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REVIEW

The physiological mechanism underlying root elongation in response to nitrogen deficiency in crop plants

Xichao Sun[1] · Fanjun Chen[1] · Lixing Yuan[1] · Guohua Mi[1]

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Abstract

Main conclusion **In response to low nitrogen stress, multiple hormones together with nitric oxide signaling pathways work synergistically and antagonistically in crop root elongation.**

Abstract Changing root morphology allows plants to adapt to soil nutrient availability. Nitrogen is the most important essential nutrient for plant growth. An important adaptive strategy for crops responding to nitrogen deficiency is root elongation, thereby accessing increased soil space and nitrogen resources. Multiple signaling pathways are involved in this regulatory network, working together to fine-tune root elongation in response to soil nitrogen availability. Based on existing research, we propose a model to explain how different signaling pathways interact to regulate root elongation in response to low nitrogen stress. In response to a low shoot nitrogen status signal, auxin transport from the shoot to the root increases. High auxin levels in the root tip stimulate the production of nitric oxide, which promotes the synthesis of strigolactones to accelerate cell division. In this process, cytokinin, ethylene, and abscisic acid play an antagonistic role, while brassinosteroids and auxin play a synergistic role in regulating root elongation. Further study is required to identify the QTLs, genes, and favorable alleles which control the root elongation response to low nitrogen stress in crops.

Keywords Nitrogen · Root elongation · Cell division · Cell elongation · Hormones · Nitric oxide

Abbreviations

N	Nitrogen
C	Carbon
NO_3^-	Nitrate
NH_4^+	Ammonium
CTK	Cytokinin
NO	Nitric oxide
NR	Nitrate reductase
ABA	Abscisic acid
SLs	Strigolactones
BRs	Brassinosteroids
IAA	Indole-3-acetic acid
NAA	1-Naphthaleneacetic acid
NPA	*N*-1-Naphthylphthalamic acid
6-BA	6-Benzylaminopurine
SHY2	Short hypocotyl 2
ACS	1-Aminocyclopropane-1-carboxylic acid synthase
BG1	β-Glucosidase
SCR	SCARECROW
ABI4	ABA INSENSITIVE 4
ABI5	ABA INSENSITIVE 5
NOS	Nitric oxide synthase
XOS	Xanthine oxidase
SNP	Sodium nitroprusside
TZ	Transition zone
DMSO	Dimethyl sulfoxide
GWAS	Genome-wide association study

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✉ Guohua Mi
miguohua@cau.edu.cn

[1] College of Resources and Environmental Sciences, National Academy of Agriculture Green Development, Key Laboratory of Plant-Soil Interactions, Ministry of Education, China Agricultural University, Beijing 100193, China

Introduction

Nitrogen (N) is one of the main nutrients limiting crop production. Crop yields have increased dramatically over the past 50 years due to the use of N fertilizers (Jiao et al. 2016). However, the average N recovery rate of crops is only 30–40% in the growing season (Chen et al. 2014a). N not absorbed by the crop may be fixed by the soil, leached to deep soil layers, or volatilized to the atmosphere in the form of ammonia or nitrogen oxides (Ju et al. 2009). Loss of N increases the cost of agricultural production and accelerates environmental degradation (Chen et al. 2014b). Optimizing N fertilizer input technology can effectively improve N fertilizer utilization rates. In addition, breeding of new N-efficient crop cultivars may achieve higher yields with low N (van Bueren et al. 2017), which promotes sustainable agricultural development (Lynch 2019).

Lynch (2007) proposed that the improvement of root traits will be the second green revolution in agricultural production. The root is the primary organ for acquisition of nutrients and water from the soil, and thus root system architecture plays a central role in adaptation to various abiotic stresses including nutrient deficiency, drought, waterlogging, and salinity (Mi et al. 2010; Mickelbart et al. 2015; Koevoets et al. 2016; Mariani et al. 2017). Large genotypic variation exists in root characteristics not only among different species, but also within a certain crop (Laperche et al. 2006; Phung et al. 2016; Fan et al. 2018; Sanchez et al. 2018). To improve root breeding strategies, it is necessary to recognize the root response to different stress conditions, determine desirable root traits, and understand the physiological mechanism underlined.

Nitrate (NO_3^-) and ammonium (NH_4^+) are the major inorganic N forms required for plant growth. While NO_3^- is mobile in soils, NH_4^+ is strongly fixed by soil particles and later released. About 2/3 of fertilizer N is fixed into soil by various chemical and biological processes. As a result, organic N is the most abundant N form in soil (Havlin et al. 2016). With increasing N deficiency, crop plants utilize more organic N resources, which are mineralized slowly and controlled by soil microbes (Mengel et al. 1982). Even in fertilized arable soil, more than half of the N required by crops comes from soil mineralization (Mikkelsen and Hartz 2008; Kirkby et al. 2014). Therefore, of the N available for crop plant uptake, only a small fraction comes from the mass flow of water, while a large portion originates from the slow mineralization of organic N. A corresponding adaptive strategy for plants is changing root morphology to explore more soil spaces for N resources. This may explain why a large root system is positively correlated to high N use efficiency in crop plants (Yu et al. 2015).

The model plant *Arabidopsis* has been demonstrated to have a root response to changing N supplies (see reviews in Zhang and Forde 2000; Forde 2014; Kiba and Krapp 2016). However, the root system of a crop (such as maize, wheat, and rice) is much more complex than that of *Arabidopsis* (Gruber et al. 2013; Atkinson et al. 2014; Bellini et al. 2014). *Arabidopsis* has a taproot system with one primary root from which several orders of lateral roots emerge. Cereal crops have a fibrous root system, which consists of roots derived from the embryo (embryonic roots) and those formed after germination from existing roots or shoots (postembryonic roots). The embryonic roots include a primary root and a number of seminal roots. The postembryonic roots arising from tissues other than roots are termed adventitious roots (Steffens and Rasmussen 2016). Both embryonic and postembryonic shoot-borne roots have the capacity to form highly branched lateral roots (Yu et al. 2016; Hochholdinger et al. 2018). The primary, seminal, shoot-borne roots are also termed axial roots (Rich and Watt 2013). The response of crop root systems to N deficiency can thus be quite different from that of *Arabidopsis*.

In crop-soil systems, NO_3^- and NH_4^+ are the predominant inorganic N forms. The average concentrations of NO_3^- and NH_4^+ in the soil are 6.0 mmol dm^{-3} and 0.77 mmol dm^{-3}, respectively (Wolt 1994). NH_4^+ content in the soil under aerobic conditions is very low, only 1/1000–1/10 of that of NO_3^- (Marschner 2012). Most applied NH_4^+ fertilizer is transformed into NO_3^- within several days (Stevenson 1982; Legg and Meisinder 1982). Therefore, NO_3^- is the main inorganic N form absorbed and utilized by most crop plants (Bloom 2015). In experimental conditions, a high supply of NH_4^+ inhibits root and shoot growth in most crops (maize, Cramer and Lewis 1993; rice, Hirano et al. 2008; wheat, Cox and Reisenauer 1973), and a mixed supply of NO_3^- and NH_4^+ can improve root and shoot growth (Guo et al. 2007; Li et al. 2013; Wang et al. 2018, 2019). In this review, we focus on root elongation in response to low NO_3^- supply. For the response of plant root elongation to NH_4^+-N supply, or the differential effect of NO_3^--N and NH_4^+-N on root growth, readers may refer to recent reviews (Britto and Kronzucker 2002; Li et al. 2014; Liu and von Wirén 2017; Jia and von Wirén 2020).

Root growth response to N supply in crop plants

Root-to-shoot ratio

Plant roots sense external environmental signals and have plastic responses morphologically and physiologically. The root responses likely reflect different strategies to cope with N availability. Under mild N deficiency, *Arabidopsis* plants adopt a “systemic foraging strategy” with a characteristic increase in root size. In severe N-deficient environments, *Arabidopsis* plants adopt a “survival strategy” of inhibited root growth (Gruber et al. 2013; Giehl and von Wirén 2014). This similar strategy is shown in crop plants like rice (Shi et al. 2005) and maize (Fig. 1). In maize, shoot biomass increased with increasing NO_3^- supply, reached the maximum value at optimal N supply (about 10 mM in the specific experiment), and maintained this maximum for a range of N (about 10–20 mM), and then decreased when the N supply was further increased (Fig. 1). When N supply was reduced from the optimum (10 mM) to mild N deficiency (about 1 mM), shoot biomass decreased, but the root biomass increased. This root response can be considered as a “positive foraging strategy”. Root biomass was reduced when the N supply was further reduced. This root response can be considered a “negative survival strategy”. Decreasing N supply always resulted in an increase in the root-to-shoot ratio (Fig. 1). The increase in root growth seems to be controlled by the internal N status signal of plants. In *Arabidopsis*, Scheible et al. (1997) showed that the root-to-shoot ratio is controlled by shoot N status, indicated by NO_3^- concentration.

Under low N conditions, plants allocate a larger proportion of assimilated N and carbon (C) to the roots. Transport of C from shoot to the root via the phloem is enhanced (Gao et al. 2015; Wang and Ruan 2016). Rufty et al. (1988) showed that, in N-deficient leaves, 52% of the assimilates are used for root respiration, thereby promoting root growth and providing sufficient energy for the absorption and assimilation of N. Under high N conditions, 40% of photosynthetic products are transported to the roots, and 25% of the assimilates are used for respiratory consumption of C metabolism (Cai et al. 2012; Irving 2015). N supply changes carbohydrate metabolism. Under low N conditions, sucrose concentration in the root decreases, but hexose and starch concentrations increase (Aloni et al. 1991). Stitt and Feil (1999) show that the concentration of sugar in tobacco roots decreased with increasing supply of NO_3^- (0.2–10 mM).

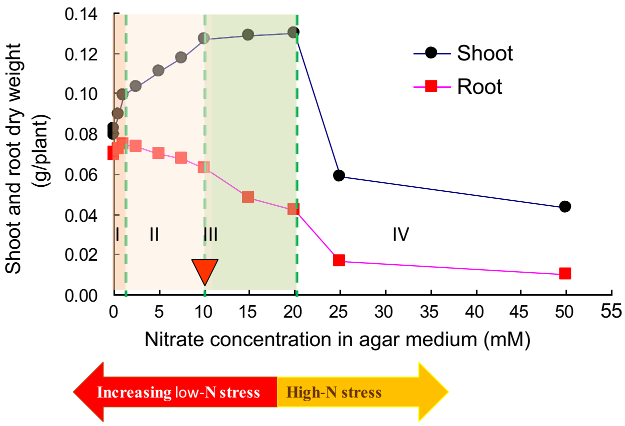

Fig. 1 Response of shoot and root growth to N supply in a large regime. In extreme N deficiency (Phase I), both shoot and root growth are inhibited with decreasing N supply, which indicates a “survival strategy” for the plants. In moderate N deficiency (Phase II), root growth is increased while shoot growth is inhibited with decreasing N input, which indicates a “morphological acclimation strategy”. In sufficient N supply (Phase III), root growth is enhanced while shoot growth is unaffected with decreasing N supply, indicating the potential for root growth to acquire N to meet the demand of the shoot at low N input, providing a physiological basis for N fertilization optimization. In supra-optimal N supply (phase IV), both shoot and root growth are inhibited with increasing N supply, indicating overuse of N has a harmful effect on plant growth. Triangle: the optimum N supply level for plant growth. The figure is modified from Guo 2004

Plant hormone cytokinin (CTK) plays a central role in the regulation of N on substance allocation between the shoot and the root. When the external NO_3^- level is 3–22 mM, increasing NO_3^- supply does not affect the biomass, but the root-to-shoot ratio is significantly decreased, and the trans-zeatin-type CTK and root-to-shoot ratio are significantly negatively correlated (Wagner and Beck 1993). When N supply is low, CTK concentration in the root is reduced, resulting in a decrease in CTK transport from root to leaf. The decrease of CTK in the leaves reduces leaf cell division and leads to the inhibition of sucrose loading from the phloem to the growing cells, resulting in a decrease in sucrose supply in the leaves, which in turn leads to an increase in phloem turgor, so that more carbohydrates are distributed to the roots (Van der Werf and Nagel 1996).

Axial root elongation

Primary root elongation in *Arabidopsis* responds to N supply (Zhang and Forde 2000; Tian et al. 2009). Recently, Jia et al. (2019) found that mild N deficiency can increase the primary root length of *Arabidopsis* by 16%. In maize, the typical response of root growth to mild low-N stress is to reduce crown root number, and to increase the elongation of the axial roots, including the primary, seminal, and crown roots (Tian et al. 2005, 2008; Wang et al. 2005; Gaudin et al. 2011; Gao et al. 2015; Li et al. 2015) (Fig. 2a). Elongation of the primary root is enhanced even at severe low-N stress (Fig. 2b). Compared to sufficient N supply conditions, low N supply can increase axial root length by about 40%. The elongation of axial roots begins to increase after low N treatment (Fig. 3a). Gao et al. (2015) found that axial root elongation is significantly promoted when shoot N concentration decreased by more than 30% or the C to N ratio increased by more than 40%. This indicates that the shoot N concentration and C to N ratio may reflect the N status in the plant and play a regulatory role in the change of root morphology.

The promotion effect of low-N stress on root elongation is also found in rice (Sun et al. 2014, 2016, 2018a, b) and

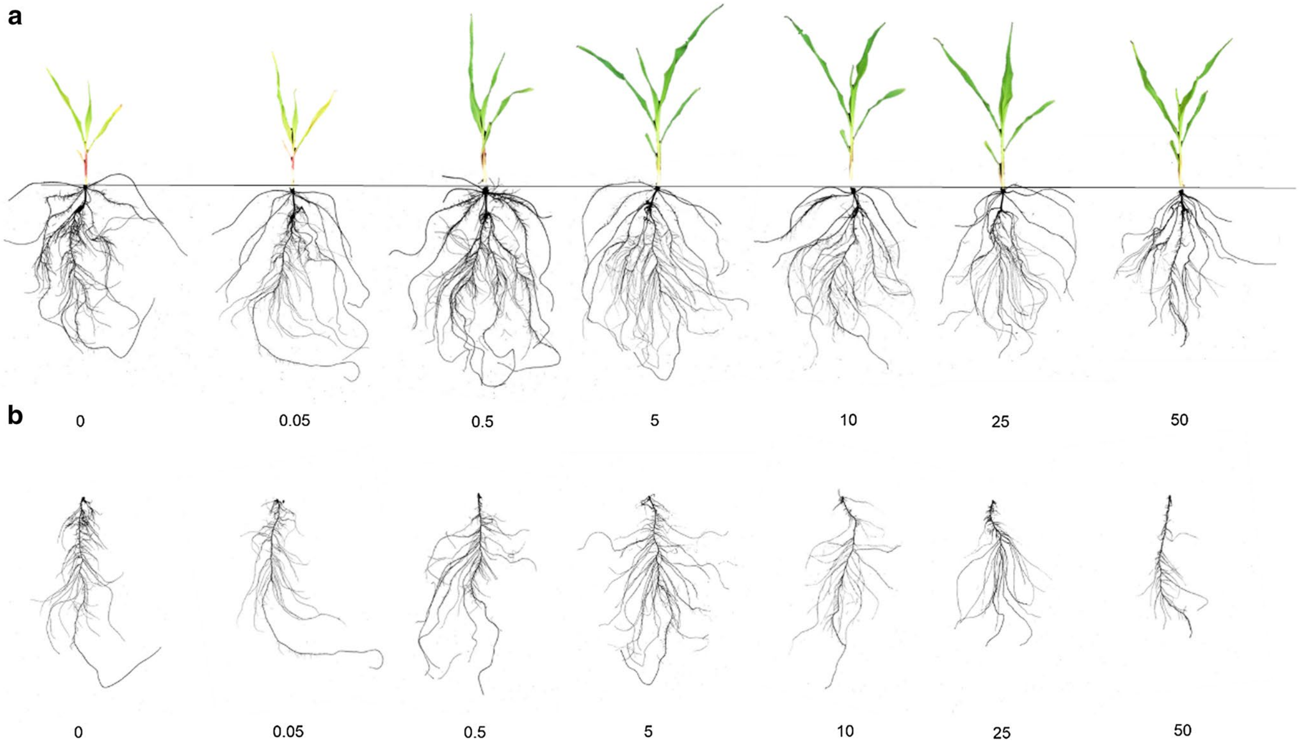

Fig. 2 Root morphology of maize in response to a wide range of N supplies. **a** Whole maize plant. **b** Maize primary root. Maize plants cultured in solution for 9 days supplied with NO_3^- concentrations of 0, 0.05, 0.5, 5, 10, 25, and 50 mM

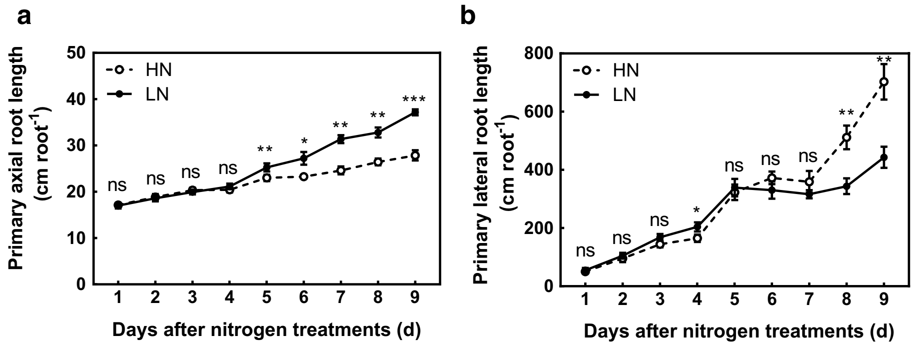

Fig. 3 Time course of the length of the primary axial root and the primary lateral root in response to low N (0.05 mM) and high N (5 mM) treatments. **a** Primary axial root length. **b** Primary lateral root length. Data are means ± SE of six biological replicates. *, ** and *** indicate significance at $P < 0.05$ and $P < 0.01$ and $P < 0.001$, respectively. Ns indicates not significant

wheat (Ren et al. 2017, 2018; Xu et al. 2019). In rice, Sun et al. (2014) showed that, compared to normal N supply, seminal root length increased by about 22% at N concentration of 0.1 mM, and increased by 33% when N supply was further decreased to 0.01 mM and 0.02 mM. In wheat, Xu et al. (2019) found that an NO_3^- supply at 0.2 mM N or lower concentration significantly promoted the primary root growth of XY54 by 44.1%.

Lateral root growth

In crop plants, N deficiency has been found to promote lateral root growth (Sun et al. 2014), or inhibit lateral root growth (Gao et al. 2015). This discrepancy may be related to the degree of N deficiency (Fig. 2). In one experiment with maize, N deficiency did not affect lateral root length during the first 3 days of treatment (Fig. 3b). After 4 days, lateral root length was promoted under the low N treatment. With lasting N deficiency, lateral root growth began to be inhibited 8 days after the low-N treatment. These data suggest that, upon sensing N deficiency, the first response of maize is to promote lateral root growth in an attempt to obtain more N from the soil. With lasting N deficiency, when the plants cannot obtain sufficient N to support shoot growth, they adjust by reducing lateral root growth to reduce C and N consumption, but maintain elongation of the axial root to explore larger soil spaces (Fig. 3a). Therefore, as found in *Arabidopsis*, crop plants promote lateral root growth under mild N deficiency, and inhibit lateral root growth under severe N deficiency.

Physiological mechanism underlying root elongation in response to low-N stress

Cell division and elongation in the root apex

The longitudinal structure of the root consists of the root cap, apical meristem zone, elongation zone, and root hair mature zone (Ishikawa and Evans 1995). Root growth rate is regulated by two coordinated processes: cell division and expansion (Baskin 2013). In wheat, the enhanced root elongation observed in low N conditions is related to increased cell length (Bosemark et al. 2006). This is also observed in maize (Tian et al. 2008). However, high N concentration can also inhibit cell division in maize (Greef and Geisler 1991). Gao et al. (2014) used kinetics to analyze the mechanism of low N promoting the elongation of maize roots, and found that cell division and elongation in the root tip are equally important for root elongation. Low N promotes cell production rate, accelerates maximum elongation rate, increases the length of the elongation zone, and increases mature cell length. The response of the primary root and crown roots to low N is similar. It is not yet clear by which mechanism N regulates cell division and/or elongation. However, there is evidence that plant hormones auxin, CTK, ethylene, abscisic acid (ABA), brassinosteroids (BRs), strigolactones (SLs), as well as the nitric oxide (NO) signal pathway, are involved in the process (Takatsuka and Umeda 2014; Pacifici et al. 2015; Steffens and Rasmussen 2016).

Auxin

Auxin regulates cell expansion according to the acid growth hypothesis (Rayle et al. 1992). This suggests that auxin initiates an acidification mechanism, possibly a membrane-bound H$^+$ pump, and the pH of the solution in the matrix of the cell wall decreases. Some yet-undescribed enzyme(s) related to wall loosening are activated by the lowered apoplast pH. The wall is loosened, and cell enlargement takes place under the direct force of the cell's own turgor pressure. Ultimately, cell elongation is promoted. In maize, Tian et al. (2008) found that indole-3-acetic acid (IAA) level in the roots increased with decreasing N supply, and that exogenous application of 1-naphthaleneacetic acid (NAA) and IAA restored primary root elongation under high N conditions. They also found that shoot-to-root IAA transport by phloem is higher at low N supply conditions. This was confirmed in another study with labeled IAA (Liu et al. 2010). Similar results have been reported in soybean (Caba et al. 2000). IAA concentration in soybean roots grown in 8 mM KNO$_3$ was about 4 times less than those grown in 1 mM KNO$_3$. When shoot-to-root IAA transport is inhibited by applying N-1-naphthylphthalamic acid (NPA) at the junction between the shoot and the root in maize, the promotion effect of low-N on root elongation is severely inhibited (Fig. 4). These results further confirm that low N induces shoot-to-root auxin transport and thereby enhances root elongation. Supporting this, Li et al. (2017) overexpressed the auxin efflux transporter *ZmPIN1a* in maize and found that the shoot-to-root transport of auxin was enhanced. As a result, carbohydrate level in the roots increased and root growth increased, with longer seminal roots and denser lateral roots. In rice, however, Sun et al. (2014) obtained an opposite result. They found that low NO$_3^-$ supply reduced auxin concentration in the roots. Similarly, in N-starved maize plants, localized supply of NO$_3^-$ to the transition zone in root can increase local auxin accumulation to increase root elongation (Manoli et al. 2016). These findings suggest the complexity of the regulatory network and imply that auxin may interact with other factors to regulate root elongation under low N conditions.

Cytokinin

Cytokinin (CTK) promotes cell division and differentiation (Schaller et al. 2014). CTK mediates the transduction of the N signal from root to shoot (Gu et al. 2018). N supply significantly affects CTK concentration in barley (Samuelson and Larsson 1993), tobacco (Singh et al. 1992), and *Urtica dioica* (Wagner and Beck 1993). Collier et al. (2003) found that NO$_3^-$ increased phloem CTK levels. Recovery of the N supply led to increased CTK content in maize xylem sap (also in shoots and roots) (Takei et al. 2001). In *P. chinensis*, exogenous treatment with CTK can overcome the growth restriction effect caused by low N supply (Kuiper 1988). Compared to NH$_4^+$ supply, the stimulating effect of NO$_3^-$ on

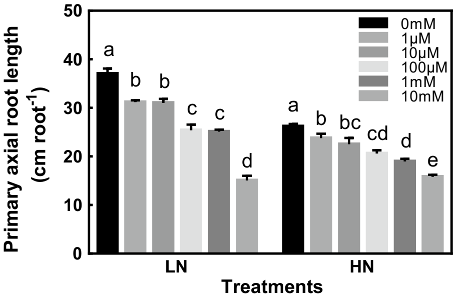

Fig. 4 Length of the primary axial root under low-N (0.05 mM) and high-N (5 mM) treatments after applying different concentrations of NPA at the junction between the shoot and root of maize. Data are means ± SE of six biological replicates. Significant differences at $P < 0.05$ are shown with different letters

shoot growth in tobacco was associated with increased levels of CTK in leaves and xylem (Walch-Liu et al. 2000).

However, high CTK level in the roots is not favorable for root elongation. In regulating root development, CTK negatively regulates the effect of auxin on cell division and cell differentiation, and ultimately affects the size of root meristem (Sozzani and Iyer-Pascuzzi 2014; Chandler and Werr 2015). Exogenous application of CTK or overexpression of the IPT gene (promoting CTK synthesis) significantly inhibited root elongation, while overexpression of the CKX gene (promoting CTK breakdown) promoted root growth (Kuderová et al. 2008; Werner et al. 2010; Köllmer et al. 2014; Xu et al. 2016; Ivanov and Filin 2018). Given that N levels are closely related to CTK levels in roots, CTK is likely to play a role in the root architecture in response to N availability (Kiba et al. 2011). By comparing two maize inbred lines with differential root elongation responses to low N stress, Tian et al. (2005) found that the change in CTK content in roots was negatively related to root elongation induced by low N. In genotype Ye478, in which root elongation is more sensitive to high N supply, root CTK level was strongly increased by high N application. High N supply reduced the distance from the root apex to the first visible lateral root, similar to the phenotype caused by the exogenous addition of CTK. Moreover, exogenous CTK 6-benzylaminopurine (6-BA) completely reversed the stimulation of root elongation by low NO_3^- supply. Considering the observed complementary effects of auxin and CTK (Schaller et al. 2015), regulation of root elongation by N supply may be accomplished through its effect on the balance between CTK and auxin levels (Mi et al. 2008). In *Arabidopsis* primary roots, the short hypocotyl 2 (SHY2) gene links the auxin and CTK signaling pathways and regulates the balance between cell division and differentiation (Ioio et al. 2008).

Ethylene

As an important gaseous phytohormone, ethylene is widely involved in regulating a variety of biological processes such as plant growth, development, and response to environmental stress (Dubois et al. 2018). Exogenously applied ethylene reduces root elongation in various cereals (Smith et al. 1971; Kim et al. 1997; Alarcón et al. 2009; Ma et al. 2014; García et al. 2015; Vandenbussche and Van Der Straeten 2018). In *Arabidopsis*, ethylene reduces proton efflux and controls cell elongation (Ruzicka et al. 2007; Hahn et al. 2008; Strader et al. 2009; Staal et al. 2011), and this regulation process is closely related to auxin levels (Ruzicka et al. 2007; Stepanova et al. 2007; Swarup et al. 2007; Hu et al. 2017). Ethylene plays an important regulatory role in plant response to N availability (Iqbal et al. 2015). In some species, high NO_3^- supply promotes ethylene production in the root (Ligero et al. 1986, 1987; Caba et al. 1998). In maize roots, N deficiency reduces ethylene production, but increases root sensitivity to ethylene and induces aerenchyma formation (Drew et al. 1989; He et al. 1992). Gao et al. (2017) found that NO_3^- increases ethylene production and cortical aerenchyma formation in rice roots under water stress.

Early studies showed that an exogenous supply of CTK increased the production of ethylene (Stenlid 1982; Bertell et al. 1990). Cytokinin induces ethylene synthesis by modulating the activity of 1-aminocyclopropane-1-carboxylic acid synthase (ACS), a key enzyme in ethylene synthesis (Lee and Yoon 2018). Therefore, under high NO_3^- conditions, CTK content in the root is increased, which can both antagonize the action of auxin and promote the production of ethylene, thereby inhibiting root elongation (Mi et al. 2008).

Abscisic acid

Abscisic acid (ABA) is a stress hormone essential for plant abiotic and biological stress responses (Verma et al. 2016; Zhu 2016). Under water stress, accumulation of ABA in maize promotes the synthesis of proline in the root tip, increasing the net accumulation of osmotic substances, and maintains the growth of the root (Sharp et al. 1990). ABA also helps to activate the root antioxidant system and keep reactive oxygen species in a suitable concentration range to promote root growth (Sharp et al. 2002). In *Medicago truncatula*, ABA plays a similar role (Zhang et al. 2014). NO_3^- supply has a role in regulating ABA synthesis and/or functions. In *Arabidopsis*, increasing NO_3^- supply increases ABA levels in the root tip. NO_3^- stimulates the release of ABA from the inactive ABA-GE pool in the root tip by β-glucosidase (BG1). ABA can regulate root growth interactions with the GRAS transcription factor SCARECROW (SCR), which is a central player controlling cell division in the root meristem. Exogenously applied ABA inhibits the expression of SCR in the root tip. Cui et al. (2012) demonstrated that SCR inhibits the expression of ABA-INSENSITIVE 4 (ABI4) and ABI5 in root tip meristems, controlling root elongation. NO_3^- supply can increase ABA level in the root tip and inhibit the expression of SCR, stimulate the expression of ABI4 and ABI5, and regulate root growth (Cui et al. 2012; Ondzighi-Assoume et al. 2016).

In *Medicago truncatula*, Morère-Le et al. (2012) show that the dual-affinity NO_3^- transporter MtNPF6.8 can also transport ABA. MtNPF6.8 is expressed in the root tip and expression is stimulated by ABA. Root elongation in the MtNPF6.8 mutant is insensitive to the inhibition of high NO_3^- supply. However, ABA treatment can inhibit root elongation of the mutant under high NO_3^-. This indicates that ABA acts downstream of the NO_3^- transporter MtNPF6.8 and regulates root growth in the NO_3^- signaling pathway

(Pellizzaro et al. 2014). It was previously demonstrated that inhibition of root elongation by high NO_3^- is altered in another high-affinity NO_3^- transporter MtNPF1.7 mutant, which has different ABA sensitivity (Harris and Dickstein 2010; Yendrek et al. 2010; Bagchi et al. 2012). Exogenous application of ABA restored the root phenotype of the MtNPF1.7 mutant (Liang et al. 2007). Pellizzaro et al. (2014) suggest that, in *Medicago truncatula*, the NO_3^- signaling pathway regulating primary root growth is dependent on ABA involvement and involves two NO_3^- transporters. MtNPF6.8 acts upstream of ABA, and MtNPF1.7 acts downstream of ABA to regulate the organization and activity of the root meristem (Liang and Harris 2005; Liang et al. 2007; Yendrek et al. 2010).

Nitric oxide

NO is a small gaseous molecule that is soluble in both water and lipids. In plants, NO can be produced by four pathways: nitric oxide synthase (NOS), nitrate reductase (NR), xanthine oxidase (XOS), and non-enzymatic reactions (Astier et al. 2018). NO acts as a signaling molecule that plays a key regulatory role in adventitious root formation (Wen et al. 2016), lateral root development (Sun et al. 2015), root hair formation (Liu et al. 2017), and primary root elongation (Zhao et al. 2007; Fernández-Marcos et al. 2011; Bai et al. 2014; Manoli et al. 2014). High levels of NO reduced the activity of the root meristem zone (Fernández-Marcos et al. 2011). A decrease in NO level reduced the elongation of the primary root and size of the root meristem in the NO-deficient mutant (Sanz et al. 2014). NO inhibits root elongation by reducing auxin transport in *Arabidopsis* and rice, suggesting an interaction of auxin and NO in regulating root growth (Fernández-Marcos et al. 2011; Sun et al. 2018a). In maize, Zhao et al. (2007) found that high NO_3^- supply reduced root elongation by reducing the NO level in the root tip. Application of sodium nitroprusside (SNP) and IAA weakened this inhibition. Low N increases IAA content, which in turn leads to an increase in NO content, and ultimately promotes elongation of the primary root. Similarly, the enhanced elongation of rice roots under N deficiency is also regulated by NO (Sun et al. 2016). NOS inhibitors reduce primary root elongation in maize grown under low NO_3^- conditions, suggesting that NOS activity may be inhibited in plants grown in high-NO_3^- solutions, resulting in reduced levels of endogenous NO (Zhao et al. 2007).

Under limited N supply, relatively more NO_3^- is reduced in the root (Marschner 2012), which can lead to more NR-dependent NO production and, therefore, contribute to the stimulation of root elongation. Sun et al. (2015) found that, in rice, NO produced by the NR pathway plays a key role in increasing N uptake capacity by regulating lateral root formation and inorganic N uptake. Subsequent studies indicated that, compared to the NH_4^+ supply, NO produced by the NR pathway under NO_3^- supply further induces seminal root elongation by regulating auxin transport (Sun et al. 2018a). In addition, when the N-starved maize plant is resupplied with NO_3^-, the promotion rate of root elongation is also dependent on NO function. When the NR inhibitor (tungstate) was added externally, no root elongation was observed in NO_3^--fed plants. However, adding SNP to N-starved plants can increase root elongation rate (Manoli et al. 2014).

Strigolactones

Strigolactones (SLs) are a class of carotenoid-derived hormones which have multiple functions in plant growth and development (Waters et al. 2017). SLs were originally identified as highly active germination stimulants of parasitic plant seeds (Cook et al. 1966), and subsequent studies found that SLs are closely related to plant branching (Waldie et al. 2014; Conn et al. 2015). They can be synthesized in both roots and shoots (Kretzschmar et al. 2012; Hou et al. 2016). In *Arabidopsis* SL-deficient mutants (*max1* and *max4*) or the SL-insensitive mutants (*max2*), the length of the primary root was significantly shorter, and the number of cells in the meristem zone was significantly reduced. Exogenous application of low concentrations of the SL analog GR24 increased the number of cells in the meristem zone and promoted the length of the primary root in the wild-type and SL-deficient mutants, but had no effect on the SL-insensitive mutants. High concentrations of GR24 inhibited the elongation of the primary root, reducing the intensity of PIN1, PIN3 and PIN7 green fluorescent protein in the root, indicating that SLs regulate the growth of the primary root by controlling the flux of auxin (Ruyter-Spira et al. 2011). Biosynthesis and exudation of SLs are increased under N deficiency (Marzec et al. 2013; Yoneyama et al. 2013). Yoneyama et al. (2015) found that shoot-derived signals other than auxin are involved in systemic regulation of SL production in roots, but the nature of the shoot-derived signals requires further study. In rice, N deficiency induction of root elongation is explained by the increased production of SLs, mediated by increasing expression of the SL biosynthesis genes D10, D17, and D27 (Sun et al. 2014). Exogenous application of GR24 restores the root phenotypes of SL-deficient mutants (*d10* and *d27*), with no effect on the SL-insensitive mutants (*d3*). SLs can also act to inhibit the elongation of the primary root (Manoli et al. 2016). In maize, recovery of the NO_3^- supply after N deficiency results in elongation of the primary root within 2 h, concurrent with decreased expression of the genes involved in the biosynthesis and transport of SLs in the root transition zone (TZ). The application of a SL biosynthesis inhibitor (TIS108) promoted root elongation of N-deficient plants.

SLs and NO can synergistically regulate root elongation under N stress (Sanz et al. 2015). In SL mutants *d3* and *d10*, increased NO levels in the apical meristem zone did not result in root elongation. Application of abamine (strigolactone inhibitor) reduced SNP-induced root elongation. NO induced degradation of D53, an inhibitor of the SL signaling pathway. This suggests that the elongation of rice seminal roots is induced by NO under N deficiency, and that NO is dependent on the participation of SLs (Sun et al. 2016).

Brassinosteroids

Brassinosteroids (BRs) are sterol growth-promoting hormones in plants, where effects include promoting cell elongation, affecting cell division and differentiation, promoting the development of reproductive organs, regulating morphogenesis, increasing resistance to stress, increasing biological yield, and interacting with other signals to regulate growth and development processes (Fariduddin et al. 2014). Low concentrations of BRs promote root growth, while high concentrations of BRs inhibit root growth (Roddick et al. 1993; Clouse et al. 1996; Müssig et al. 2003; Wei and Li 2016). In the *Arabidopsis* BR-insensitive mutant *bri1-116* (Li and Chory 1997), the BR over-responsive mutant *bes1-D* (Yin et al. 2002), and transgenic *Arabidopsis* plants overexpressing BRI1-GFP (Friedrichsen et al. 2000), the length of the primary root is shorter than that of the wild type. BRs affect the balance of division and differentiation of apical meristem cells, which in turn affects the size of the root meristem. BRs affect the size of the meristematic tissue area by affecting the cell cycle progression in the primary root meristem region, remodeling of quiescent central cells, and the differentiation of distal stem cells (González-García et al. 2011). BRs can alter the polar transport of auxin and its distribution in roots (Bao et al. 2004; Chaiwanon and Wang 2015). Auxin-induced genes are also induced by BRs (Goda et al. 2004; Nemhauser et al. 2004). BRs interact synergistically with auxin in controlling root elongation (Vragović et al. 2015). Recently, in *Arabidopsis*, Jia et al. (2019) found that low N specifically upregulates the transcriptional level of the BR co-receptor BAK1 to activate BR signaling and stimulate primary root elongation.

Conclusions and perspectives

Root elongation allows plants to build sound root system architecture and, therefore, is an important adaptive response to low-N environments. Given the complexity of the response, it is to be expected that multiple signaling pathways are involved in the regulatory network. These varied pathways fine-tune root elongation in response to soil N availability. Based on current knowledge, we propose a model to explain how different signaling pathways interact to regulate root elongation in response to low-N stress (Fig. 5). In an N-deficient plant, a low-N status signal in the shoot leads to increased transport of auxin from the shoot to the root (Mi et al. 2008; Tian et al. 2008). Increased auxin level in the root tip accelerates cell division by stimulating the production of NO (Zhao et al. 2007; Manoli et al. 2014), which in turn stimulates the synthesis of SLs (Sun et al. 2016). Root elongation can also be stimulated by NO derived from NO_3^- reduction. Auxin may also directly stimulate cell elongation by acidifying apoplast pH in the root elongation zone (Rayle et al. 1992). Both cell division and elongation contribute to the enhanced root elongation. The low-N status signal also upregulates the transcriptional level

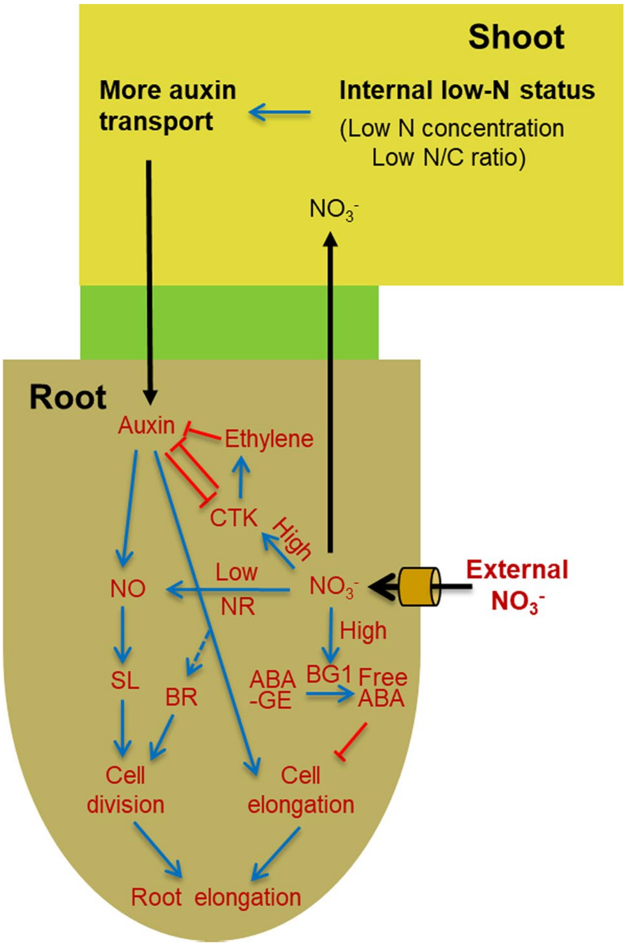

Fig. 5 A model explaining N-mediated axial root elongation. Reducing N supply leads to low internal N in the shoot, enhancing shoot-to-root auxin transport. In the root, auxin interacts with other hormonal pathways to regulate root elongation, occurring several days after low-N stress. Blue arrows, red blunted lines, and blue dashed lines indicate positive, negative, and synergistically effects, respectively. *N* nitrogen, *C* carbon, NO_3^- nitrate, *NR* nitrate reductase, *NO* nitric oxide, *BRs* brassinosteroids, *CTK* cytokinin, *SLs* strigolactones, *ABA* abscisic acid

of the BR co-receptor BAK1 to activate BR signaling and stimulate primary root elongation, possibly via a synergistic interaction with auxin (Jia et al. 2019). Under high N availability conditions in the rhizosphere, a large amount of N is absorbed into the roots. High N levels in the root leads to high CTK synthesis, which antagonizes the auxin pathway and inhibits root elongation (Tian et al. 2005; Mi et al. 2008). CTK can also induce ethylene production, which inhibits root elongation by affecting the transport of auxin in the roots (Chae 2003; Stepanova et al. 2007). Excessive N can increase the level of free ABA, which in turn inhibits cell elongation, leading to short roots (Pellizzaro et al. 2014). Therefore, a plant can effectively coordinate internal N status and external N signals and balance the different signaling pathways, and fine-tune root elongation.

The above physiological regulatory network helps to understand how root elongation is enhanced under low-N stress. However, the underlying molecular and genetic mechanisms remain to be discovered. Future research will identify the key regulatory genes which will enable improvement of the root elongation response to low-N stress in crop plants. Genotypic differences predict differential root elongation responses to low-N stress in maize (Chun et al. 2005; Tian et al. 2005; Wang et al. 2005), suggesting this trait can be improved genetically. A related QTL is associated with the response of maize root elongation to low-N stress (Liu et al. 2008). More work is required to identify the key QTLs, genes, and favorable alleles related to this response. By utilizing new generation gene discovery tools such as genome-wide association studies (GWAS) and genetic modification technologies (e.g., CRISPR/Cas9), new cultivars with greater root elongation ability will be developed to increase N use efficiency and achieve more sustainable development in agriculture (Hirel et al. 2011).

Materials and methods

Plant materials and growth conditions

Seeds of the maize inbred line Ye478 were surface sterilized in 10% (v/v) H_2O_2 for 40 min, subsequently washed with deionized water three times and then soaked for 8 h in saturated $CaSO_4$ solution. Seeds were then placed between sheets of filter paper soaked in deionized water and germinated in the dark at room temperature (20–22 °C). When the roots were approximately 2 cm long (about 1 day), uniform seedlings were placed approximately 2 cm below the top edge of the filter paper, covered with another piece of wet filter paper. The paper was rolled and placed into a plastic container filled with distilled water to allow the seeds to continue growing. When the seedlings had one expanded leaf, the endosperm was removed, then seedlings were transferred into porcelain pots (5 seedlings per pot) containing 2 L of nutrient solution. Hydroponic experiments were conducted in a growth chamber with the photosynthetic photon flux density of 300 μmol m^{-2} s^{-1} at canopy height, day/night temperature of 28/22 °C, and 60% relative humidity. The basic nutrient solution contained 0.75 mM K_2SO_4, 0.25 mM KH_2PO_4, 0.1 mM KCl, 0.65 mM $MgSO_4$, 0.13 mM EDTA–Fe, 1.0 μM $ZnSO_4$, 1.0 μM H_3BO_3, 1.0 μM $MnSO_4$, 0.1 μM $CuSO_4$, and 0.005 μM $(NH_4)_6Mo_7O_{24}$. Maize seedlings were supplied with NO_3^- concentrations of 0, 0.05, 0.5, 5, 10, 25, and 50 mM provided as $Ca(NO_3)_2$, and the Ca^{2+} concentration in N treatments lower than 5 mM was adjusted to the same level as that of the 5 mM N treatment by the supply of $CaCl_2$. The pH value of the solution was adjusted to 6.0 using KOH and HCl. The nutrient solution was renewed every 3 days and was aerated continuously by a pump. The pots were randomly placed and their positions were changed frequently.

Root phenotype

Six uniform seedlings from each treatment were sampled 1–9 days after low N (0.05 mM) and high N (5 mM) treatments. At each harvest, the primary axial root length was measured. The primary root samples were then floated in water in a transparent plastic tray and scanned with an Epson V700 scanner. Scanned root images were analyzed using the WinRHIZO software package (Pro 2014b, Canada). Total primary root length minus the primary axial root length was used to obtain the primary lateral root length.

Lanolin paste treatment of NPA

Each time the nutrient solution was changed, lanolin paste containing NPA was smeared uniformly around the junction between the shoot and the root. When preparing the lanolin paste blend, NPA was dissolved in dimethyl sulfoxide (DMSO) to make a mother liquor, and the mother liquor was thoroughly mixed into heated lanolin paste. The final working concentrations of NPA were 1, 10, 100, 1000, and 10,000 μM. Control plants were treated with lanolin paste without NPA. Six uniform seedlings from each treatment were sampled 9 days after low N (0.05 mM), high N (5 mM), and NPA treatments. At each harvest, the primary axial root length was measured.

Statistical analysis

Data were analyzed with ANOVA in SPSS Statistics 19.0 (SPSS Inc., Chicago, IL, USA). Differences were compared using the least significant difference test at the 0.05 level of probability. The data were then mapped with GraphPad Prism 7 software.

Author contribution statement XS wrote the initial draft of the manuscript; FC and GM helped with the literature review; LY and GM oversaw the preparation of the manuscript; FC, LY, and GM contributed to the various revisions of the manuscript.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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