

The presence of $FePO_4$ NPs appears to alter metal homeostasis in roots. We observeddifferential expression of a heavy metal transport/detoxification superfamily protein (HIPP/HPP) transcript (*Cucsa.257230.1*, Table 2), possibly associated with metal transport[42]. This transcriptional behaviour aligns with the observed efficiency of $FePO_4$ NPs as a P source for cucumber plants and the previously reported accumulation of excess Fe in the apoplast[29]. A role of $FePO_4$ NPs in metal homeostasis is further supported by the downregulation of two transcripts (*Cucsa.090660.1* and *Cucsa.092390.1*, Table 2) homologous to *Arabidopsis AtNAS4*. AtNAS4 is critical for the biosynthesis of the Fe chelator nicotianamine and known to be induced in roots under Fe deficiency[43,44].

Transcriptional differences between treated NP-treated and Fe-sufficient maize roots

Maize is a Strategy II species for Fe acquisition and has been shown to efficiently utilize Fe delivered by $FePO_4$ NPs[29]. Similar to our analysis on cucumber, we aimed to determine whether the role of $FePO_4$ NPs in maize was purely nutritional or if the supplied nanoparticles also impacted other processes. To validate such a role of $FePO_4$ NPs, we compared the root transcriptional profiles of plants 24 h after supplying FeNP as an Fe source with those grown in the presence of a soluble Fe form (FeEDTA) as a control (Additional file 2). No genes involved in Fe acquisition or cellular Fe homeostasis were differentially expressed between treated and non-treated plants, suggesting that NP-treated plants were adequately supplied with the micronutrient[29]. However, several DETs encode proteins involved in lateral root formation and stress response.

Plants supplied with $FePO_4$ NPs showed a reduction in the expression of two class III peroxidase transcripts (*GRMZM2G138450_T01* and *GRMZM2G015280_T01*, Table 2), possibly pointing to a less developed root apparatus. Class III peroxidases is known to play a role in lateral root emergence in *Arabidopsis*[45]. This assumption is further supported by the induction of a transcript that shows homology to the HOMEOBOX transcription factor AtHB22 of *Arabidopsis* in FeNP-treated roots (*GRMZM2G417229_T01*, Table 2). AtHB22 and its close homolog AtHB25 are involved in the feedback regulation of auxin-signalling-related SMALL AUXIN-UP RNA (SAUR) through deposition of the histone variant H2A.Z, thereby reducing IAA-induced lateral root formation[46]. The hypothesized repression of lateral root formation due to $FePO_4$ NPs supply is also supported by the upregulation of a DET (*GRMZM2G346095_T01*, Table 2*)* encoding a chalcone synthase homologous to AtTT4. AtTT4 negatively controls lateral root development by synthesizing flavonoids that scavenge superoxide ions, a driver of lateral root emergence[47]. Additionally, FeNP supply induced the expression of *GRMZM2G090381_T01* (Table 2), encoding a protein homologous to AtCNGC, which is involved in Ca^{2+} uptake and primary root growth[48]. Primary roots grow faster in the *Arabidopsis cngc1* mutant[48], suggesting that higher transcript levels of this gene in FeNP-treated plants exerts a negative control on root growth. Taken together, these results suggest that $FePO_4$ NPs predominantly repress molecular processes involved in lateral root development. Iron deficiency can enhance ectopic lateral root branching in maize[49], an observation that confirms that plants supplied with $FePO_4$ NPs were in a sufficient Fe nutritional status[29]. This assumption is further supported by the upregulation of an HIPP transcript (*AC217910.3_FGT001*, Table 2), potentially involved in metal homeostasis[42].

Regarding DETs related to stress responses, we observed increased abundance of a transcript involved in the detoxification of xenobiotics[50], the Tau GST *GRMZM2G480439_T01* (Table 2) in $FePO_4$ NP-treated roots relative to control plants (FeNP vs. C), suggesting that, similar to what has been observed in cucumber (see above), FeNP can activate biochemical pathways involved in xenobiotic detoxification in maize. We also observed an increase in the expression of a transcript corresponding to a protein homologous to SUGAR-DEPENDENT1 (SDP1) TAG lipase (AtSDP1) (*GRMZM2G004276_T01*, Table 2), which may be involved in lipid remodelling during the cold response[51]. Downregulation of two transcripts homologous to AtAOX1a (*GRMZM2G125669_T01* and *GRMZM2G074761_T01*, Table 2) in FeNP-treated roots compared to controls (FeNP vs. C) suggests that roots of plants supplied with $FePO_4$ NPs as a source of Fe did not experience oxidative stress. AtAOX1a is typically upregulated in response to various stress conditions[52].

Transcriptional differences between NP-treated and Fe-deficient maize roots

We also analysed the effects of $FePO_4$ NPs as a Fe source by comparing the root transcriptional profiles of FeNP-treated and Fe-deficient plants (FeNP vs. -Fe, Additional file 2). We observed significant changes in the abundance of transcripts potentially involved in root developmental processes and responses associated with Fe availability. Considering cell wall metabolism associated with root developmental processes, we recorded an induction of transcripts encoding a homolog of the P-type H^+-ATPase AtAHA8 (*GRMZM2G148374_T01*), a pectin lyase (*GRMZM2G177940_T01*), an extensin (*GRMZM5G888797_T01*), and two peroxidases (*GRMZM2G361475_T01* and *GRMZM2G061776_T01*, Table 2), indicative of active cell wall processes specific to root elongation and differentiation zones[36]. These changes in gene expression corroborate the observation that the root systems of FeNP-treated plants were better developed compared to those grown without Fe (Fig. 4).

NP-induced transcriptional changes that were independent of the nutritional status

Venn diagram analysis identified DETs that were commonly modulated in both cucumber comparisons (PNP vs. C and PNP vs. -P) and maize comparisons (FeNP vs. C and FeNP vs. -Fe). These DETs, totalling two in cucumber and 26 in maize (Fig. 5, Additional files 1 and 2), represent transcripts specifically affected by $FePO_4$ NPs and seem to be independent of the nutritional status of the plants. Notably, expression of these transcripts followed the same pattern when $FePO_4$ NP-treated roots were compared to both control and nutrient-deficient conditions (-P for cucumber and -Fe for maize). Thus, these transcriptome changes could be attributed to the inherent properties of the NPs. In cucumber, the two DETs were repressed by $FePO_4$ NPs and could play roles in root development. One of these DETs (*Cucsa.322910.1*, Table 3) encodes a protein homologous to AtDVL17, a member of a class of small polypeptides putatively involved in coordinating differentiation, development, and growth[53]. The other DET encodes a heavy chain-like protein (*Cucsa.048300.5*, Table 3).

Maize roots appear to be more responsive than cucumber to the $FePO_4$ NPs, a total of 26 DETs were common in the two comparisons. Within this group, we observed the upregulation of a transcript (*GRMZM2G086401_T01*) encoding a protein similar to AtSPK1. This protein is involved in the SPK1-ROP6-RIC1 pathway that is activated by auxin and inhibits the internalization of PIN2, an auxin efflux transporter crucial for the polar transport of the hormone[54]. This pathway controls the longitudinal spacing of lateral roots[55]. Notably, both the *spk1* null mutant and *pin2* knockout plants exhibited an increase in lateral root density[54]. Moreover, NP-inducted expression of a KINASE-ASSOCIATED PROTEIN PHOSPHATASE (KAPP, *GRMZM2G150608_T01*), encoding a protein with high similarity to AtRAG1, further indicates reduced lateral root development, as the *rag1-1* mutant of *Arabidopsis* exhibited enhanced lateral root formation under salt stress[56]. On the contrary, we recorded induction of a pectin lyase transcript (*GRMZM2G177940_T01*) with a putative role in cell wall metabolism in the zone of lateral root emergence[36,57]. All these observations are consistent with the phenotype shown by maize plants treated with $FePO_4$ NPs, which, after 10 d of treatment, exhibited a less developed root system compared to control plants (Fig. 4).

Other effects of the $FePO_4$ NP treatment are related to hormone signalling. Application of NPs induced a GUANINE NUCLEOTIDE EXCHANGE FACTOR (GEF) transcript (*GRMZM2G442148_T01*) with a putative role in signal transduction[58]. GEF proteins activate RHO OF PLANTS (ROPs), which in turn regulate cell growth and shape, subcellular localization of proteins, and responses to stresses[59]. ROPs are also involved in the regulation of ABA signalling as well as in the signalling and transport of auxin[59]. In relation to ABA, we also

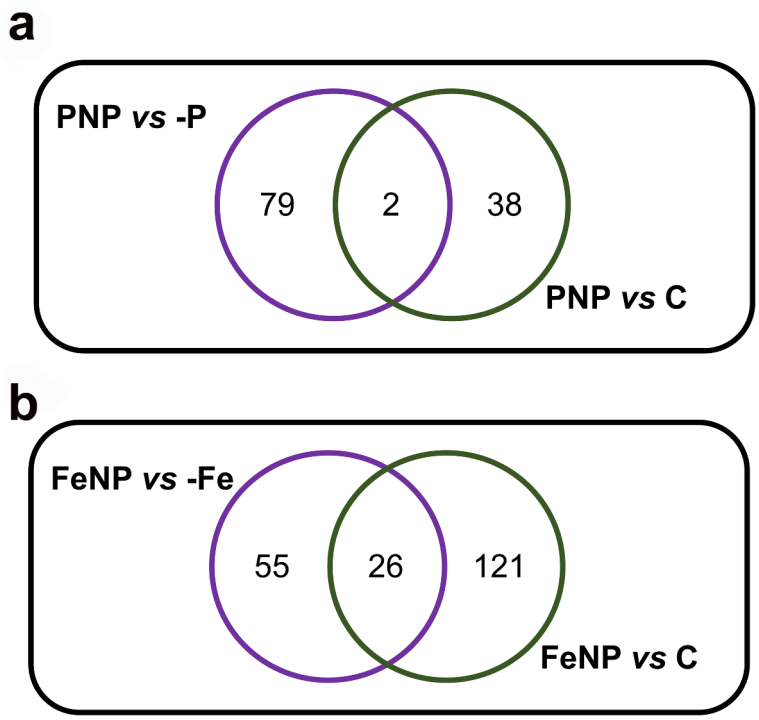

Fig. 5. Venn diagram of DETs inferred from the various comparisons. **a** Number of specific and common DETs of the transcriptional comparison PNP vs. C and PNP vs. -P in cucumber; **b** Number of specific and common DETs of the transcriptional comparison FeNP vs. C and FeNP vs. -Fe in maize.

DETs commonly modulated in PNP vs. C and PNP vs. *-P* in cucumber roots				
Transcript ID	**Description**	**TAIR10 ID**	**Fold-change PNP vs. C**	**Fold change PNP vs. *-P***
Cucsa.048300.5	Myosin heavy chain-related	AT2G34730.2	-2.279	-2.250
Cucsa.322910.1	ROTUNDIFOLIA like 6	AT4G35783.1	-2.496	-3.223

DETs commonly modulated in FeNP vs. C and FeNP vs. -Fe in maize roots				
Transcript ID	**Description**	**TAIR10 ID**	**Fold-change FeNP vs. C**	**Fold change FeNP vs. -Fe**
GRMZM2G000052_T01	Polyamine oxidase 2	AT2G43020.1	3.251	2.080
GRMZM2G086401_T01	Guanyl-nucleotide exchange factors	AT4G16340.1	8.345	4.103
GRMZM2G114503_T01	RAD-like 6	AT1G75250.1	2.805	2.638
GRMZM2G150608_T01	Kinase associated protein phosphatase	AT5G19280.1	3.171	3.880
GRMZM2G158117_T01	RAD-like 1	AT4G39250.1	3.842	5.505
GRMZM2G177940_T01	Pectin lyase-like superfamily protein	AT5G19730.1	2.449	2.582
GRMZM2G336766_T01	ABA-responsive element binding protein 3	AT3G56850.1	3.687	3.907
GRMZM2G341771_T01	Don-glucosyltransferase 1	AT2G36800.1	3.854	3.536
GRMZM2G442148_T01	RHO guanyl-nucleotide exchange factor 11	AT1G52240.1	3.500	3.933

Table 3. DETs commonly modulated in both comparisons of cucumber (PNP vs. C and PNP vs. -P) and maize roots (FeNP vs. C and FeNP -Fe) cited in the results and discussion section.

observed an upregulation of a transcript (*GRMZM2G336766_T01*) encoding a protein similar to the ABF/AREB transcription factor DPBF3, which is involved in the response to drought stress in *Arabidopsis* by remodelling the actin cytoskeleton and promoting stomatal closure[60]. In addition, $FePO_4$ NPs caused induction of two transcripts (*GRMZM2G158117_T01* and *GRMZM2G114503_T01*) encoding ABA-inducible MYB transcription factors homologous to *AtRMS2* and *AtRMS3*[61]. Also in line with the modulation of ABA responses caused by $FePO_4$ NPs is the upregulation of a transcript (*GRMZM2G000052_T01*) for a protein similar to the polyamine oxidase AtPAO2. In *Arabidopsis*, AtPAO2 appears to act antagonistically to ABA inhibition of primary and secondary root growth[62].

Brassinosteroids (BRs) are hormones involved in growth, development, and stress responses[63]. Induction of a UDP-glucosyltransferase (*GRMZM2G341771_T01*) with homology to AtUGT73C5 may hint to a role of $FePO_4$

NPs in governing BR activity. In *Arabidopsis*, AtUGT73C5 controls the level of active BRs through inactivation by conjugation[64]. Notably, it has been reported that BR and ABA pathways antagonize each another[63]. Additionally, BRs are known to promote the growth of lateral roots by enhancing the acropetal transport of auxin[65], and a reduction in active BR levels could be associated with decreased lateral root development. In summary, $FePO_4$ NPs could affect hormone signalling, regulate root development, and promote plant tolerance to abiotic stresses. This is consistent with the effects of $FePO_4$ NPs treatments of various materials applied to different plant species, including maize[66].

Conclusion

In conclusion, the data presented here confirm that Fe and P-containing $FePO_4$ NPs are good nutritional sources for cucumber and maize plants. Transcriptional profiling of NP-treated plants did not reveal indications for nutrient deficiencies, corroborating the ability of $FePO_4$-NPs to provide Fe and P that can be readily absorbed by the roots. As depicted in Fig. 6, alongside their nutritional function, these nanomaterials exert species-specific molecular actions on the root systems already 24 h post-treatment. It is notable that some of these actions are entirely independent of nutritional aspects and appear to be related to the nanoparticulate nature of the material used for the treatment. Our findings further confirm that the impact of nanomaterials on plants depends not only on the characteristics of the nanoparticles but also on the physiological and metabolic specificity of the plant species under investigation, making it challenging to derive generalizable plant responses.

Materials and methods

$FePO_4$ NPs synthesis

The $FePO_4$ nanoparticles were citrate-capped and synthesized using a batch method as described by Sega et al.[28] where extensive characterization, including TEM and FT-IR analyses, was performed. The $FePO_4$ nanoparticles used in this study originated from the same batch characterized in a previous study[7]. These NPs were spheroidal and tended to aggregate, with a size peak at 91 nm[7]. The Fe and P concentrations were 0.15 M and 0.12 M, respectively, with a Fe/P ratio of 1.25[7].

Plant growth and physiological analyses

Purchased seeds of *Cucumis sativus* Viridis F1 hybrid (Franchi Sementi S.p.A.) and of *Zea mays* L. inbred line PR33T56 (Pioneer Hybrid Italia S.p.A.) were germinated on paper soaked with 1 mM $CaSO_4$ in dark at 25 °C. Six seedlings were transferred in one 2-L pot filled with 1.8 L of continuously aerated nutrient solution. One pot was set up for each treatment. The control nutrient solution had the following composition: 0.7 mM K_2SO_4, 2 mM $Ca(NO_3)_2$, 0.5 mM $MgSO_4$, 0.1 mM KH_2PO_4, 0.1 mM KCl, 100 µM FeNaEDTA, 10 µM H_3BO_3, 0.5 µM MnSO4, 0.5 µM $ZnSO_4$, 0.2 µM $CuSO_4$, and 0.01 µM $(NH_4)_6Mo_7O_{24}$. In addition to the control, conditions were set up as follows. For cucumber, plants were grown in NS without KH_2PO_4 (-P) and in NS without KH_2PO_4 and supplied with $FePO_4$ NPs as P source (PNP). In the case of maize, plants were grown in NS without FeNaEDTA (-Fe) and in NS without FeNaEDTA and supplied with $FePO_4$ NPs as Fe source (FeNP). $FePO_4$ NPs were used in a concentration of P and Fe equivalent to 100 µM. The nutrient solution was changed twice per week. Plants were grown in a 16/8 day/light photoperiod at 25 °C and 200 µmol m^{-2} sec^{-1} PPFD (Photosynthetic Photon Flux Density). Roots of three out of six seedlings per pot (treatment) were sampled after 24 h and pooled to obtain samples for RNA extraction and further transcriptomic analyses. The remaining three cucumber and maize seedlings were grown for 13 and 10 days, respectively, in accordance with the occurrence of nutrient deficiency symptoms. For these seedlings, the SPAD index was calculated as average of all leaves of each plant. For each leaf, the average value of five measurements performed using a SPAD-502 Plus Chlorophyll meter® (Konica Minolta) was calculated. In addition, root parameters were determined from images of the roots of each seedling, acquired with an EpsonV700 perfection scanner. Images were then analysed with the WinRHIZO™ software 2015a Pro version (Regent Instruments Inc.) using the "root morphology" mode to determine the volume, length, and surface area of the roots. Three independent experiments (biological replicates) were performed.

RNA extraction, cDNA synthesis, and microarray expression analysis

Total RNA was extracted from three biological replicates (e.g. three independent growth), each consisting of pooled roots from three seedlings collected 24 h after treatment, for both cucumber and maize. Total RNA extractions were performed using 80 mg of maize and cucumber root tissue homogenized in liquid nitrogen and the Spectrum™ Plant Total RNA kit (Sigma-Aldrich). Total RNA quantity was determined using a NanoDropTM 1000 (Thermo Scientific) spectrophotometer, RNA quality was analysed with Bioanalyzer 2100 using a Bioanalyzer Chip RNA 6000 Nano kit (Agilent). The cRNA was synthesized and labelled using 200 ng of total RNA from each sample, the Low Input Quick Amp Labeling Kit, and Cyanine 3 (Cy3)-CTP fluorescent dye following the instructions of the Agilent technical manual (http://www.agilent.com). For each sample, 1.65 µg of Cy3-labeled cRNA was used for the hybridization reactions. The Cy3-labeled cRNA samples of maize and cumber were hybridized on two different 4×44k Agilent arrays according to the manufacturer's manual for 17 h at 65° C and scanned on an Agilent G2565CA Microarray Scanner System (Agilent). For cucumber, microarray analyses were carried out using a chip that allows to analyse the expression of 30,364 transcripts predicted by the cucumber (Gy14) v1 genome annotation (http://cucurbit-genomics.org/organism/7). Probe design was performed using Agilent eArray (http://www.genomics.agilent.com). A complete description of the chip is available at the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo) under the series entry (GPL34058). For maize, we used the chip described by Santi et al.[67], which allowed for analysing the expression of 39,372 maize transcripts predicted from the B73 reference genome whose description is available at the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo; GPL22578). Feature intensities were extracted

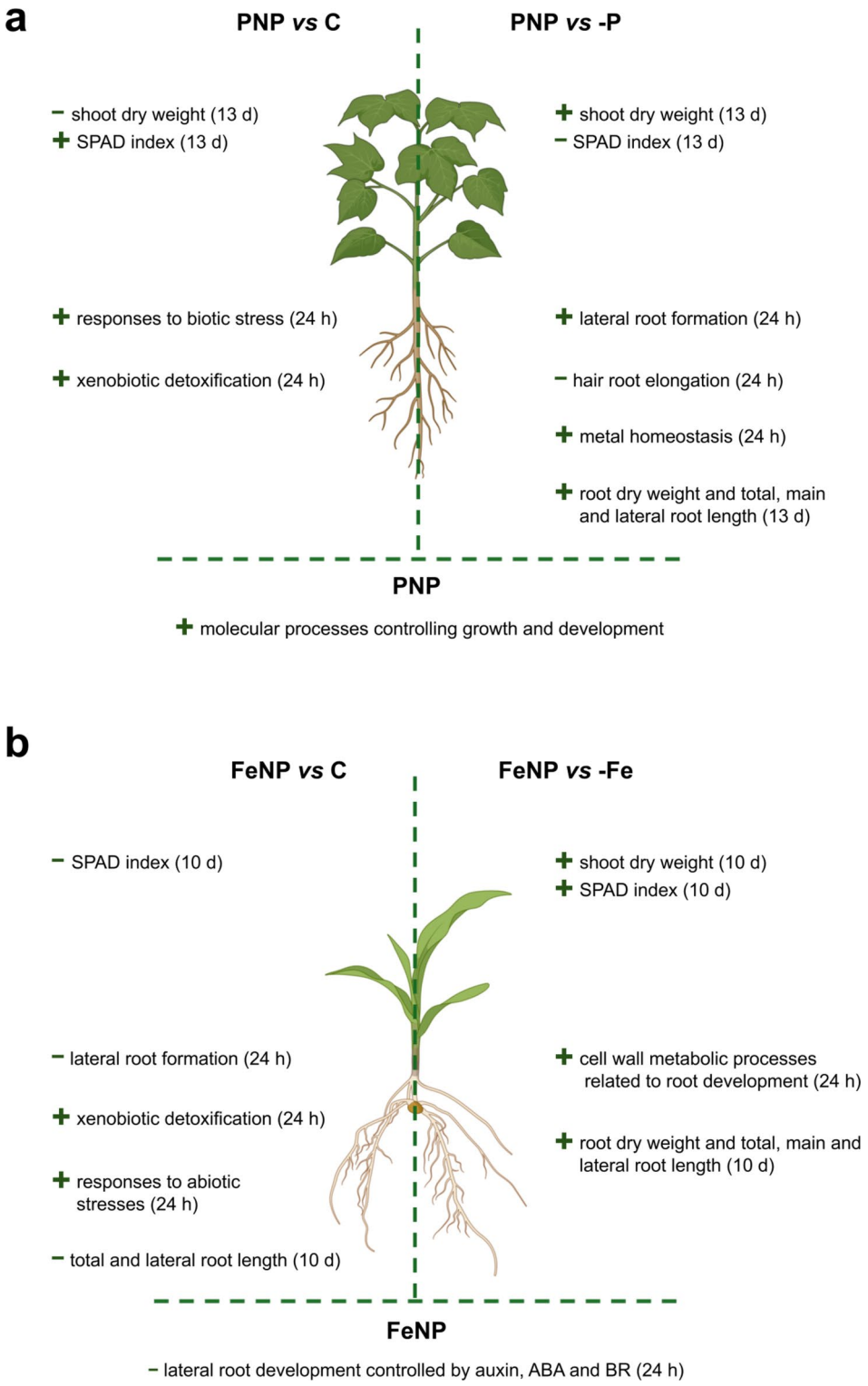

Fig. 6. Schematic diagram depicting the effects of NPs. **a** Effects of NPs as P- source for cucumber **b** Effects of NPs as Fe sources for maize. The root transcriptional changes (24 h) and shoot and root parameters (13 d for cucumber and 10 d for maize) are reported; the symbols + and – indicate processes that were enhanced or repressed by NPs, respectively.

using Agilent's Feature Extraction Software 12.0 (www.agilent.com). Hybridization data of all samples were normalized using the value of the 75th percentile and Log_2 transformed. Hierarchical clustering analyses were carried out with MeV software. After, differentially expressed transcripts (DETs) were identified through t-tests carried out using the MeV software (http://mev.tm4.org/#/welcome) for the comparisons PNP vs. -P and PNP

vs. C for cucumber, and for the comparisons FeNP vs. -Fe and FeNP vs. C for maize. T-tests were performed, setting a p-value based on permutation with a critical value of 0.05. DETs were then filtered based on fold change values (|FC|≥2). Data are available at the GEO data repository (http://www.ncbi.nlm.nih.gov/geo) with the series entries GSE252553 and GSE252443. The complete lists of inferentially expressed transcripts identified in the different transcriptional comparisons are reported in Additional file 1 for cucumber and Additional file 2 for maize, respectively. For each maize and cumber transcript, the annotation of Phytozome for *Cucumis sativus* v1.0 (Csativus_122_annotation_info.txt) and the B73 maize reference genome (Zmays_284_5b+.annotation_info.txt) was used. GO enrichment analysis of the differentially expressed transcripts was carried out on the platform PlantRegMap[68], the results were visualized with tools of the SRplot platform[69].

Quantitative reverse transcription PCR (RT-qPCR) analysis

DNase I treatment was carried out for each sample using 1000 ng of total RNA and the RQ1 RNase-Free DNase (Promega, Madison, WI, USA) according to the manufacturer's procedure. These treated samples were used to produce cDNA using the ImProm-II Reverse Transcription System (Promega). RT-qPCR analyses were performed with a final volume of 10 µL, a primer concentration of 350 nM, and 1 µL of an 1:3 solution of cDNA sample using the FastSYBR® Green Master Mix (ThermoFisher Scientific). The reaction was carried out with StepOnePlus™ (ThermoFisher Scientific) using the following reaction conditions: 20 s at 95 °C for initial denaturation, then 3 s at 95 °C, and 30 s at 60 °C for 40 cycles. The sequences of primers are reported in Table S2 and Table S3. Mean Normalized Expression (MNE)[70] was calculated for each sample. Two housekeeping transcripts were used both for cucumber (*Cucsa.313280.1* and *Cucsa.238470.1*) and maize (*GRMZM2G047204_T01* and *GRMZM2G149286_T01*) analysis. The PCR efficiency of each primer couple was calculated using the LinRegPCR software[71] based on fluorescence raw data. Mean normalized expression (MNE) was determined for each transcript and each sample using the two housekeeping transcripts[70]. Then, the geometric mean of the two MNE values obtained for each transcript and each sample was calculated[72]. Fold change (FC) values were then calculated in the MNE of samples in each considered comparison.

Data availability

Microarray data are available at the GEO (http://www.ncbi.nlm.nih.gov/geo) with the series entries GSE252553 and GSE252443. The data of the current study are available from the corresponding author on reasonable request.

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Author contributions

A. Z. and Z.V.: conceived and designed the experiments. A.C.: acquired the data. A.C. and A.Z.: analysed the data. All the authors interpreted the data. A.C. and A.Z.: wrote the original draft. A. Z.: revised the draft. Z.V.: supervised the work and provided funding.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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