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Strigolactones: New players in the nitrogen–phosphorus signalling interplay

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Abstract

Nitrogen (N) and phosphorus (P) are among the most important macronutrients for plant growth and development, and the most widely used as fertilizers. Understanding how plants sense and respond to N and P deficiency is essential to optimize and reduce the use of chemical fertilizers. Strigolactones (SLs) are phytohormones acting as modulators and sensors of plant responses to P deficiency. In the present work, we assess the potential role of SLs in N starvation and in the N–P signalling interplay. Physiological, transcriptional and metabolic responses were analysed in wild-type and SL-deficient tomato plants grown under different P and N regimes, and in plants treated with a short-term pulse of the synthetic SL analogue 2′-epi-GR24. The results evidence that plants prioritize N over P status by affecting SL biosynthesis. We also show that SLs modulate the expression of key regulatory genes of phosphate and nitrate signalling pathways, including the N–P integrators PHO2 and NIGT1/HHO. The results support a key role for SLs as sensors during early plant responses to both N and phosphate starvation and mediating the N–P signalling interplay, indicating that SLs are involved in more physiological processes than so far proposed.

KEYWORDS
nitrate starvation, nutrient deficiency, phosphate starvation, tomato

1 | INTRODUCTION

In a world with an increasing global population, one of the main challenges for modern agriculture is to enhance food production, while protecting the environment (Crist, Mora, & Engelman, 2017). Crops are constantly exposed to biotic and abiotic stresses which greatly impact their productivity, with nutrient deficiency being one of the most important limiting factors (Nair, 2019). Therefore, in order to face such drawback, intensive agriculture relies on a massive use of chemical fertilizers and pesticides to maintain high yield crop production

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(Majeed, 2018; Savci, 2012). However, the abuse of such agrochemicals contaminates soils and groundwater, negatively impacting the environment and human health (Elahi, Weijun, Zhang, & Nazeer, 2019; Mahmood, Imadi, Shazadi, Gul, & Hakeem, 2016). Thus, there is a need to find more eco-friendly strategies to reduce agrochemicals input without compromising yield and food quality. In this sense, breeding of plants that are more efficient in the use of natural resources and able to perform better when grown under poor nutrient environments is a promising alternative (Qaim, 2020). Phosphorus (P), in the form of inorganic phosphate (Pi), and nitrogen (N), as nitrate and ammonium, are among the most important macronutrients for plant development, and their coordinated use is essential for optimal plant growth and yield (Hu & Chu, 2020; Oldroyd & Leyser, 2020). Nitrate, the preferred N source, tends to leach from the soil and Pi is relatively immobile; therefore plants can only use 30–40% of the N and less than 30% of the Pi sources applied as fertilizers, which results in both Pi and N deficiency in agricultural soils (Nasr et al., 2021; Oldroyd & Leyser, 2020). Nowadays, the massive use of fertilizers is costly and it is leading to an increased N and P leaching into the biosphere, with the consequent negative impact on the environment. Therefore, understanding how plants sense, signal and respond to Pi and N shortage is essential to optimize and reduce the use of chemical fertilizers, alleviating agricultural costs and the excessive consumption of these non-renewable resources.

Nitrogen and Pi are also signalling molecules triggering downstream N and Pi responses, which are critical for plant adaptation to environments with variable nutrient availability (Raghothama, 2000). Therefore, sensing nutrient availability and signalling to coordinate appropriated responses is crucial for plant performance. To cope with P and N deficiency, plants have developed an array of adaptations that affects their growth and development, collectively known as Pi and N starvation responses (PSR and NSR, respectively). In Pi- and N-deficient environments, overall plant growth is reduced, but the root system is generally increased to favour nutrient foraging, thus increasing the root-to-shoot ratio (Hu & Chu, 2020; Oldroyd & Leyser, 2020). PSR requires a fine-tuned coordination of plant responses in which a number of genes and signalling molecules are involved (Figure 1a). Here, the transcriptional activator PHOSPHATE STARVATION RESPONSE 1 (PHR1) plays a key role in the expression of most Pi starvation-induced genes (Bustos et al., 2010; Ham, Chen, Yan, & Lucas, 2018; Puga et al., 2017). Although PHR1 expression is not transcriptionally regulated, its activity is modulated by the plant Pi status, being negatively regulated by the SYG1/Pho81/XPR1 (SPX)-domain proteins. Under Pi limitation, the complex SPX-PHR1 becomes weak releasing PHR1, inducing the expression of high-affinity Pi transporters (PHTs) and facilitating Pi-acquisition (Figure 1a). PHR1 also promotes the expression of the microRNA miR399 (Pant, Buhtz, Kehr, & Scheible, 2008), which reduces the number of PHO2 transcripts, encoding an ubiquitin-conjugating E2 enzyme involved in protein degradation (Lin et al., 2008). Subsequently, down-regulation of PHO2 prevents the degradation of PHO1, a Pi transporter involved in Pi transport into the aerial tissues (Liu et al., 2012). Therefore, miR399/PHO2 is an important component of the Pi signalling network operating downstream of PHR1 (Bari, Pant, Stitt, & Scheible, 2006). PHR1 also promotes miR399 levels, it also induces the expression of IPS1, a non-protein coding gene involved in miR399 sequestration (Franco-Zorrilla et al., 2007) (Figure 1a). Therefore, Pi acquisition and homeostasis is regulated by PHR1 and the triad ISP1-miR399-PHO2 (Franco-Zorrilla et al., 2007; Puga et al., 2017).

As for Pi, N signalling is also precisely fine-tuned, but this occurs through several interconnected signalling pathways (O'Brien et al., 2016). The primary nitrate response (PNR) corresponds to a rapid and nitrate-specific activation of sentinel genes, including the sensor NRT1.1 (Figure 1b) (Maghiaoui, Gojon, & Bach, 2021; Wang et al., 2018). In Arabidopsis, this gene encodes a nitrate transporter with dual affinity (Ho, Lin, Hu, & Tsay, 2009; Wang et al., 2018). NRT1.1 has the capacity to switch between low- and high-affinity in response to external nitrate. At

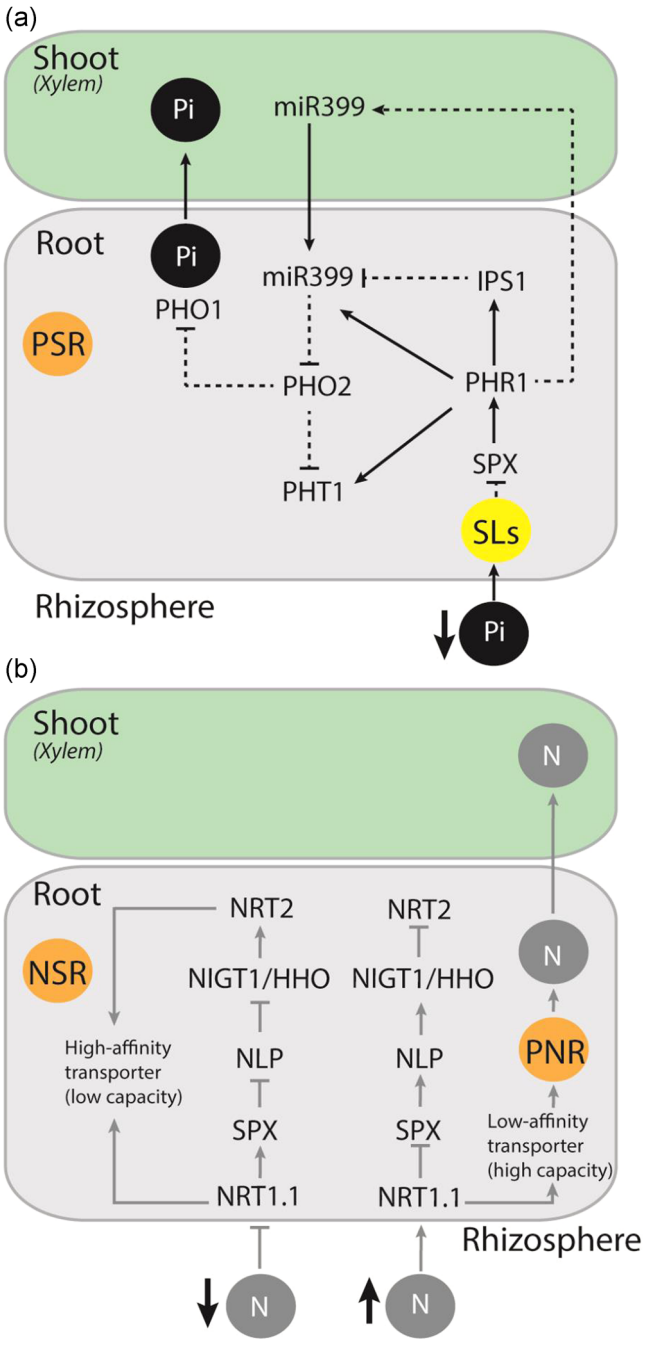

FIGURE 1 (See caption on next page)

high concentrations, it works as a low-affinity (high capacity) transporter, triggering high-level nitrate responses and facilitating N transport to the aerial tissues. Under these conditions, the family of transcription factors NINE-LIKE PROTEIN (NLP) is also activated. NLPs are master regulators of nitrate responses initiating transcriptional cascades as the induction of the NIGT1/HHO family, a group of G2-like GARP-type transcription factors. Then, NIGT1/HHOs repress the expression of the transporters of the family NRT2 (Hu & Chu, 2020). When environmental nitrate is limited, NRT1.1 is phosphorylated acting as a high-affinity (low capacity) transporter and triggering nitrate starvation responses (NSR; Figure 1b). Here, NLPs become inactive, negatively affecting the expression of NIGT1/HHO repressors, which facilitates a slow activation of high-affinity transporters NRT2, probably to increase nitrate uptake. In Arabidopsis, it has been shown that the expression of NTR2 transporters, especially NRT2.1 and NRT2.4, is regulated by the NIGT1/HHO repressors in a NRT1.1-dependent manner (Figure 1b) (Kiba et al., 2018; Maeda et al., 2018; Medici et al., 2015). Some studies have suggested that NRT2.1 may also act as a sensor for root development, although its exact role in nitrate signalling is not yet clarified (Wang et al., 2018). Finally, the third pathway includes long-distance signals where cytokinin biosynthesis, C-terminally encoded peptides and glutaredoxins are involved (Ohkubo, Tanaka, Tabata, Ogawa-Ohnishi, & Matsubayashi, 2017; Tabata et al., 2014).

Recent evidence shows that there is interplay between Pi and nitrate starvation signalling pathways, which is regulated at different levels (Hu & Chu, 2020; Medici et al., 2019; Ueda & Yanagisawa, 2019). It has been shown that NRT1.1 regulates the nitrate-activated PSR in a PHR1-dependent manner, and that PHO2 levels are reduced under Pi starvation in the presence of nitrate (Medici et al., 2019). Here, SPX proteins play a critical role as NRT1.1 can interact with specific SPX proteins promoting its degradation and allowing PHR1 activation. SPX proteins can also interact with NLPs. Therefore, the formation of the NRT1.1-SPX module allows NLP activation at high nitrate conditions (Hu & Chu, 2020). On the other hand, it was shown that nitrate uptake is reduced by Pi starvation via PHR1 (Maeda et al., 2018). An important role for AtNIGT1/HHOs in the integration of N-P plant responses has been also shown in Arabidopsis. The expression levels of *AtNIGT1/HHOs* are promoted by nitrate and by Pi starvation, but here only under high nitrate conditions, which is under control of both AtNLP7 and AtPHR1 (reviewed in Ueda & Yanagisawa, 2019; Hu & Chu, 2020). Despite these recent findings, the regulatory mechanisms and compounds involved in the N–P signalling interplay are still poorly characterized.

Plant adaptation to nitrogen and Pi availability is also regulated by phytohormones. It is widely accepted that strigolactones (SLs) are an ancient and major class of endogenous plant growth regulators. They modulate, in coordination with other phytohormones, shoot branching, internode elongation, root architecture, secondary growth, leaf senescence and reproductive development (Kohlen et al., 2012; Waters, Gutjahr, Bennett, & Nelson, 2017). Accordingly to their role as growth regulators, SL production is promoted by plants in response to Pi and N deficiency as adaptation to such stress conditions (López-Ráez et al., 2008; Yoneyama et al., 2012). In addition to act as phytohormones, SLs have a key role as chemical signals in the rhizosphere favouring plant association with beneficial microorganisms as arbuscular mycorrhizal fungi and rhizobia (Al-Babili & Bouwmeester, 2015; López-Ráez, Shirasu, & Foo, 2017).

We have recently shown that SLs are early modulators of plant responses during Pi limitation, promoting the expression of key regulatory genes in the PSR and regulating metabolic changes to cope with Pi deficiency (Gamir et al., 2020). So far, the role of SLs in N starvation has not been investigated. In the present work, using tomato (*Solanum lycopersicum*) as a model, we assess the potential role of SLs as regulators of N starvation signalling. Moreover, we test whether they are also involved in the interplay between PSR and NSR. For that, we analyse the transcriptional and metabolic responses in wild-type and in the SL-deficient *SlCCD8*-RNAi L09 tomato plants grown under different Pi and nitrate regimes, and in plants treated with a short-term pulse of the synthetic SL analogue 2′-*epi*-GR24. We show that PSR is controlled by N status in tomato, and that SLs play a role in the regulation of the N-P interplay.

FIGURE 1 Schematic models of the core elements involved in the regulation of Pi and nitrate signalling pathways in plants. (a) Under Pi deficiency, SLs biosynthesis is promoted. Through as yet unknown mechanism, SLs affect the complex SPX-PHR1, which becomes unstable releasing the master regulator PHR1. Then, PHR1 promotes the expression of Pi transporters from the PHT1 family in the roots, thus increasing Pi uptake. PHR1 also induces the expression of the microRNA miR399, which negatively regulates the repressor PHO2, and that of the non-protein coding gene IPS1. PHO2 down-regulation prevents degradation of the Pi exporter PHO1, allowing Pi xylem loading and subsequent transport into the shoots. On the other hand, IPS1 can interact and block miR399 transcripts, preventing miR399-PHO2 binding and degradation of PHO2. Adapted from Puga et al. (2017). (b) N signalling pathway is mainly regulated by the transceptor NRT1.1. This sensor has the capacity to switch between low- and high-affinity depending on the external nitrate provision. Under optimal N conditions (N), NRT1.1 expression is induced activating the PNR facilitating N transport to the shoots via xylem. Under these conditions, NRT1.1 interacts with specific SPX proteins, promoting its degradation and allowing the activation of the family of transcription factors NIN-LIKE PROTEIN (NLP), which are master regulators of nitrate responses. Subsequently, NLPs initiate transcriptional cascades by activating the expression of the GARP-type transcription factors NIGT1/HHO. NIGT1/HHOs act mainly as repressors, reducing the expression of the high-affinity transporters NRT2. Under nitrate deficiency, NRT1.1 is phosphorylated acting as a high-affinity (low capacity) transporter and triggering nitrate starvation responses (NSR). Under these conditions, NLPs become inactive, negatively affecting the expression of NIGT1/HHO repressors, which facilitates a slow activation of NRT2 transporters

2 | MATERIALS AND METHODS

2.1 | Plant growth, conditions and treatments

Two independent experiments were performed in pot experiments. In Experiment 1, tomato (*S. lycopersicum* L) cv. MoneyMaker plants were

used. In Experiment 3, tomato cv. Craigella (LA3247) and the SL-deficient line *SlCCD8*-RNAi L09 (Kohlen et al., 2012) were used. Seeds were surfaced-sterilized in 4% sodium hypochlorite for 10 min, washed with sterile demiwater and sown in trays containing sterile zeolite: sand (1:1) for germination at 25°C in darkness. Seedlings with two true leaves were transplanted individually into plastic pots (0.5 L) with a mixture of sterile zeolite and sand (1:1). The experimental design included two factors: P (2 levels: high [HP], 1.3 mM and low [LP], 0.3 mM) and N (2 levels: [HN], 20 mM and [LN], 5 mM). Ten plants per treatment were grown. Plants were watered twice a week with 50 ml of the corresponding Hewitt nutrient solution (Hewitt, 1966), modified depending on the treatments as detailed in Table S1. Plants were grown for six weeks under greenhouse conditions at 25/19°C with 16/8 hr photoperiod and a relative humidity of 50–60%. Before harvest, root exudates from each plant were collected individually as described below. At harvest, shoots and roots were collected, weighed, snap-frozen in liquid nitrogen and kept at −80°C until analysis.

For the experiment in hydroponics (Experiment 2), tomato (cv. MoneyMaker) seeds were surface-sterilized in 4% sodium hypochlorite for 10 min, washed with sterile demiwater for 10 min, and germinated in a plate on moistened filter paper at 25°C in darkness. After 2 days, seeds were sown in 1.5 ml Eppendorf tubes filled with 0.5% phytoagar and grown hydroponically in 3 L plastic containers with Hewitt nutrient solution (Hewitt, 1966) with 0.8 mM of Pi and constant aeration for 4 weeks. Growth conditions were 25/19°C with 16/8 hr photoperiod and a relative humidity of 50–60%. Nutrient solution was replaced once a week. In this case, the experimental design included two factors: Pi (2 levels: with [+P] and without [−P, 0%]) and 2′-*epi*-GR24 (2 levels: with [GR24] and without [C]). After 4 weeks, half of the plants were transferred to nutrient solution without Pi (−P) and grown for an additional week. The other half was kept under the same Pi conditions as during the pre-cultivation (+P). Then, 10 nM of the active diasteroisomer 2′-*epi*-GR24 (a synthetic analogue of SLs) was applied to half of the plants of each treatment (GR24) in the nutrient solutions (+ and −Pi) for 1 hr. After the treatment, plants were kept for 24 hr with the corresponding nutrient solution (+ or −Pi) without 2′-*epi*-GR24 to allow them to respond to the treatment. Each of the four treatments comprised six replicates. Roots were collected, weighed, snap-frozen in liquid nitrogen and kept at −80°C until use.

2.2 | Determination of mineral nutrients in leaves

Phosphorus and other element concentrations were analysed by inductively coupled plasma optical emission spectrometry (ICP-OES; Varian ICP 720-ES) after acid digestion of the samples. Total C and N content were determined using an Elemental Analyser (Leco Truspec CN), according to standard procedures. For the measurements, frozen shoots were ground into a fine powder and lyophilized. A 200 mg aliquot of dry tissue was used per sample. Six biological replicates per treatment were analysed.

2.3 | Searching for tomato SlNIGT1/HHO genes

The family of the GARP-type transcription factors NIGT1/HHO has not been characterized in tomato. We searched the putative orthologue of the Arabidopsis AtNIGT1.4/HHO1 (also known as HRS1) gene (At1g13300) in the tomato genome using BLAST on the platform Sol Genomics Network. A sequence with an open reading frame (ORF) of 1,303 bp (Solyc05g009720), encoding a predicted 400 amino acids protein was found. The sequence showed a 74% identity with a 25% of query cover to AtNIGT1.2/HHO2 at nucleotide level and a 46% identity with an 84% of query cover at amino acid level. Specific primers for real-time quantitative PCR (qPCR) analysis of this gene were designed (Table S2).

2.4 | RNA isolation and gene expression analysis by qPCR

RNA extraction and purification, synthesis of the corresponding cDNA and qPCR was performed as described in Gamir et al. (2020). Specific primers for genes involved in SL biosynthesis, and Pi and nitrate signalling pathways were used (Table S2). Six independent biological replicates were analysed per treatment. Relative quantification of specific mRNA levels was performed using the comparative $2^{-\Delta(\Delta Ct)}$ method (Livak & Schmittgen, 2001). Expression values were normalized using the housekeeping genes *SlEF*, encoding for the tomato elongation factor 1a, or *SlActin*, encoding for the tomato actin (Table S2).

2.5 | Root exudate collection and purification of strigolactones

Root exudates were collected from each pot individually and used for further analysis by liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS). For exudate collection, the substrate was rinsed with 1 L of tap water to remove the compounds accumulated during the plant growth. Subsequently, 50 ml of the corresponding Hewitt nutrient solution (Table S1) was added to each pot to reconstitute the treatments. Plants were kept for 48 hr in the greenhouse and the 'fresh' exudates were collected individually by applying 1 L of tap water to each pot. The crude exudates were filtered through glass fibre filters by vacuum, and concentrated and purified by solid phase extraction through Telos C18 (EC) SPE columns (Octadecyl 500 mg/3 ml) (Kinesis) using a SPE vacuum manifold (Supelco). For that, SPE columns were first pre-equilibrated with 5 ml of 100% acetone. Then, 1 L of each exudate solution was loaded onto the pre-equilibrated columns. Each column was washed with 5 ml of sterile demiwater, and the exudates were eluted with 5 ml of 100% acetone and collected in 10 ml amber tubes. Purified root exudates were stored at −80°C until use. Before LC–MS/MS analysis, the exudates were normalized to the same ratio of millilitre of exudate per gram of root fresh weight.

2.6 | Strigolactone analysis by LC–MS/MS

SL quantification was performed by LC–MS/MS as described by Rial et al. (2019). Samples were collected and purified as described above. Of note, 5 µl of each sample were directly injected into the equipment. The samples were analysed on a Bruker EVOQ Triple Quadrupole Mass Spectrometer (Bruker), using an electrospray (ESI+) as ionization source.

2.7 | Statistical analyses

All variables were subjected to analysis of variance (ANOVA) with Pi and N (Experiments 1 and 3) or Pi and 2′-*epi*-GR24 (Experiment 2) as the main factors including the interaction term. Data were checked for normality and homogeneity of variance before statistical analyses. Data from root fresh weight and Pi content were transformed using logarithms to remove the normality error. The statistical analysis was performed using the software Infostat (Di Rienzo et al., 2013) and its interface with the software R. The Tukey test ($p \leq .05$) was carried out when suited to compare means a posteriori.

3 | RESULTS

3.1 | P and N deficiency affect plant growth and development differentially

In order to determine the influence of P and N availability on plant growth, and how their individual deficiency affects the perception of the other, tomato plants were grown with different P-N combinations (Experiment 1). As expected, plant growth was significantly reduced in plants grown under P and/or N deficiency as compared to those grown under 'optimal' control conditions (HPHN) (Figure 2a). Shoot fresh weight was reduced by 62 and 73% under Pi (LPHN) or nitrate (HPLN) limitation, respectively. When both nutrients were deficient (LPLN), a reduction of 77% was detected (Figure 2b). A similar pattern was observed in the roots. Hereto, a reduction of 36 and 46% was detected under Pi and nitrate deficiency, respectively, and 49% when both nutrients were limited as compared to 'optimal' conditions (Figure 2b). Plants always performed better under high N conditions, independently of the Pi application, with both shoot and root weight being improved under these conditions (Figure 2a,b). However, root-to-shoot ratio was higher under N deficiency (Figure S1a). Root length also increased upon N deficiency, but not by Pi starvation (Figure S1b). Leaves from plants grown under low Pi (LPHN) showed the characteristic dark green colour in the upper surface (adaxial) and a purple tone on the lower surface (abaxial), as a consequence of the increase in anthocyanins; a phenotype almost absents when both nutrients were low (LPLN) (Figures 2a and S2).

As expected, P and N concentrations were reduced by 30 and 45% in leaves from plants subjected to low P and N, respectively (Figure 2c,d). Conversely, the concentration of the two nutrients increased when the other nutrient was deficient. That is, P levels increased by 93% when grown under N deficiency and N levels increased by 50% under Pi limitation over the control growing conditions (HPHN; Figure 2c,d). The observed higher P and N concentration under low nitrate (HPLN) and Pi (LPHN) conditions, respectively, is likely related to the limitation of plant growth by deficiency of the other nutrient. This may lead to an increase of the concentration of a non-limiting nutrient in plant tissues, as has been reported in Medicago and pea (Bonneau, Huguet, Wipf, Pauly, & Truong, 2013; Nasr et al., 2021). Indeed, there was a significant interaction of the two factors (P and N availability) on shoot and root biomass, as well as P and N content (Table S3). Carbon (C) is another primary element involved in plant growth and development, and it has been shown that the C:N ratio is a good indicator of N use efficiency (Zhang et al., 2020). Therefore, we also measured C levels in tomato leaves and calculated the corresponding C:N ratios for the different treatments (Table 1). Carbon content was homogeneous among the treatments except for LPHN, where a slight reduction was observed. The C:N ratio significantly increased in plants under low N, independently of the P status (HPLN and LPLN). Plants with a higher C:N ratio improved N use efficiency under N deficiency to ensure survival instead of growth (Zhang et al., 2020). In addition, other macro- and micro-nutrients were analysed by ICP-OES. In general, N deficiency reduced important macro and micronutrients as Fe, Cu and Na, while they slightly increased under Pi limitation (Table S4), supporting the idea that N limitation has a higher impact in plant growth than P.

3.2 | Promotion of SL biosynthesis by Pi deficiency depends on N provision

SL biosynthesis is promoted by P and N deficiency (López-Ráez et al., 2008; Yoneyama et al., 2012). SLs are derived from carotenoids synthesized by the sequential action of several enzymes, such as the β-carotene isomerase (D27) and two carotenoid cleavage dioxygenases (CCD7 and CCD8; Waters et al., 2017; Yoneyama et al., 2018). Here, we assess how different combinations of P and N levels affect SL biosynthesis in tomato. The expression of the biosynthetic genes—*SlD27*, *SlCCD7* and *SlCCD8*—were analysed by qPCR. Nitrate deficiency under optimal P provision (HPLN) significantly increased the expression of the three biosynthetic genes as compared to control conditions (HPHN). However, the highest induction for all genes was observed by P limitation under optimal N provision (LPHN) (Figure 3a–c). Interestingly, this higher induction by P limitation was not observed under N deficiency (LPLN), suggesting that their expression seems to be ruled by the N status.

A similar pattern was observed by the analytical quantification of the characterized tomato SLs solanacol and orobanchol (López-Ráez et al., 2008). None of the two SLs were detected in the root exudates under optimal nutrient conditions. Remarkably, they were also not detected under nitrate deficiency. The higher promotion of solanacol and orobanchol levels was detected by Pi deficiency under normal N conditions (LPHN) with solanacol levels being 35 times higher than

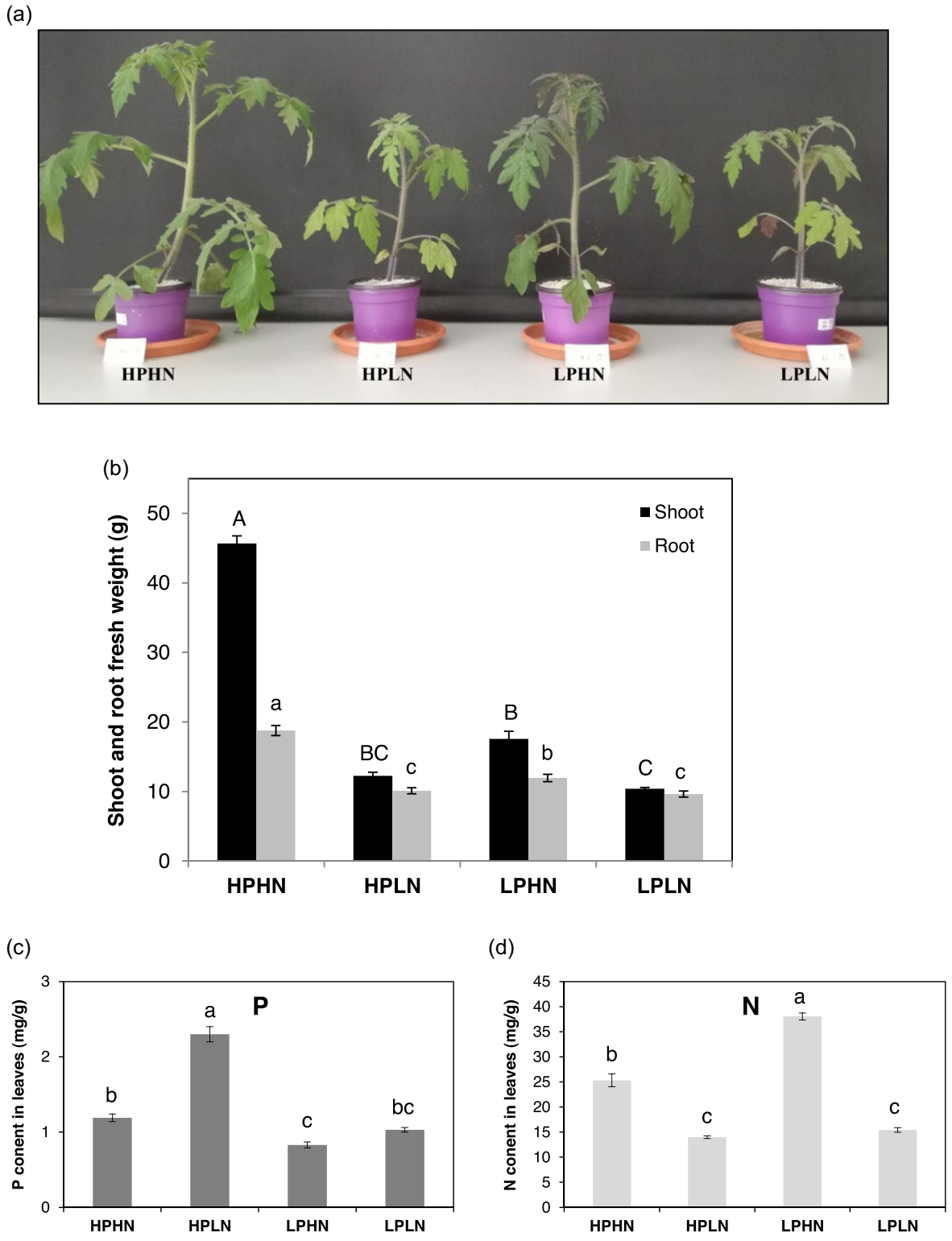

FIGURE 2 Effect of different Pi and nitrate regimes on tomato (cv. MoneyMaker) growth and performance. (a) Phenotypic comparison of 6-week old plants grown under optimal (control) Pi and nitrate conditions (HPHL), optimal Pi and limiting nitrate (HPLN), limiting Pi and optimal nitrate (LPHN) and deficiency of both nutrients (LPLN). Shoot and root fresh weight (b), phosphorus (P) (c) and nitrogen (N) (d) content in tomato leaves. Bars represent the means of 10 (b) and six (c and d) independent replicates (±*SE*). Data not sharing a letter in common differ significantly ($p < .05$) according to the Tukey test

TABLE 1 Carbon (C), nitrogen (N) levels and C:N ratios in tomato leaves from plants grown on different phosphorus (P) and nitrogen (N) combinations: high P and high N (HPHN), high P and low N (HPLN), low P and high N (LPHN) and low P and low N (LPLN)

	Nutrient content (mg/g)		
Treatment	C	N	C:N
HPHN	425.00 ± 1.98a	25.32 ± 1.27b	17.02 ± 0.96b
HPLN	426.67 ± 2.50a	14.00 ± 0.26c	30.31 ± 0.56a
LPHN	410.50 ± 2.45b	38.06 ± 0.70a	10.80 ± 0.18c
LPLN	420.83 ± 2.55a	15.42 ± 0.46c	27.42 ± 0.82a

Note: Values present the means of six independent replicates (±*SE*). Data not sharing a letter in common differ significantly ($p < .05$) according to the Tukey test.

those of orobanchol (Figure 3d,e). When the availability of both nutrients was limited (LPLN), the promotion observed by Pi deficiency under normal N conditions was strongly reduced; solanacol levels were half of those in low Pi, and orobanchol fell below the detection limit (Figure 3d,e).

3.3 | Pi starvation signalling depends on plant's N status

The results on SLs biosynthesis and content support the proposed crosstalk between Pi and nitrate signalling pathways (Hu & Chu, 2020; Medici et al., 2019; Ueda & Yanagisawa, 2019). To further explore the mechanisms and the compounds regulating such interplay, we addressed the influence of nitrate levels on P-related signalling by analysing the expression of key genes regulating Pi starvation signalling, the triad ISP1-miR399-PHO2 (Figure 1a). Transcript levels of *SlPHO2* were down-regulated by Pi deficiency only under optimal N conditions (LPHN; Figure 4a). Remarkably, the down-regulation was suppressed when the two nutrients (Pi and nitrate) were limited (LPLN). Conversely, *SlPHO2* levels were slightly but significantly promoted by N deficiency under optimal Pi conditions (HPLN; Figure 4a). The opposite expression pattern was observed for SlmiR399 and *LeTPSI1*, the tomato homologue to IPS1 (Liu, Muchhal, & Raghothama, 1997). Their transcript levels were increased by Pi limitation and reduced by N deficiency (Figure 4b,c). In this case, when both nutrients were limited the expression of SlmiR399 and *LeTPSI1* was down-regulated, being the N deficiency effect predominant over that shown by Pi deficiency. Strikingly, the same pattern was shown for the Pi transporter LePT2, which belongs to the PTH1 family of PHTs and it is transcriptionally regulated by the plant Pi status (Gamir et al., 2020; Nagy et al., 2005). Indeed, its expression was induced by Pi limitation, but down-regulated by N deficiency and when both nutrients were limiting (Figure 4d).

The influence of nitrate and Pi supply in the expression of regulatory genes of the N signalling pathways (PNR and NSR; Figure 1b) was also investigated. So far, five genes encoding nitrate transporters belonging to the NRT1 and NRT2 families have been characterized in tomato (Albornoz, Gebauer, Ponce, & Cabeza, 2018). Two of them—LeNRT1.1 and LeNRT1.2—belong to the NRT1 family, encoding for high-capacity and low-affinity nitrate transporters. The other three—LeNRT2.1, LeNRT2.2 and LeNRT2.3—belong to the NRT2 family, encoding for low-capacity and high-affinity transporters. The expression of the high-capacity transporters *LeNRT1.1* and *LeNRT1.2* was repressed by N deficiency (HPLN; Figure 5a,b). However, the two genes showed a different regulation pattern under Pi limitation (LPHN): Transcript levels of *LeNRT1.1* decreased, while those of *LeNRT1.2* increased (Figure 5a,b). When both Pi and N were deficient (LPLN), *LeNRT1.1* was similarly reduced, but *LeNRT1.2* was not detected. Low-capacity transporters (NRT2) also showed a differential expression pattern. Under Pi deficiency, the three genes *LeNRT2.1*, *LeNRT2.2* and *LeNRT2.3* were down-regulated as compared to the control conditions (Figure 5c–e). However, no significant changes in the expression of *LeNRT2.1* and *LeNRT2.2* were detected under N deficiency, and only a slight reduction was observed when both nutrients were limiting (Figure 5c,d). Conversely, the expression of *LeNRT2.3* increased under N limitation. Moreover, this induction was maintained when both nutrients were deficient (Figure 5e).

The expression of the NRT2 transporters is regulated by the NIGT1/HHO repressors (Figure 1b) (Kiba et al., 2018; Maeda et al., 2018; Medici et al., 2015), which play an important role in the N–P signalling interplay (Hu & Chu, 2020; Ueda & Yanagisawa, 2019; Wang et al., 2020). The expression of one of the putative tomato NIGT1/HHO genes, *SlNIGT1.2/HHO2* (Solyc05g009720), was analysed. Its transcript levels were repressed by N limitation (HPLN) and induced by Pi starvation (LPHN) as compared to control conditions (HPHN; Figure 5f). When both nutrients were deficient (LPLN), the expression of *SlNIGT1.2/HHO2* was also reduced, thus prevailing the N starvation effect. Overall, the gene expression data support the crosstalk between the PSR and NSR signalling pathways, and that plants generally prioritize the response to N starvation when both nutrients are limited.

3.4 | Exogenous application of SLs affects Pi and N signalling

We show that SLs promotion under Pi limitation depends on N provision, and that Pi signalling is affected by the N status (Figures 3 and 4). Previously, we demonstrated that SLs modulate PSR by transcriptionally regulating the regulatory genes ISP1-miR399-PHO2 (Gamir et al., 2020). Therefore, we aimed to assess whether SLs are also involved in NSR signalling and in the crosstalk between PSR and NSR. For that, we exogenously applied SLs in plants subjected to Pi deprivation and analysed the impact on NSR regulatory genes (Experiment 2). Plants were grown hydroponically under optimal Pi conditions (+P) or exposed to Pi shortage for the last week of growth (−P). Then, a 1 h-pulse with 10 nM of the synthetic SL analogue 2′-*epi*-GR24 (orobanchol-type) was applied to half of the plants of each treatment (GR24). The expression of the five nitrate transporters described in tomato—*LeNRT1.1*, *LeNRT1.2*, *LeNRT2.1*, *LeNRT2.2*

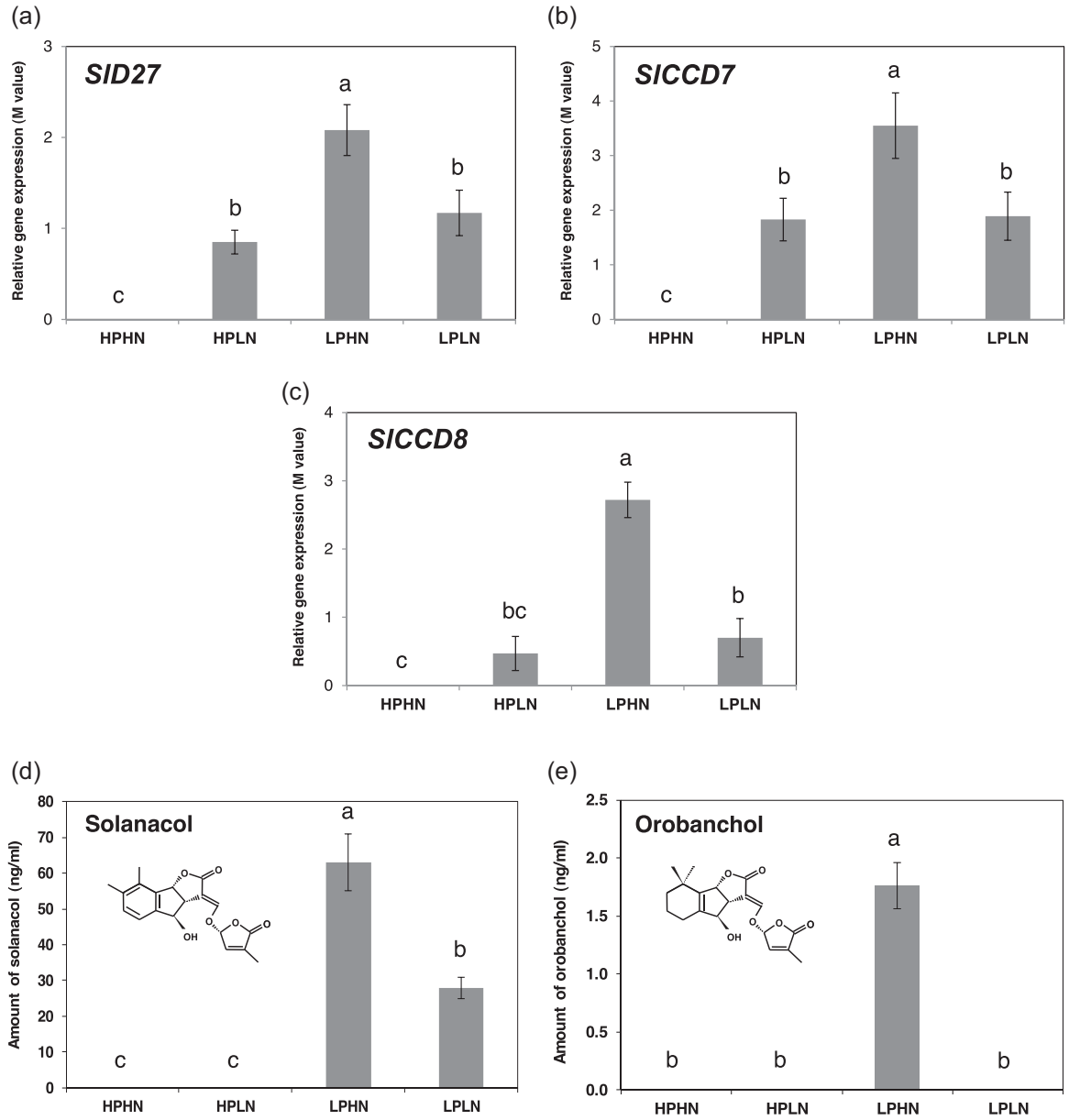

FIGURE 3 Effect of Pi and nitrate deficiency on SL biosynthesis. Tomato plants (cv. MoneyMaker) were grown under different nutrient regimes, as described in the legend to Figure 2. Gene expression analysis (*M* value) of the SL biosynthesis genes *SlD27* (a), *SlCCD7* (b) and *SlCCD8* (c) in roots from 6-week old plants. Expression values were normalized using the housekeeping gene *SlEF*. *M* value (log2 ratio) is zero if there is no change; '+1' or '−1' indicate two-fold change induction or repression, respectively. Content of the SLs solanacol (d) and orobanchol (e) in root exudates. Bars represent the means of six independent replicates (±*SE*). Data not sharing a letter in common differ significantly ($p < .05$) according to the Tukey test

and *LeNRT2.3*—and *SlNIGT1.2/HHO2* was analysed by qPCR. As in Experiment 1, the expression of *LeNRT1.1* was significantly reduced by Pi starvation, whereas that of *LeNRT1.2* was induced (Figure 6a,b). Under these conditions, the expression of genes from the NRT2 family also showed the same pattern as in the previous experiment, except for *LeNRT2.2*, whose transcripts could not be detected upon 35 cycles of PCR. That is, *LeNRT2.1* and *LeNRT2.3* transcripts were reduced by Pi limitation (Figure 6c,d). *SlNIGT1.2/HHO2* also showed the same pattern as in the previous experiment, being induced by Pi deficiency (Figure 6e). Interestingly, the same expression pattern was observed for all the genes upon application of 2′-*epi*-GR24 under optimal Pi conditions (Figure 6). Therefore, SLs mimic the effect of Pi starvation on N-related signalling in the absence of Pi limitation.

3.5 | The SL-deficient line SlCCD8-RNAi L09 is partially altered in N signalling

To further assess the potential role of SLs as signals in N starvation and in the N-P signalling interplay, the response to P and N levels was compared in the SL-deficient line *SlCCD8*-RNAi L09 and the corresponding wild-type cv. Craigella (Experiment 3). *SlCCD8*-RNAi L09

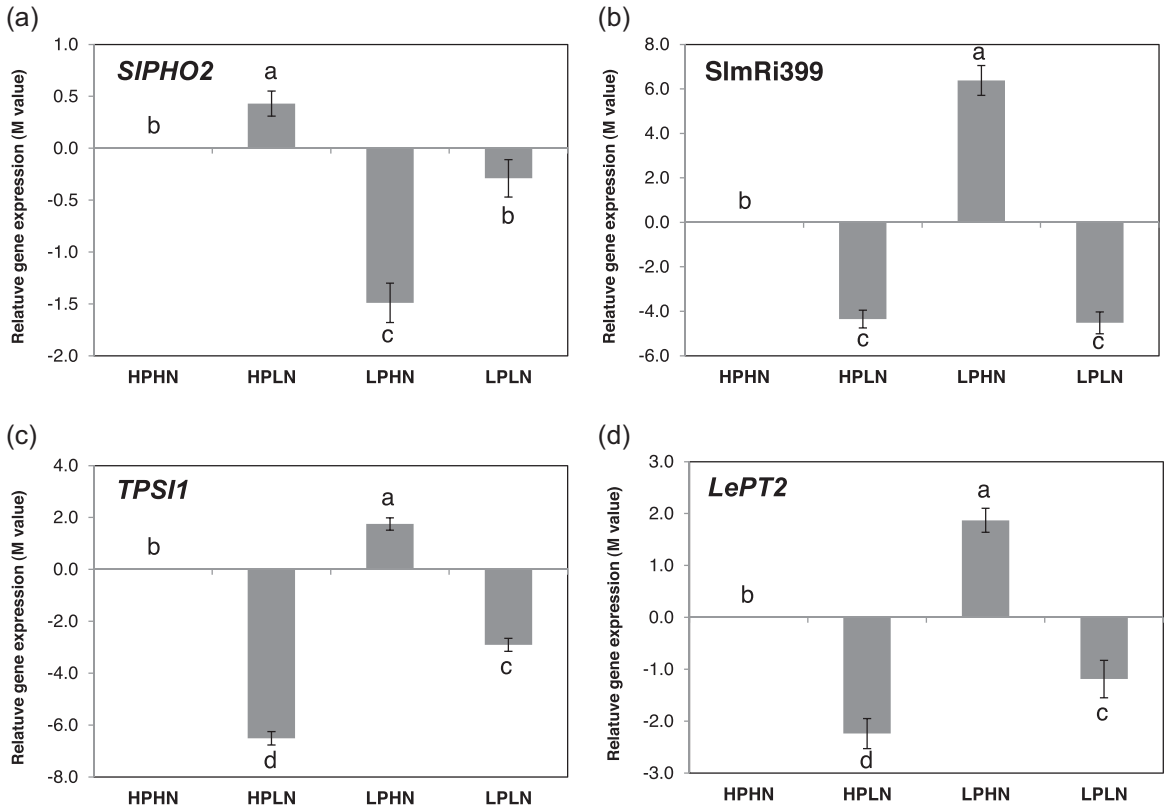

FIGURE 4 Expression analysis of genes associated to Pi signalling and homeostasis pathways. Effect of Pi and nitrate deficiency on the expression (*M* value) of genes encoding for the Pi signalling regulators *SlPHO2* (a), SlmiR399 (b), *LeTPSI1* (c) and the Pi transporter *LePT2* (d) in tomato roots. Bars represent the means of six independent replicates (±*SE*). For data analysis, statistics and nutrient regimes see legends in Figures 2 and 3

displays a 95% reduction in SL levels (Kohlen et al., 2012) (Figure S3). The effect of nutrient availability on plant growth was similar in both genotypes. As in Experiment 1 with MoneyMaker (Figure 2), shoot and root fresh weights were reduced under P and/or N deficiency as compared to the 'optimal' conditions (HPHN; Figure S4a,b). Remarkably, *SlCCD8*-RNAi L09 showed a higher root-to-shoot ratio than the wild-type in all the P–N combinations (Figure S4c). The expression of the PSR signalling genes also showed a similar pattern to that observed previously for MoneyMaker (Figure 4). The expression of *SlPHO2* was induced by N limitation and repressed by Pi deficiency in the wild type, a reduction that was diminished when both nutrients were limited (Figure 7a). Interestingly, a different behaviour was observed for the SL-deficient line. *SlPHO2* expression was induced by N deficiency, as in the wild type; however, it did not respond to Pi starvation (Figure 7a). The expression of the genes SlmiR399 and *LeTPSI1*, and *LePT2* also showed a similar pattern to that observed previously. Their transcript levels were reduced by N deficiency and promoted by Pi deprivation, being the induction abolished when both nutrients were limited (Figure 7b–d). Remarkably, the effect by N and Pi deficiency was significantly lower in *SlCCD8*-RNAi L09 than in the wild-type, characterized by a decreased response to Pi deficiency (Figure 7b–d).

As for PSR, the NSR regulatory genes also showed a similar pattern to that observed in Experiment 1 with MoneyMaker (Figures 5 and 8). *LeNRT1.1* and *LeNRT1.2* were repressed by N limitation, but Pi deficiency differentially affected their expression. Transcript levels of *LeNRT1.1* were decreased under Pi starvation, while those of *LeNRT1.2* were increased (Figure 8a,b). When both nutrients were deficient, the expression of *LeNRT1.2* was down-regulated (Figure 8b). Regarding the NRT2 genes, N deprivation induced the expression of *LeNRT2.1* and *LeNRT2.3*, while no changes were detected in *LeNRT2.2* (Figure 8c–e). Conversely, Pi deficiency repressed the expression of the three genes, although this effect was reduced when both nutrients were limiting (Figure 8c–e). The expression of *SlNIGT1.2/HHO2* was down-regulated by N deficiency and induced by Pi starvation compared to control conditions (Figure 8f). In the SL deficient line, the effects of N and Pi deficiency were generally in the same direction than in the wild type, but significant differences were found between both genotypes, mainly under low N (Figure 8b–f) and low Pi (Figure 8a, b and e) conditions. Overall, the changes were less pronounced in *SlCCD8*-RNAi L09 than in the wild-type, supporting a role of SLs in the regulation of the key regulatory genes of NSR.

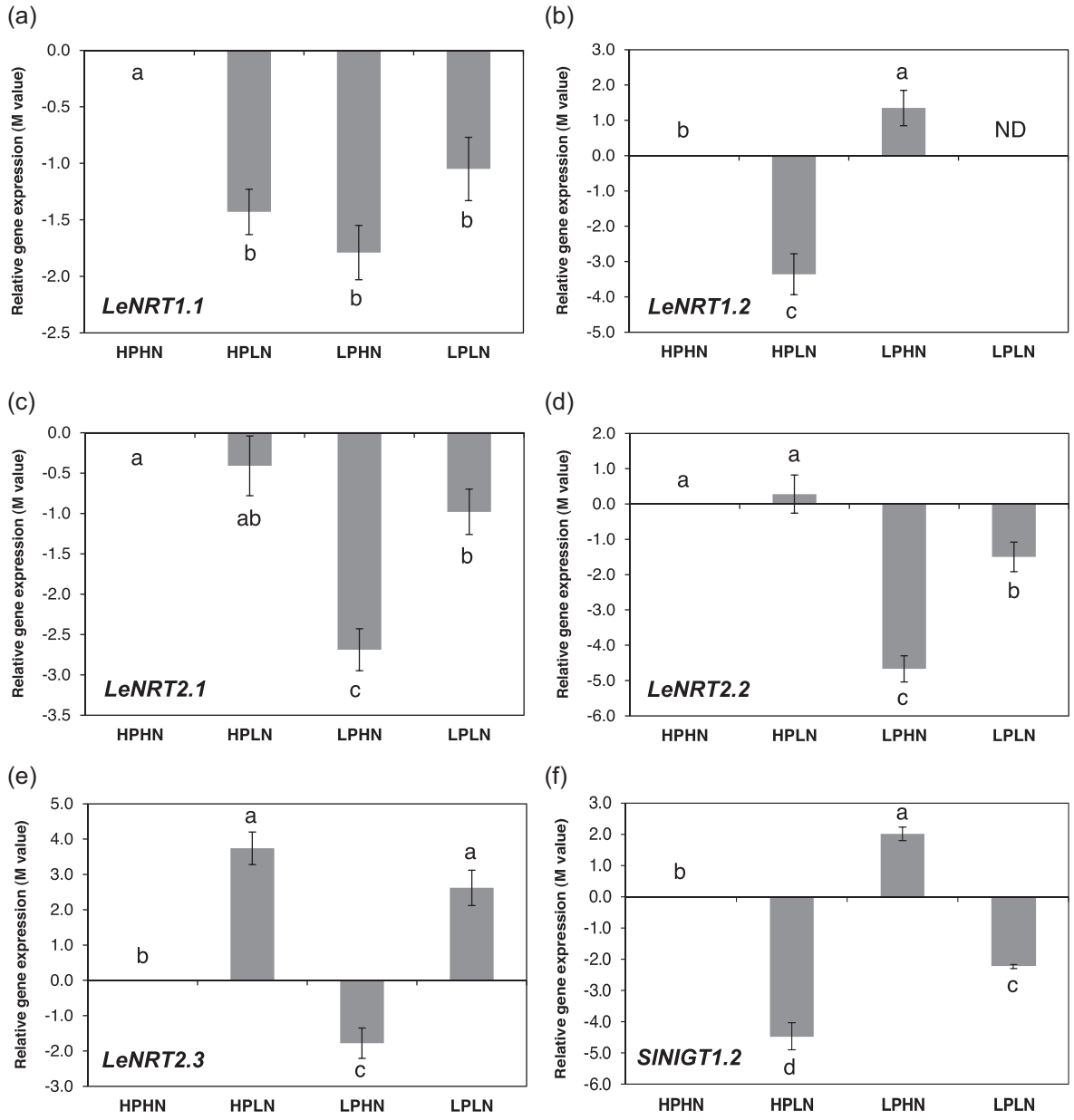

FIGURE 5 Expression analysis of genes associated to the nitrate signalling pathway. Effect of Pi and nitrate deficiency on the expression (*M* value) of the nitrate signalling pathway genes *LeNRT1.1* (a), *LeNRT1.2* (b), *LeNRT2.1* (c), *LeNRT2.2* (d), *LeNRT2.3* (e) and *SlNIGT1.2/HHO2* (f) in tomato roots. Bars represent the means of six independent replicates (±*SE*). For data analysis, statistics and nutrient regimes, see legends in Figures 2 and 3

4 | DISCUSSION

Nitrogen as nitrate and/or ammonium and P in the form of Pi are the two most abundant macronutrients used by plants, being their coordinated use essential for optimal plant growth and maximal crop production (Hu & Chu, 2020; Nasr et al., 2021; Oldroyd & Leyser, 2020). Understanding how plants sense, signal and respond to N and Pi deficiency is crucial to optimize the use of these nutrients and reduce the need of fertilizers, alleviating agricultural costs and the excessive consumption of non-renewable resources. Under nutrient shortage, plants have the ability to optimize N and Pi uptake and use through a number of physiological adaptations. Thus, their overall growth is reduced, although the root system is usually expanded to facilitate nutrient foraging, increasing the root-to-shoot ratio (Hu & Chu, 2020; Oldroyd & Leyser, 2020). This is what we observed in this study with tomato plants grown under different nitrate and Pi regimes. The root-to-shoot ratio increased in plants subjected to nutrient deficiency, but the effect was stronger under N starvation. Plants grown under limiting N conditions performed worst in terms of growth and nutrient content independently of the Pi levels, suggesting that N status has a higher influence on plant growth and development than P status, as previously observed in Arabidopsis and rice (Medici et al., 2019).

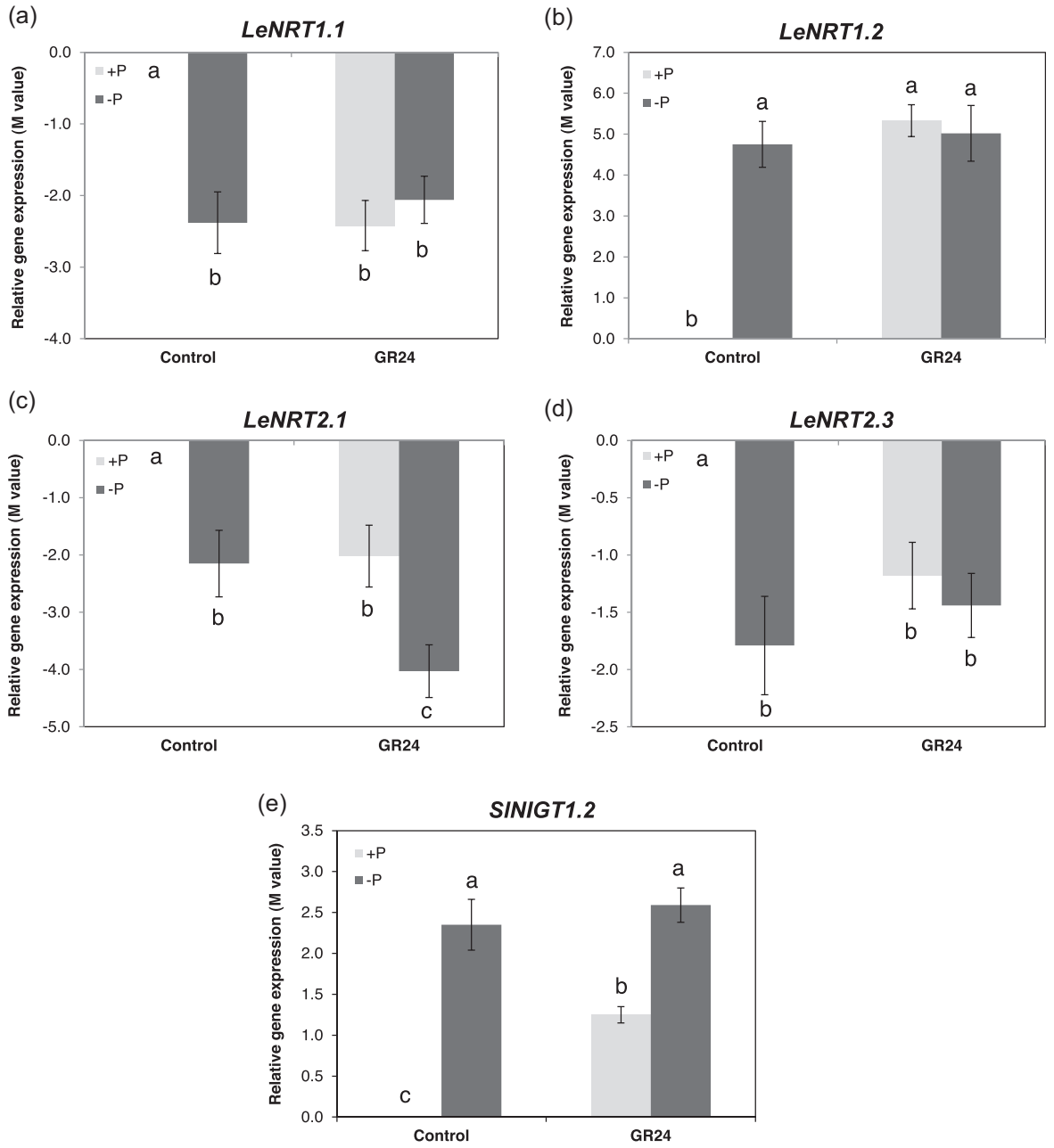

FIGURE 6 Effect of SLs on the expression of genes associated to the nitrate signalling pathway. Effect of the synthetic SL analogue 2′-*epi*-GR24 under normal (+P; grey bars) or deficient (−P; closed bars) Pi conditions in the expression (*M* value) of genes encoding for the nitrate signalling genes *LeNRT1.1* (a), *LeNRT1.2* (b), *LeNRT2.1* (c), *LeNRT2.3* (d) and *SlNIGT1.2/HHO2* (e) in tomato roots. Plants were untreated (Control) or treated with 2′-*epi*-GR24 (GR24). Gene expression values were normalized using the housekeeping gene *SlActin*. Bars presents the means of five independent replicates (±*SE*). For statistics see legend in Figure 3

SLs are phytohormones modulating plant growth under nutrient deficiency and stress conditions. It is well known that under nutrient limitation, mainly Pi, SLs modulate the coordinated development of roots and shoots to optimize nutrient uptake and use (Andreo-Jiménez et al., 2015; Santoro et al., 2021; Sun et al., 2014; Waters et al., 2017). Accordingly, SL biosynthesis is promoted by Pi starvation (López-Ráez et al., 2008; Yoneyama et al., 2012). In the present work, the highest promotion of the SLs solanacol and orobanchol was observed under Pi deficiency, but under sufficient N provision (LPHN). Under N deficiency, neither solanacol nor orobanchol were detected despite the induction of some biosynthetic genes. It might be that some SLs or SL-like compounds non-characterized so far were specifically promoted by nitrate deprivation. Interestingly, when both N and Pi were limiting, the increase in SLs triggered by Pi starvation was reduced (Figure 3). A similar pattern was observed in alfalfa, where N deprivation supressed the promotion by P limitation of the SLs orobanchol and orobanchyl acetate (Peláez-Vico, Bernabéu-Roda, Kohlen, Soto, & López-Ráez, 2016). These results suggest that N status influences SL

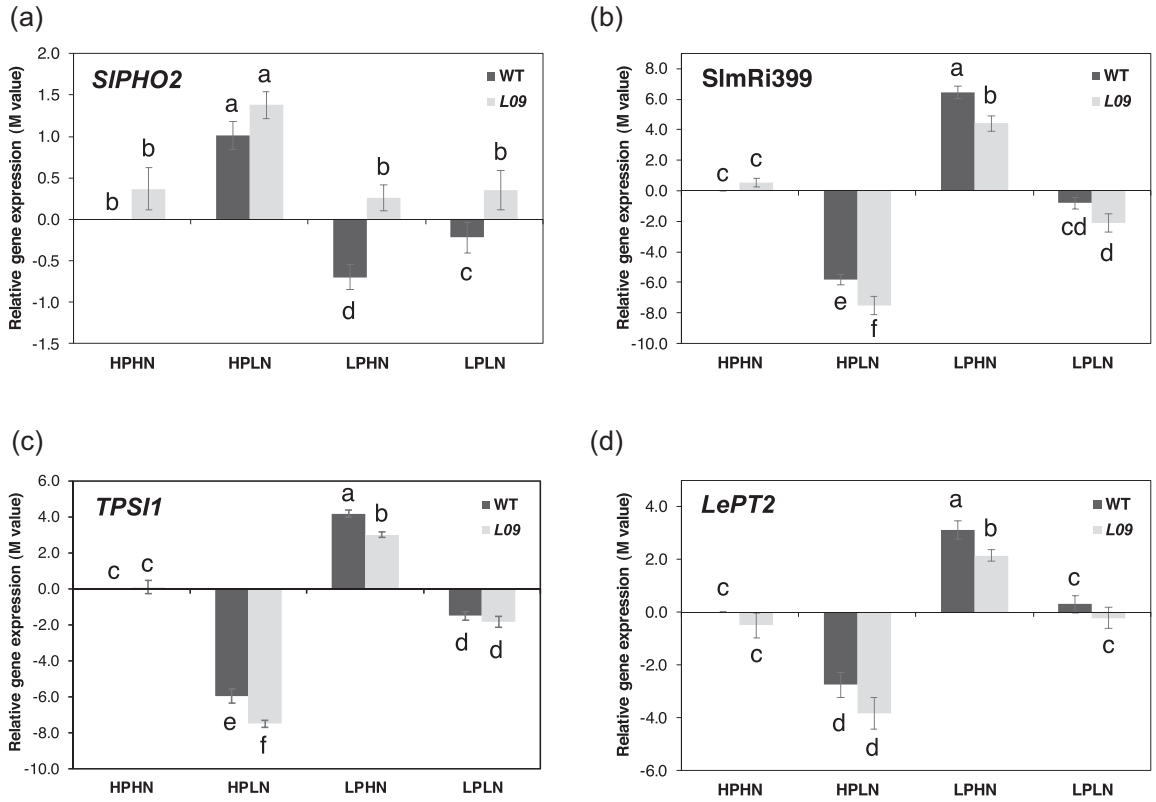

FIGURE 7 Expression analysis of genes associated to Pi signalling and homeostasis pathways in wild-type plants and in the SL-deficient line *SlCCD8*-RNAi L09. Effect of Pi and nitrate deficiency on the expression (*M* value) of genes encoding for the Pi signalling regulators *SlPHO2* (a), SlmiR399 (b), *LeTPSI1* (c) and the Pi transporter *LePT2* (d) in tomato roots. Bars represent the means of six independent replicates (±*SE*). For data analysis, statistics and nutrient regimes, see legends in Figures 2 and 3

levels, and that N deficiency has a negative impact in their induction by Pi starvation. Supporting this idea, no promotion of SLs was detected by N deficiency under sufficient Pi provision (Figure 3), as previously reported in tomato and other plant species such as alfalfa and red clover (López-Ráez et al., 2008; Peláez-Vico et al., 2016; Yoneyama, Yoneyama, Takeuchi, & Sekimoto, 2007). A stimulatory effect of N starvation in SL biosynthesis has been reported in some plant species such as pea, sorghum and lettuce (Foo & Reid, 2013; Yoneyama et al., 2007, 2012). However, this effect was considerably weaker than that observed for Pi starvation. In line with this, it was suggested that P but not N levels have a regulatory effect on SL biosynthesis (Yoneyama et al., 2012).

It is known that SLs act as sentinel molecules during Pi deficiency modulating the expression of key regulatory genes of PSR such as the triad IPS1-miR399-PHO2 in tomato and wheat (Figure 1a) (Campos et al., 2018; Gamir et al., 2020). Here, we have confirmed the role of SLs in PSR signalling and addressed their involvement in NSR signalling. First, we have shown that SL biosynthesis and PSR signalling depend on plant N status. The expression of SlmiR399 and *LeTPSI1* was promoted under Pi deficiency, but their transcript levels were down-regulated by N starvation, a repression that was also observed when both N and Pi were limiting. Remarkably, the effect by N and Pi deficiency in the SL-deficient line *SlCCD8*-RNAi L09 was significantly lower to that observed in the wild-type. The opposite pattern was observed for the PSR repressor *SlPHO2*, whose expression was repressed by Pi starvation and induced by N limitation in wild-type plants. The down-regulation of *SlPHO2* by Pi deficiency was absent in the SL-deficient line, confirming a deficiency on the regulation of PSR in the SL-deficient line and supporting the role of SLs in this response. The repression of *SlPHO2* under Pi limitation was abolished when both nutrients were deficient (Figure 4); showing again that the effect of N limitation overrules that of Pi limitation. An induction of *PHO2* levels by N deprivation has been previously shown in Arabidopsis (Medici et al., 2019). These authors also showed a de-repression of the PSR signalling genes in the *pho2* mutant, and proposed PHO2 as the integrator of the PSR and NSR signalling pathways (Medici et al., 2019). We found here that the expression of the triad LeTPSI1-miR399-PHO2 under the different P-N regimes agreed with that of SL levels, showing an interplay between the two signalling pathways, which depend on the plant's N status and where SLs seems to play a key role.

In agreement with this hypothesis, the expression of the N status sentinel genes NRT1, NRT2 and NIGT1/HHO was also regulated by SLs. The gene *LeNRT1.2* showed the same expression pattern as the PSR signalling genes SlmiR399 and *SlTPSI1*, and correlated with that of SL levels (Figures 3–8). *LeNRT1.2* was also induced by the exogenous application of the synthetic SL analogue 2′-*epi*-GR24 under optimal Pi conditions, mimicking the effect observed in Pi starvation and showing a SL dependency. LeNRT1.2 is homolog to the

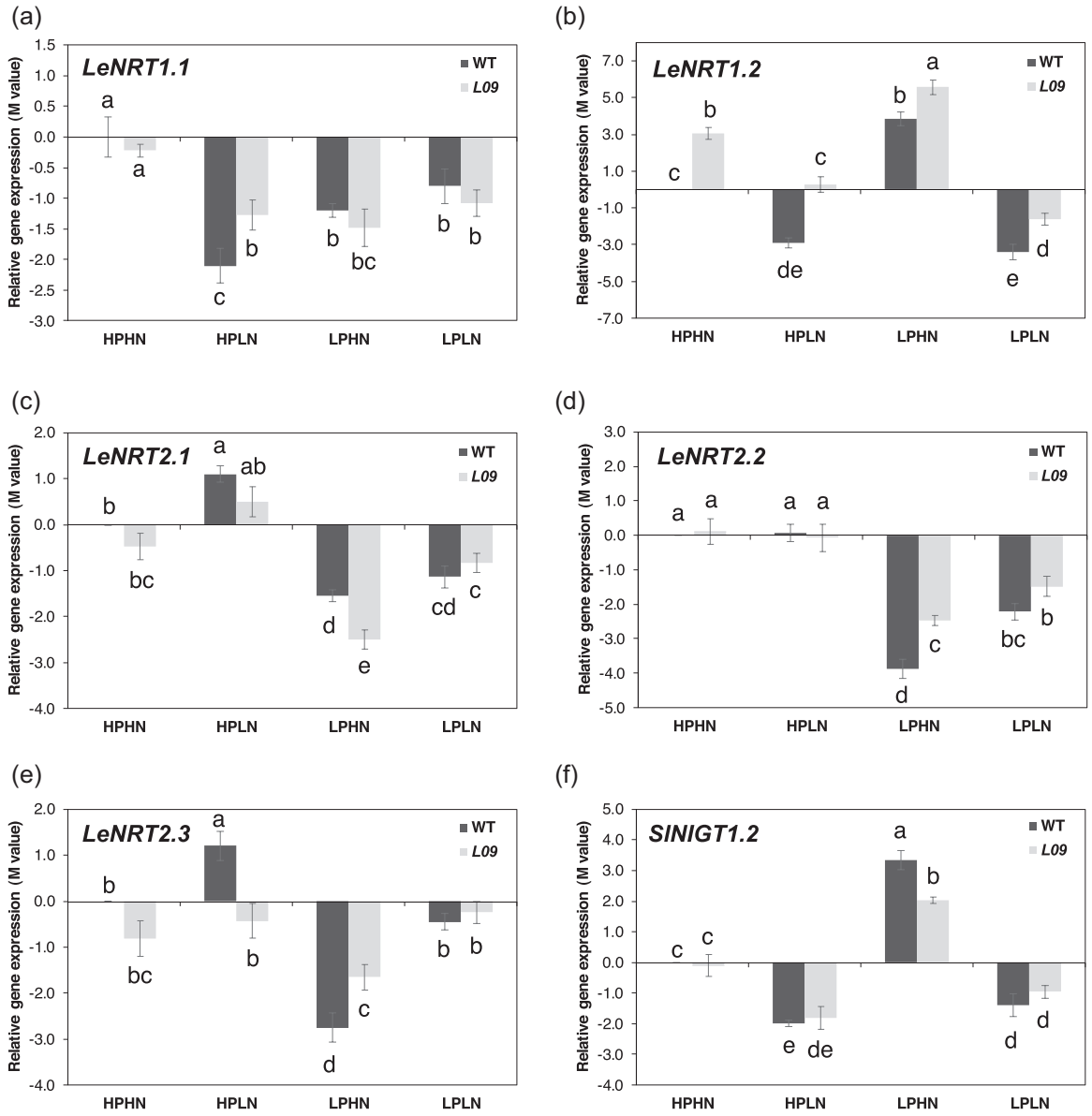

FIGURE 8 Effect of SLs on the expression of genes associated to the nitrate signalling pathway. Effect of the synthetic SL analogue 2′-*epi*-GR24 under normal (+P; grey bars) or deficient (−P; closed bars) Pi conditions in the expression (*M* value) of genes encoding for the nitrate signalling genes *LeNRT1.1* (a), *LeNRT1.2* (b), *LeNRT2.1* (c), *LeNRT2.3* (d) and *SlNIGT1/HHO* (e) in tomato roots. Plants were untreated (Control) or treated with 2′-*epi*-GR24 (GR24). Gene expression values were normalized using the housekeeping gene *SlActin*. Bars presents the means of five independent replicates (±*SE*). For statistics, see legend in Figure 3

Arabidopsis nitrate transceptor (protein with transport and sensing function) AtNRT1.1, which triggers the PNR and NSR signalling pathways (Hu & Chu, 2020; Medici et al., 2019). This sensor shows a dual nitrate affinity depending on N availability (Ho et al., 2009; Wang et al., 2018). Here, a dual expression pattern was observed for the tomato nitrate transporter *LeNRT1.2*, which could be associated to a dual nitrate affinity. Transcript levels of *LeNRT1.2* were increased under low Pi and optimal N conditions, suggesting a low affinity and high-capacity activity. Conversely, its expression was reduced by N deficiency under optimal Pi conditions, suggesting a high-affinity and low-capacity activity. This suggests that the tomato LeNRT1.2 could act as a nitrate transceptor during PNR similarly to AtNRT1.1 in Arabidopsis. Remarkably, the expression pattern of *LeNRT1.2* was opposite to that observed for the repressor *SlPHO2*. Since PHO2 integrates PSR and NSR signalling pathways under nutrient deficiency (Medici et al., 2019), and its expression is regulated by SL levels, we propose that SLs could modulate nitrate and Pi signalling through PHO2 by the regulation of the NRT1 sensors.

One of the mechanisms by which AtNRT1.1 modulates NSR signalling is through the regulation of some high-affinity transporters of the family NRT2, thus connecting PNR and NSR (Figure 1b; Maghiaoui et al., 2021; Medici et al., 2019). NRT2 transporters are involved in root nitrate influx, being their expression generally induced by N starvation (O'Brien et al., 2016). However, their transport capacity is

low, so they are considered nitrate transceptors rather than transporters (Ho et al., 2009; Medici et al., 2015; O'Brien et al., 2016; Wang et al., 2018). Here, we show that the expression of the tomato *LeNRT2.3* was promoted by N deficiency, but repressed by Pi starvation (Figures 5 and 8). This expression pattern was opposite to that observed for SlmiR399 and *SlTPSI1*, and for SL levels. In addition, the repression under Pi deficiency was lower in *SlCCD8*-RNAi L09 than in the corresponding wild-type, suggesting a role for *LeNRT2.3* in both signalling pathways and the involvement of SLs in such regulation. Expression of *LeNRT1.2*, the proposed homologous to *AtNRT1.1*, was also opposite to that of *LeNRT2.3*. In Arabidopsis, the expression of *AtNRT2.1* is induced under N deficiency in an AtNRT1.1-dependent manner (Maghiaoui et al., 2021; Medici et al., 2019). Thus, the duo LeNRT1.2-LeNRT2.3 in tomato could play a similar role to AtNRT1.1-AtNRT2.1 in Arabidopsis, acting as nutrient sensors and connecting NSR and PSR signalling pathways through SL biosynthesis. Further studies are required to confirm this hypothesis.

Recently, the NIGT1/HHO family has been described as new player in the N-P signalling interplay. These transcriptions factors modulate Pi and nitrate uptake in order to maintain the P-N balance in plants. In Arabidopsis, the expression of NRT2 transceptors is regulated by AtNIGT1/HHO repressors in an AtNRT1.1-dependent manner (Figure 1b) (Kiba et al., 2018; Maeda et al., 2018; Medici et al., 2015; Wang et al., 2020). In agreement with this, the expression of the putative tomato *SlNIGT1.2/HHO2* was repressed by N starvation. Therefore, it is tempting to speculate that this will release the repression of *LeNRT2.3* to optimize N use under nitrate deficiency. Conversely, *SlNIGT1.2/HHO2* expression was induced by Pi starvation, which correlated with a repression of *LeNRT2.3*, probably to prioritize Pi uptake (Figures 5 and 8). An induction of NIGT1/HHO genes under Pi deficiency was previously found in Arabidopsis and maize, coordinating Pi and nitrate uptake by targeting PHT1 Pi transporters and NRT1.1 (Wang et al., 2020). AtNIGT1/HHO can also target the Pi starvation signalling repressor AtPHO2 under Pi deficiency, activating Pi uptake and use (Kiba et al., 2018). Here in tomato, the induction of *SlNIGT1.2/HHO2* under Pi starvation also correlated with a reduction of *SlPHO2*, supporting a conserved regulatory mechanism across plant species. When Pi and N were limited, the expression of *SlNIGT1.2/HHO2* was also reduced, abolishing the induction by Pi starvation and indicating, once again, the priority for the plant of N over P status. The induction of *SlNIGT1.2/HHO2* by Pi deficiency was lower in *SlCCD8*-RNAi L09 compared to the wild-type, and it was induced by 2′-*epi*-GR24 under optimal Pi conditions, showing that its expression is regulated by SLs. The regulation by Pi starvation of all the analysed key elements in NSR was reduced in the SL-deficient line *SlCCD8*-RNAi L09 and mimicked under Pi sufficient conditions by exogenous SL application, confirming that SLs act as signals for Pi starvation. Since NIGT1/HHO transcription factors and NRT transceptors are important players integrating N–P signals, and their expression is regulated by endogenous SLs levels, we propose that SLs are key signals regulating the N-P interplay during fluctuating nutritional conditions.

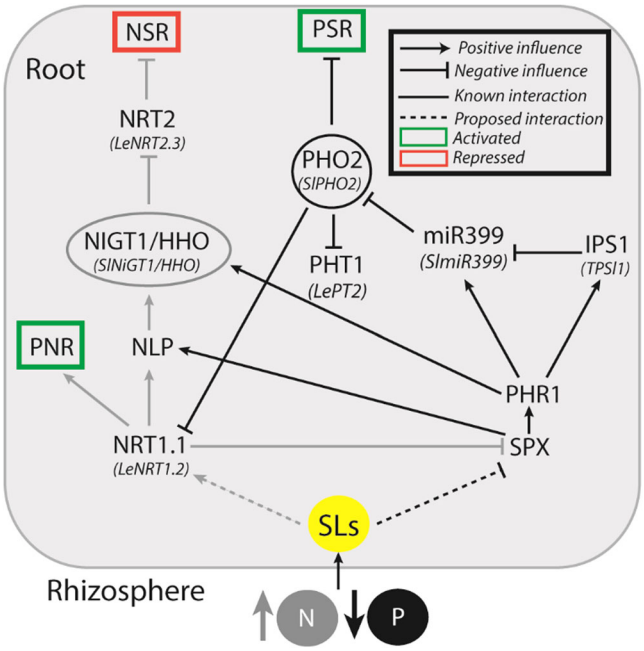

FIGURE 9 Proposed model for the regulation of plant responses to Pi and nitrogen deficiency and the potential role of SLs. Under Pi deficiency and optimal N conditions, SL biosynthesis is promoted, releasing PHR1 (via SPX degradation) and inducing the expression of miR399 and *IPS1* (*TPSI1*). In turn, miR399 reduces the levels of the suppressor PHO2, activating the PSR pathway. Regarding N signalling, SLs would promote the expression of *NRT1.1* (*LeNRT1.2*), either directly or in a PHO2-dependent manner, activating nitrate transport through the PNR pathway. At the same time, NRT1.1 (via NLPs) and PHR1 would induce the expression of the repressors *NIGT1/HHOs*, blocking the expression of the high-affinity transporters/sensors NRT2 (*LeNRT2.3*) and inactivating the NSR pathway

Based on the present results, we propose an integrative model for the regulation of plant responses to nitrate and Pi deficiency (Figure 9). Under Pi deficiency and optimal N conditions, SL biosynthesis is induced. In the presence of SLs, PHR1 is released inducing the expression of miR399. In turn, miR399 reduces the levels of the repressor PHO2, activating the PSR pathway. SLs would also promote the expression of NRT1.1 (LeNRT1.2), either directly or in a PHO2-dependent manner, activating the PNR pathway. Subsequently, NRT1.1 and PHR1 would induce the expression of NIGT1/HHO repressors, blocking the expression of the high-affinity transporters/sensors NRT2 (LeNRT2.3) and inactivating the NSR pathway. A different scenario takes place under nitrate starvation, independently of Pi status, Here, SL biosynthesis is not promoted, just maintaining basal levels for normal plant growth. The absence of SLs gives rise to an up-regulation of PHO2, which blocks PSR signalling responses even when Pi levels are scarce. Low levels of SLs would also repress the expression of NRT1.1 (directly or through PHO2) inhibiting PNR, and those of NIGT1/HHOs. At the same time, this repression would allow the expression of the high-affinity NRT2 (LeNRT2.3) transceptors, activating NSR signalling.

Overall, our results provide evidences showing that SLs are early modulators of plant responses to Pi and nitrate starvation, acting as key signals in the N-P interplay. They modulate the expression of key regulatory genes of both signalling pathways and that of the N-P integrators such as the PHO2 and NIGT1/HHO repressors. The fact that the regulation of these genes is not completely abolished in SL-depleted plants indicates that other(s) regulatory mechanism(s), in addition to SLs, may also be involved in the N-P interplay. Further research is required to decipher these other mechanisms/molecules. We also show that plants prioritize responses to N over P limitation, N deficiency influencing strongly Pi starvation responses, probably through the regulation of SL biosynthesis. This knowledge will help to develop new strategies to optimize plant N and P uptake and usage, alleviating cost and reducing the excessive use of chemical fertilizers in agriculture.

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CONFLICT OF INTEREST

We declare that we do not have any conflict of interest.

DATA AVAILABILITY STATEMENT

All data generated or analysed during this study are included in this published article (and its Supporting Information files).

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