An Acad Bras Cienc (2020) 92(3): e20190254 DOI 10.1590/0001-3765202020190254
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

AGRARIAN SCIENCES

Low auxin sensitivity of *diageotropica tomato* mutant alters nitrogen deficiency response

LUIZ C.N. DOS SANTOS, LUCAS A. GAION, RENATO M. PRADO,
RAFAEL F. BARRETO & ROGÉRIO F. CARVALHO

Abstract: Plant responses to nitrogen supply are dependent on auxin signaling, but much still remains to be elucidated regarding N deficiency in tomato. Thus, the objective of this work was to evaluate how low auxin sensitivity regulates the responses of tomato plants to N deficiency. For this purpose, we used the tomato *diageotropica* mutant, with low auxin sensitivity, and a near isogenic line cv. Micro-Tom grown in nutrient solutions under absence and presence of nitrogen. Plant height, stem diameter, root and shoot dry mass, area and root density, number of lateral roots, leaf area, chlorophylls and carotenoids content, nitrogen accumulation and nitrogen use efficiency were evaluated. We observed a clear interaction between the tomato genotype and nitrogen. When the plants were grown with nitrogen, 'Micro-Tom' showed higher growth than the *diageotropica* mutant. Under nitrogen deficiency condition, the mutant showed improved growth, nitrogen use efficiency and higher contents of pigments. In general, the low sensitivity to auxin in *diageotropica* caused reduced growth in both shoot and root. However, the *diageotropica* tomato showed a positive regulation of the nitrogen use efficiency under nitrogen deficiency. In general, our data revealed that the reduced sensitivity to auxin increased the adaptive capacity to the nitrogen deficiency.

Key words: auxin, cyclophilin protein, plant hormone, plant nutrition, *Solanum lycopersicum* L.

INTRODUCTION

Nitrogen (N) deficiency (-N) is a limiting factor in plant growth and development (Koohkan & Maftoun 2016). Further, low N availability induces a sequence of signaling cascade events stimulating complex molecular changes, evolving to subcellular modifications that lead to cellular alterations and tissue disorganization (Kiba & Krapp 2016). Additionally, an increase in N use efficiency (NUE) is observed in plants under -N conditions, which is dependent on changes in root architecture, N uptake and root-to-shoot transport and also N utilization (Garnett et al. 2009, Xu et al. 2012, Zhu et al. 2015). These adaptive responses involve countless molecules transported long-distance to mediate organ-to-organ communication, including peptides, microRNAs and plant hormones (Okamoto et al. 2013, Tabata et al. 2014, Nguyen et al. 2015). Among those molecules, plant hormones have a crucial role controlling uptake and transport of ions and in morphophysiological responses to nutrient cues (Krouk 2016).

Plant hormones can modulate the activity of high affinity nitrate transporters, induce enzyme activity that facilitates nutrient remobilization from organic or inorganic sources and stimulate the growth of organs, such as roots, directly involved in nutrient acquisition (Bittsánszky et al. 2015, Ferraro et al. 2015, Li et al. 2018). Furthermore, auxins (AUXs) are one of the

most prominent phytohormones involved in plant responses to -N (Ma et al. 2014). When produced in the shoot, AUXs can be transported long-distance and control the development of the root system, including lateral root (LR) formation (Ivanchenko et al. 2015). Nevertheless, accumulated NO_3^- in the shoot can reduce root branches by inhibition of AUX synthesis or its transport to the root (Krouk 2016). This N effect on AUX translocation and accumulation in the root has been observed in different species, such as *Brassica caulorapa* (Avery et al. 1937), *Glycine max* (Caba et al. 2000), *Triticum aestivum* (Chen et al. 1998), *Ananas comosus* (Tamaki & Mercier 2007), *Zea mays* (Liu et al. 2010) and *Arabidopsis thaliana* (Krouk et al. 2010, Mounier et al. 2014, Yang et al. 2015). Together, these findings reveal the complexity of hormonal crosstalk and underlying mechanisms regulating plant growth under nutritional deficiency.

The use of mutant plants has been an interesting tool in order to better understand the relationship between AUXs and N supply (Krouk et al. 2010). For example, the Arabidopsis knockout mutant for *NPF6.3* (*NRT1.1*), a nitrate high affinity transporter with dual affinity, showed accumulation of AUXs in LRs and an increase in LR growth under low N availability (Krouk et al. 2010, Wang et al. 2018). The authors propose that the NPF6.3 represses LR growth by promoting basipetal AUX transport from these roots, at least under -N conditions. However, the molecular mechanisms through which the nitrate transporter affects the distribution of AUXs when NO_3^- is absent require further studies. Additionally, AUXs can be a key factor in the NO_3^- signaling pathway mediating the adaptive response of plants to soil NO_3^- availability (Mounier et al. 2014, Bouguyon et al. 2015). Recently, the putative N sensor NRT2.1 was identified, which apparently controls root development in response to N availability; however, the physiological and biochemical changes involved in N perception by NRT2.1 are not fully elucidated (Jacquot et al. 2017).

Moreover, the interaction between N and AUXs remains poorly understood largely because the AUX signaling pathway shows a complex regulatory network dependent on intricate mechanisms of AUX transport and perception. For example, the tomato *diageotropica* (*dgt*) mutant, with low AUX sensitivity due to a single mutation in the *Cyclophilin1* gene, exhibits a pleiotropic phenotype that includes lack of geotropism, abnormal xylem structure, elevated shoot-to-root ratio and particularly, lack of LRs (Ivanchenko et al. 2015, Spiegelman et al. 2017). The mutation of a member of the Aux/IAA protein family, transcriptional repressors of AUX-mediated gene expression, can recover the capacity of *dgt* plants to initiate LRs (Ivanchenko et al. 2015). Likewise, the grafting between *dgt* tomato and the wild type restored the normal development of shoot and root (Ivanchenko et al. 2015). This last result demonstrates the existence of a mobile signal regulating the AUX responses, possibly dependent on a PIN-FORMED protein, a family of AUX efflux transporters, suggesting that a cyclophilin protein is transported through the vascular bundles of the plant and regulates AUX transport/signaling (Ivanchenko et al. 2015, Spiegelman et al. 2017). As *dgt* plants are expected to show a differential response to N supply, using the *dgt* mutant can be an important tool to study the relation between AUXs and N metabolism. In this work, we grow *dgt* and tomato cv. Micro-Tom (MT) plants under N sufficiency or deficiency, to provide breakthroughs about the underlying mechanisms implicated in the interactions between AUXs signaling and N uptake and use.

MATERIALS AND METHODS

Experimental design and treatments

To evaluate the role of the AUX hormones in response to -N, a completely randomized design was used, with six replicates in a 2 x 2 factorial scheme, corresponding to two tomato genotypes (*dgt* and MT) grown in the presence (+N) and in the absence of N (-N) in nutrient solution. In the +N treatments, 5 mM nitrate was supplied via calcium nitrate. Ca was balanced for all treatments with calcium chloride. Each experimental unit consisted of four potted tomato plants.

Plant material and growth conditions

The *dgt* mutant tomato, which presents low sensitivity to AUXs due to a defective gene for the biosynthesis of a cyclophilin protein (Oh et al. 2006), and a near isogenic line cv. Micro-Tom were used. To propagate the genotypes, seeds were germinated in boxes containing a mixture of 1:1 (v/v) commercial pot mix (BioPlant, Brazil) and vermiculite, supplemented with 1 g dm^{-3} of an NPK formulation (10:10:10). The plants were grown in a growth chamber and 10 days after germination, the seedlings were transferred to polypropylene pots with 180 mL capacity filled with a nutrient solution of Hoagland and Arnon (1950) at 25% of the ionic strength. After 8 days of transplanting (DAT), the ionic strength was increased to 50%. From 16 DAT, half of the previously described plants were submitted to a period of 10 days without N, whereas the remaining plants continued to receive the same nitrate concentration until the end of the experiment. The pH value of the nutrient solution was controlled daily and maintained at 6.0±0.5 by adding solutions of NaOH and HCl (10 %).

Growth analysis

Plant height was obtained using a graduated ruler and the stem diameter was measured with a digital caliper at the height of the plant lap. The leaf area was measured using an Image Analysis System (Delta-T Devices, Cambridge, UK). For the dry matter evaluation, plants were separated into roots and shoots. Then, plant material was dried in a forced-air oven at 65 ± 5°C for 96 h to reach a constant weight. After drying, the dry weights of shoots and roots were determined using an analytical balance (Denver Instrument Company AA-200). Additionally, the sum of the shoot and root weights was obtained as the plant dry matter. In order to determine root area and root density, the Delta-T Devices LTD analysis system was used. The root system remained in methylene blue solution for approximately 2 minutes, and then the root was scanned using a Hewlett Packard 125C digitizer. The number of LR was counted using a magnifying glass (10×).

Physiological analysis

The root and shoot N contents were determined following the methods described by Bataglia et al. (1983), based on the classical Kjeldahl method. Taking into account the N content and the dry matter, the accumulation of N in roots and shoot (mg per plant) was calculated. From the accumulation of N in roots and shoot, the N use efficiency (NUE) was estimated (Fageria & Baligar 2005):

$$NUE = [(\text{dry matter of the organ})^2 / (\text{N accumulation in the organ})] \quad (1)$$

In addition, the components of NUE, the nitrogen uptake efficiency (NUpE) and the nitrogen utilization efficiency (NUtE) were also calculated according to Schneider-Canny et al. (2019):

$$Nav\ (mg) = [Nf\ (mg) + Nt\ (mg)] \quad (2)$$

$$NUpE\ (\%) = [Nup\ (g)/Nav\ (g)] \times 100 \quad (3)$$

$$NUtE\ (g.g^{-1}) = biomass\ (g)/Nup\ (g) \quad (4)$$

where, Nup is N accumulation in the plant biomass. The amount of N available (Nav) in the nutrition solution was quantified as the sum of the N from fertilizer applied (Nf) plus the N uptake by plant tissues (Nt) in pots without N supply.

For the leaf chlorophylls and carotenoids content, four 0.35 cm^2 disks were collected and conditioned in a 2 mL tube containing 1.5 mL of methanol. Then, the samples were shaken at 4°C for 48 h under low light conditions. Subsequently, leaf tissues were removed and the absorbance of the extraction solution containing the pigments was read at 663, 647, and 470 nm. Pigment concentrations were estimated according to (Lichtenthaler 1987) and expressed as µg cm^{-2}.

Statistical analysis

The results were submitted to analysis of variance (ANOVA) by the F-test, followed by Tukey's test ($P<0.05$), using the Sisvar software (Ferreira 2011).

RESULTS AND DISCUSSION

Based on clear evidence of interaction between N availability and AUX transport (Krouk et al. 2010), we used a tomato mutant (*dgt*) with low sensitivity to AUXs to further evaluate the underlying mechanisms of crosstalk between AUXs and N. The *dgt* tomato mutant containing a single mutation in the *Cyclophilin1* gene, which

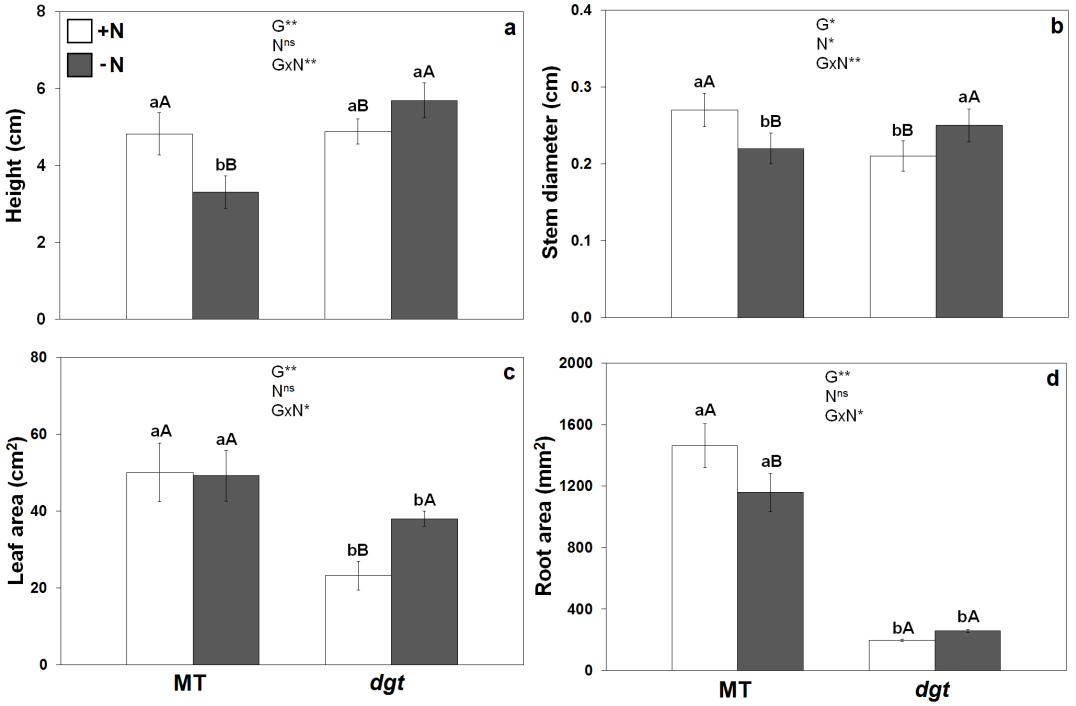

Figure 1 - Height (a), stem diameter (b), leaf area (c) and root area (d) of plants of two genotypes of tomato, MT (control) and *dgt*, grown in nutrient solution under deficiency (-N, dark gray bars) and sufficiency of nitrogen (+N, white bars). **; *; and ns = significant (P <0.01); significant (P <0.05); and not significant, respectively, by the F-test. Bars are the standard error of each treatment. Means followed by different uppercase letters differ in the presence of N within the genotypes MT and *dgt*; and for those followed by different lowercase letters, the genotypes differ in the same treatments, by Tukey's test (P ≤ 0.05).

triggers an auxin-insensitive response, exhibits a pleiotropic phenotype that includes the lack of geotropism, abnormal xylem structure, elevated shoot-to-root ratio and particularly, lack of LRs (Ivanchenko et al. 2015, Spiegelman et al. 2017). Thus, the *dgt* tomato mutant and a near isogenic line (MT) were grown in the presence (+N) and absence (-N) of N.

Under +N condition, *dgt* and MT plants exhibited similar height (Figure 1a). However, *dgt* plants showed reduced stem diameter and leaf and root area in comparison with MT when grown under +N condition (Figure 1b, c, d). Indeed, less growth of the *dgt* mutant was reported relative to MT plants. For example, the reduced leaf area observed in *dgt* is apparently associated with the small area of *dgt* epidermal cells (Carvalho et al. 2011). These results are consistent with the role of AUXs on cell growth processes, such as cell division and expansion (Saini et al. 2017). Actually, Ivanchenko et al. (2015) demonstrated that the absence of functional cyclophilin proteins, encoded by the *diageotropica* gene, modifies the AUX distribution pattern, negatively affecting the growth of the entire plant. The cyclophilin protein can act as a long-distance mobile signal regulating PIN-FORMED AUX efflux carrier (Ivanchenko et al. 2015, Spiegelman et al. 2015, 2017). When grown under -N condition, as expected, MT plants exhibited reductions in height, stem diameter and root area compared with the corresponding +N samples (Figure 1). However, we verified that the height, stem diameter and leaf area of the *dgt* mutant increased when the plants were cultivated without N supply compared with those in the +N condition (Figure 1a, b, c). Under -N, the height and stem diameter were greater in *dgt* than in MT plants (Figure 1a, b).

When adequately supplied with N, MT plants had higher root density, dry mass of the shoot (DMS), roots (DMR) and whole plant (DMWP) and number of LR than those of the *dgt* mutant (Figure 2). Although -N caused reductions in root density, DMS, DMR and DMWP in MT plants, the low AUX sensitivity *dgt* plants showed increased DMS, DMWP and LR under -N condition compared with those in the corresponding +N samples (Figure 2). These results differ from those reported previously, in which nitrogen deficiency rapidly reduced plant growth (Petropoulos et al. 2008, Reddy & Matcha 2010, Zhu et al. 2014). However, despite the positive response of *dgt* to -N, the root density, DMR and LR were lower than MT plants (Figure 2).

Thus, the increase in number of LRs in the *dgt* mutant under -N condition, compared with that in plants that received N, diverges from literature results in which main root growth increases and LRs are inhibited under low N availability (Vidal & Gutiérrez 2008, Krouk et al. 2010, Giehl et al. 2014). Based on the current knowledge, *NPF6.3* genes repress the accumulation of AUXs in the LR apices of Arabidopsis plants cultivated under low NO_3^- concentration (Krouk et al. 2010). However, the question of how the NO_3^- carrier can affect the localization of the hormone remains unanswered. Thus, the complexity of the interaction between AUXs and NO_3^- increases based on the opposite response observed in the *dgt* under -N conditions. In fact, root growth and development are well known to be under intricate control of hormonal signaling. Further, it is not surprising that NO_3^- also controls the biosynthesis and transport of the hormone (Krouk 2016). This result obtained under -N condition is unprecedented and even more surprising (Figure 2e) because increased production of LRs for the *dgt* tomato was not observed even with the application of exogenous AUXs (Ivanchenko et al. 2006, 2015). Indeed, Ivanchenko et al. (2015) confirm that the *dgt* mutation causes changes related to the transport of AUXs as well as the lack of a component of its signaling pathway.

The *dgt* mutant naturally showed dark green leaves, indicating high chlorophyll content (Coenen & Lomax 1998) (Figure 3). However, compared with MT, the increased pigmentation in *dgt* mutant might be a consequence of reduced cell area caused by the AUX mutation (Mignolli et al. 2012). Thus, although leaf chlorosis is one of the most evident symptoms of -N, we propose that the increase in chlorophyll retention in *dgt* plants under -N was due to reduced cell expansion. Nevertheless, the metabolism of the *dgt* mutant under -N remains of interest as the mutant did not present symptoms of nutritional deficiency at the tissue level.

Under +N condition, we observed a lower N content and NUE in *dgt* plants in both shoot and root as compared to MT plants (Figure 4). Moreover, MT plants exposed to -N condition showed reduced N accumulation and NUE, independently of the organ (Figure 4). However, although no difference was observed in root N accumulation, the NUE of *dgt* plants increased under -N condition compared with that of the *dgt* genotype under +N condition. Despite this result, the N accumulation and shoot NUE were higher in MT plants than those in the *dgt* mutant even under -N, and only root NUE was higher in *dgt* plants (Figure 4). Similarly to NUE, the *dgt* plants exhibited lower NUpE in comparison to MT plants when grown under +N, whereas both genotypes showed pronounced increase of NUpE under -N condition (Figure 4e). On the other hand, the *dgt* plants had an increased NUtE when compared to MT plants under -N condition (Figure 4f).

Because increases in root area and density are two important root traits for N acquisition (Ju et al. 2015), the decrease in NUE of MT plants after exposure to -N could be associated with a reduction in the root density (Figure 2a). However, Abenavoli et al. (2016) studying the NUE in different tomato genotypes reports that NUE may be more dependent on N utilization than N uptake from the soil. In fact, this dependence on N utilization was also observed herein, especially on *dgt* plants under -N (Figure 4).

The nitrogen deficiency generally results in a decrease of plant growth, and the limitation is often associated with the acceleration of leaf senescence (Flores et al. 2016, Koohkan & Maftoun 2016, Yong et al. 2010). The reduced accumulation of N in *dgt* (Figure 4a, b) might be related to the role of AUXs. In fact, Li et al. (2018) showed that the application of exogenous AUX increased the N content and AUX inhibitors decreased the amount of N absorbed in rice plants. Therefore, the increased growth of the *dgt* mutant under the N deficient condition showed that this mutation apparently allowed a different development strategy under low N availability (Figures 1 and 2). Apparently, *dgt* plants are able to assimilate a greater amount of carbon per unit of N absorbed under N deficiency condition when compared to MT (Figure 4); although the molecular mechanisms related to this response have not yet been elucidated.

Therefore, this hormonal and nutrient crosstalk requires better elucidation, particularly the intricate mechanisms through which N modulates AUX signaling and vice-versa. Thus, the question is raised of how the AUX signaling modulates plants response to N supply. The results presented in this work can greatly contribute to the advance of modern agriculture, demonstrating an increase in productivity using less nitrogen fertilizer and improving the NUE of crops such as tomato. In the present work, the differences in N accumulation, plant growth, N utilization efficiency, and root architecture exhibited by AUX tomato mutant were postulated to be directly attributed to disruption of the AUX signaling pathway. In the +N condition, the reduced sensitivity to AUX in tomato plants resulted in less root and shoot growth and of the

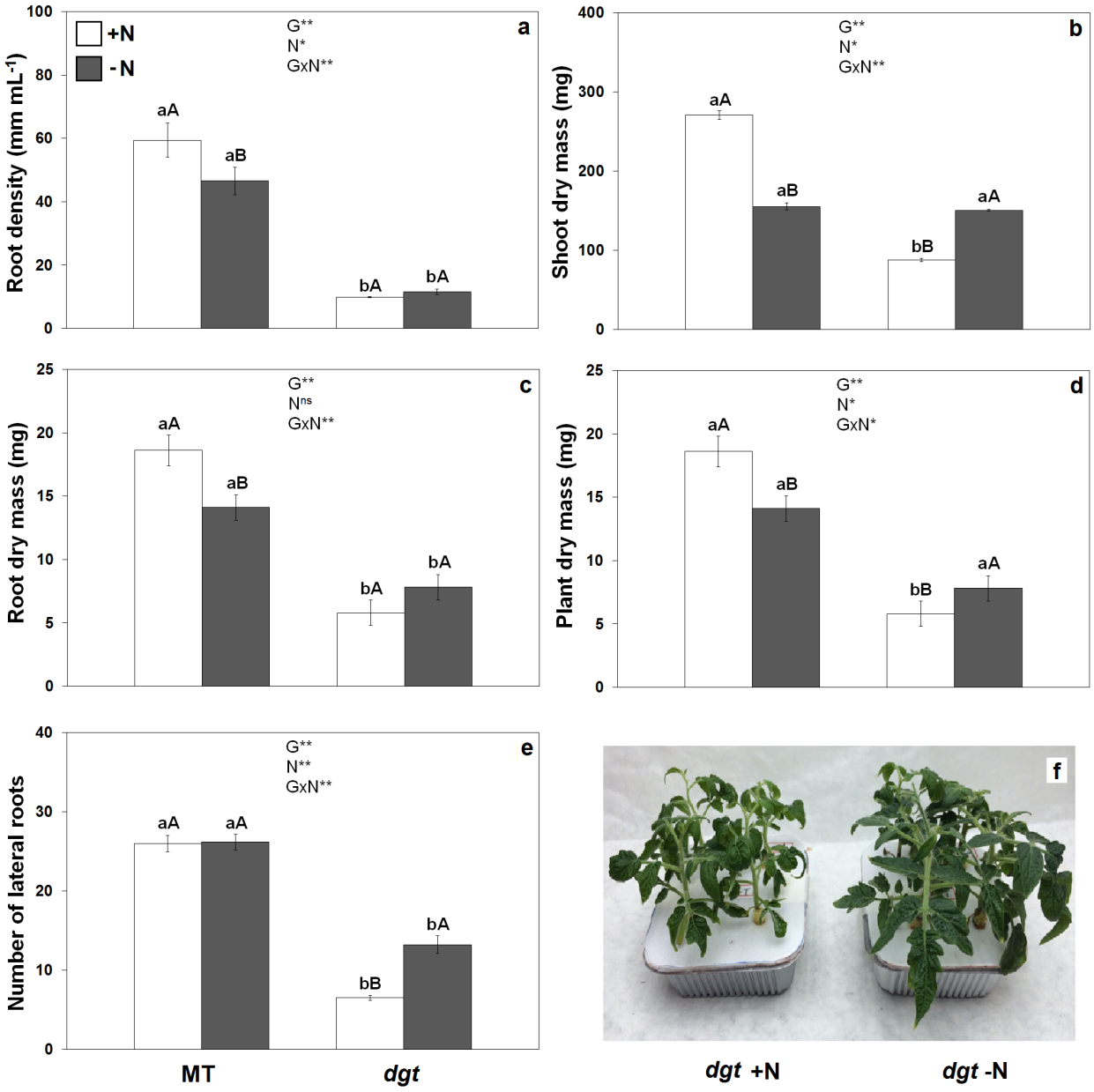

Figure 2 - Root density (a), shoot dry mass (b), root dry mass (c), total plant dry mass (d) and number of lateral roots (e) of plants of two genotypes of tomato, MT (control) and *dgt* (low sensitivity to AUXs), grown in nutrient solution under nitrogen deficiency (-N, dark gray bars) and sufficiency of nitrogen (+N, white bars). Note that *dgt* shows a vigorous appearance in -N when compared with *dgt* + N (f). **; *; and ns = significant (P <0.01); significant (P <0.05); and not significant, respectively, by the F-test. Bars are the standard error of each treatment. Means followed by different uppercase letters differ in the presence of N within the genotypes MT and *dgt*; and for those followed by different lowercase letters, the genotypes differ in the same treatments, by Tukey's test (P ≤ 0.05).

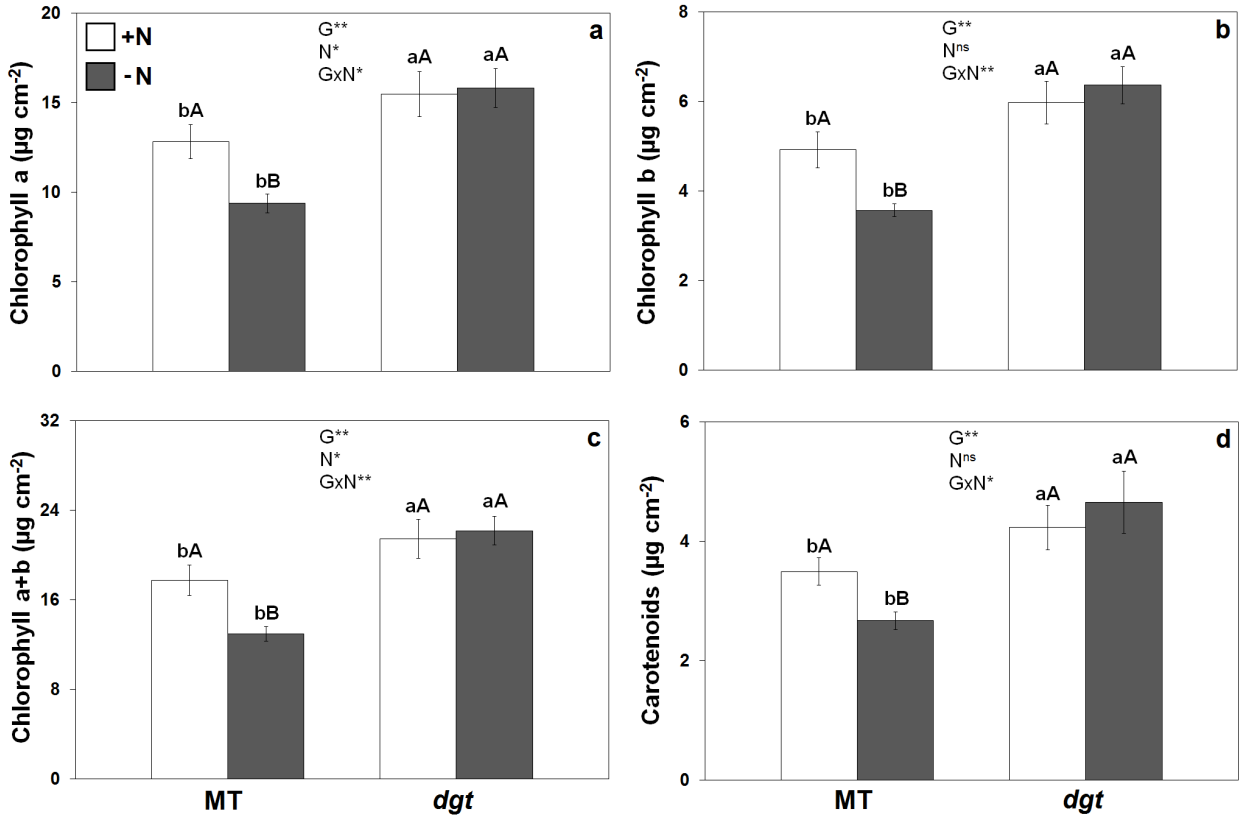

Figure 3 - Chlorophyll *a* (a), chlorophyll *b* (b), chlorophyll *a* + *b* (c) and carotenoids (d) of plants of two genotypes of tomato, MT (control) and *dgt* (low sensitivity to AUXs), grown in nutrient solution under nitrogen deficiency (-N, dark gray bars) and sufficiency of nitrogen (+N, white bars). **; *; and ns = significant (P <0.01); significant (P <0.05); and not significant, respectively, by the F-test. Bars are the standard error of each treatment. Means followed by different uppercase letters differ in the presence of N within the genotypes MT and *dgt*; and for those followed by different lowercase letters, the genotypes differ in the same treatments, by Tukey's test (P ≤ 0.05).

whole plant (Figures 1 and 2). However, under -N supply in the nutrient solution, *dgt* tomato exhibited positive modifications in N nutrition (Figure 2f), with an increase in N accumulation and dry matter production and NUE.

CONCLUSIONS

In short, the results demonstrated an important role of AUX signaling on N deficiency responses. In fact, although the relationship between N starvation and auxin signaling and metabolism is still an initial matter, there is a specific modulation of N accumulation and use efficiency under N deficiency that results in a positive adjustment of plant growth. Recently Nadeem et al. (2018) have found in Foxtail Millet (*Setaria italica*) that low N led to lower chlorophyll contents and N concentrations, but higher root/shoot and C/N ratios and N utilization efficiencies under hydroponic culture. These results indicate that the modification of AUX signaling could be used as an alternative to cope with reduced concentrations of N in the growing conditions for tomato plants, because the plants are capable of changing the root architecture and N utilization to improve the NUE (Zhu et al. 2015). However, the molecular issue

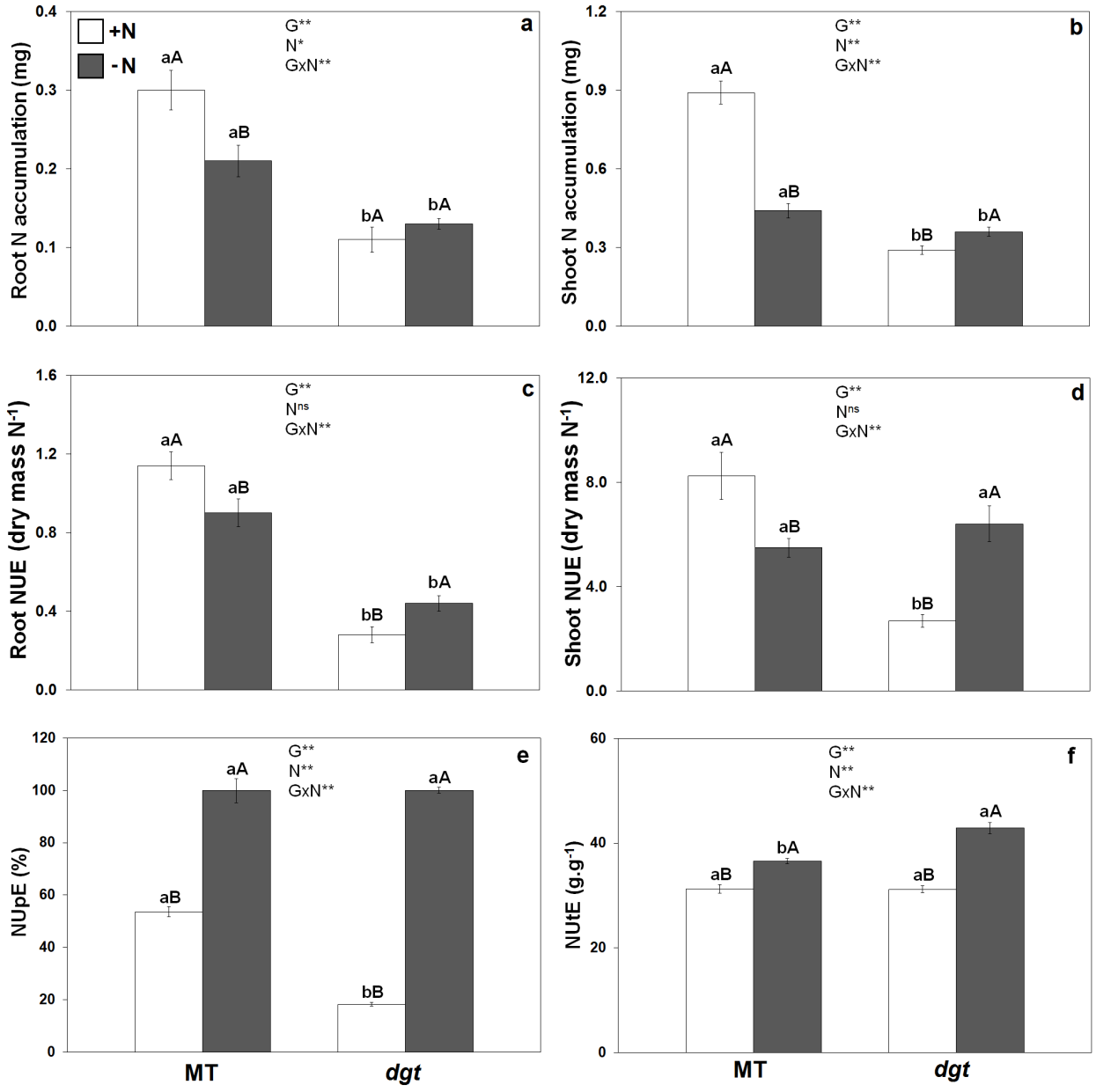

Figure 4 - Nitrogen accumulation in root (a) and shoot (b), nitrogen use efficiency (NUE) in root (c) and shoot (d), nitrogen uptake efficiency (e) and nitrogen utilization efficiency (f) of plants of two genotypes of tomato, MT (control) and *dgt* (low sensitivity to AUXs), grown in nutrient solution under N deficiency (-N, dark gray bars) and sufficiency of nitrogen (+N, white bars). **; *; and ^{ns} = significant (P <0.01); significant (P <0.05); and not significant, respectively, by the F-test. Bars are the standard error of each treatment. Means followed by different uppercase letters differ in the presence of N within the genotypes MT and *dgt*; and for those followed by different lowercase letters, the genotypes differ in the same treatments, by Tukey's test (P ≤ 0.05).

of these responses remains to be elucidated. Certainly, this is an enormous potential for the manipulation of AUX sensitivity in crop plants in order to maximize the use of N fertilizers.

Acknowledgments

We are grateful to the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) for the direct or indirect support provided.

REFERENCES

ABENAVOLI MR, LONGO C, LUPINI A, MILLER AJ, ARANITI F, MERCATI F, PRINCI MP & SUNSERI F. 2016. Phenotyping two tomato genotypes with different nitrogen use efficiency. Plant Physiol Biochem 107: 21-32.

AVERY GS, BURKHOLDER PR & CREIGHTON HB. 1937. Nutrient deficiencies and growth hormone concentration in Helianthus and Nicotiana. Am J Bot 24: 553-557.

BATAGLIA OG, FURLANI AMC, TEIXEIRA JPF, FURLANI PR & GALLO JR. 1983. Métodos de análises químicos de plantas. Instituto Agronômico, Campinas.

BITTSÁNSZKY A, PILINSZK K, GYULAI G & KOMIVES T. 2015. Over coming ammonium toxicity. Plant Sci 231: 184-190.

BOUGUYON E, BRUN F, MEYNARD D, KUBEŠ M, PERVENT M, LERAN S & HOYEROVÁ K. 2015. Multiple mechanisms of nitrate sensing by Arabidopsis nitrate transceptor NRT1. 1. Nat Plants 1: 15015.

CABA JM, CENTENO ML, FERNÁNDEZ B, GRESSHOFF PM & LIGERO F. 2000. Inoculation and nitrate alter phytohormone levels in soybean roots: differences between a supernodulating mutant and the wild type. Planta 211: 98-104.

CARVALHO RF, CAMPOS ML, PINO LE, CRESTANA SL, ZSÖGÖN A, LIMA JE, BENEDITO VA & PERES LEP. 2011. Convergence of developmental mutants into a single tomato model system: 'Micro-Tom' as an effective toolkit for plant development research. Plant Methods 7(1).

CHEN JG, CHENG SH, CAO W & ZHOU X. 1998. Involvement of endogenous plant hormones in the effect of mixed nitrogen source on growth and tillering of wheat. J Plant Nutr 21: 87-97.

COENEN C & LOMAX TL. 1998. The *Diageotropica* gene differentially affects auxin and cytokinin responses throughout development in tomato. Plant Physiol 117: 63-72.

FAGERIA NK & BALIGAR VC. 2005. Enhancing nitrogen use efficiency in crop plants. Adv Agron 88: 97-185.

FERRARO G, D'ANGELO M, SULPICE R, STITT M & VALLE EM. 2015. Reduced levels of NADH-dependent glutamate dehydrogenase decrease the glutamate content of ripe tomato fruit but have no effect on green fruit or leaves. J Exp Bot 66: 3381-3389.

FERREIRA DF. 2011. Sisvar: a computer statistical analysis system. Ciência e Agrotecnologia 35: 1039-1042.

FLORES RA, BORGES BMMN, ALMEIDA H & PRADO RDM. 2016. Growth and nutritional disorders of coffee cultivated in nutrient solutions with suppressed macronutrients. J Plant Nutr 39: 11578-1588.

GARNETT T, CONNECTICUT V & KAISER BN. 2009. Root based approaches to improving nitrogen use efficiency in plants. Plant Cell Environ 32: 1272-1283.

GIEHL RFH, GRUBER BD & VON WIRÉN N. 2014. It's time to make changes: modulation of root system architecture by nutrient signals. J Exp Bot 65: 769-778.

HOAGLAND DR & ARNON DI. 1950. The water-culture method for growing plants without soil. - Circular. Calif Agric 347.

IVANCHENKO MG, COFFEEN WC, LOMAX TL & DUBROVSKY, J.G. 2006. Mutations in the *Diageotripica* (*dgt*) gene uncouple patterned cell division during lateral root initiation from proliferative cell division in the pericycle. Plant J 46: 436-447.

IVANCHENKO MG ET AL. 2015. The cyclophilin A DIAGEOTRIPICA gene affects auxin transport in both root and shoot to control lateral root formation. Development 142: 712-721.

JACQUOT A, LI Z, GOJON A, SCHULZE W & LEJAY L. 2017. Post-translational regulation of nitrogen transporters in plants and microorganisms. J Exp Bot 68: 2567-2580.

JU C, BURESH RJ, WANG Z, ZHANG H, LIU L, YANG J & ZHANG J. 2015. Root and shoot traits for rice varieties with higher grain yield and higher nitrogen use efficiency at lower nitrogen rates application. Field Crops Res 175: 47-55.

KIBA T & KRAPP A. 2016. Plant nitrogen acquisition under low availability: regulation of uptake and root architecture. Plant Cell Physiol 57: 707-714.

KOOHKAN H & MAFTOUN M. 2016. Effect of nitrogen-boron interaction on plant growth and tissue nutrient concentration of canola (*Brassica napus* L.). J Plant Nutr 39: 922-931.

KROUK G. 2016. Hormones and nitrate: a two-way connection. Plant Mol Biol 91: 599-606.

KROUK G ET AL. 2010. Nitrate-regulated auxin transport by NRT1.1 defines a mechanism for nutrient sensing in plants. Dev Cell 18: 927-937.

LI X, ZHOU J, XU RS, MENG MY, YU X & DAI CC. 2018. Auxin, cytokinin, and ethylene involved in rice N availability improvement caused by endophyte *Phomopsis liquidambari*. J Plant Growth Regul 37: 128-143.

LICHTENTHALER HK. 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods Enzymol 148: 350-382.

LIU J, AN X, CHENG L, CHEN F, BAO J, YUAN L, ZHANG F & MI G. 2010. Auxin transport in maize roots in response to localized nitrate supply. Ann Bot 106: 1019-1026.

MA W, LI J, QU B, HE X, ZHAO X, LI B, FU X & TONG Y. 2014. Auxin biosynthetic gene TAR2 is involved in low nitrogen mediated reprogramming of root architecture in Arabidopsis. Plant J 78: 70-79.

MIGNOLLI F, MARIOTTI L, LOMBARDI L, VIDOZ ML, CECCARELLI N & PICCIARELLI P. 2012. Tomato fruit development in the auxin-resistant *dgt* mutant is induced by pollination but not by auxin treatment. J Plant Physiol 169: 1165-1172.

MOUNIER E, PERVENT M, LJUNG K, GOJON A & NACRY P. 2014. Auxin-mediated nitrate signalling by NRT1.1 participates in the adaptive response of Arabidopsis root architecture to the spatial heterogeneity of nitrate availability. Plant Cell Env 37: 162-174.

NADEEM F, WANG R, HAN J, SHEN Q, CHANG F, DIAO X, ZHANG F & LI X. 2018. Foxtail Millet [*Setaria italica* (L.) Beauv.] grown under low nitrogen shows a smaller root system, enhanced biomass accumulation, and nitrate transporter expression. Front Plant Sci 9: 205.

NGUYEN GN, ROTHSTEIN SJ, SPANGENBERG G & KANT S. 2015. Role of microRNAs involved in plant response to nitrogen and phosphorous limiting conditions. Front Plant Sci 6: 629.

OH K, IVANCHENKO MG, WHITE TJ & LOMAX TL. 2006. The *diageotripica* gene of tomato encodes a cyclophilin: a novel player in auxin signaling. Planta 224: 133-144.

OKAMOTO S, SHINOHARA H, MORI T, MATSUBAYASHI Y & KAWAGUCHI M. 2013. Root-derived CLE glycopeptides control nodulation by direct binding to HAR1 receptor kinase. Nat Commun 4: 2191.

PETROPOULOS SA, OLYMPIOS CM & PASSAM HC. 2008. The effect of nitrogen fertilization on plant growth and the nitrate content of leaves and roots of parsley in the Mediterranean region. Sci Hortic 118: 255-259.

REDDY KR & MATCHA SK. 2010. Quantifying nitrogen effects on castor bean (*Ricinus communis* L.) development, growth, and photosynthesis. Ind Crops Prod 31: 185-191.

SAINI K, MARKAKIS MN, ZDANIO M, BALCEROWICZ DM, BEECKMAN T, VEYLDER L, PRINSEN E, BEEMSTER GTS & VISSENBERG K. 2017. Alteration in auxin homeostasis and signaling by overexpression of PINOID kinase causes leaf growth defects in *Arabidopsis thaliana*. Front Plant Sci 8: 1009.

SCHNEIDER-CANNY R, CHEKHOVSKIY K, MUÑOZ P, KWON S & SAHA MC. 2019. Characterization of bermudagrass (*Cynodon dactylon* L.) germplasm for nitrogen use efficiency. Euphytica 215: 40.

SPIEGELMAN Z, HAM B, ZHANG Z, TOAL T, BRADY S, ZHENG Y & FEI Z. 2015. A tomato phloem-mobile protein regulates the shoot-to-root ratio by mediating the auxin response in distant organs. Plant J 83: 853-863.

SPIEGELMAN Z, OMER S, MANSFELD BN & WOLF S. 2017. Function of Cyclophilin1 as a long-distance signal molecule in the phloem of tomato plants. J Exp Bot 68: 953-964.

TABATA R, SUMIDA K, YOSHII T, OHYAMA K, SHINOHARA H & MATSUBAYASHI Y. 2014. Perception of root-derived peptides by shoot LRR-RKs mediates systemic N-demand signaling. Sci 346: 343-346.

TAMAKI V & MERCIER H. 2007. Cytokinins and auxin communicate nitrogen availability as long-distance signal molecules in pineapple (*Ananas comosus*). J Plant Physiol 164: 1543-1547.

VIDAL EA & GUTIÉRREZ RA. 2008. A systems view of nitrogen nutrient and metabolite responses in Arabidopsis. Curr Opin Plant Biol 11: 521-529.

WANG W ET AL. 2018. Expression of the nitrate transporter gene *OsNRT1.1A/OsNPF6.3* confers high yield and early maturation in rice. Plant Cell 30: 638-651.

XU G, FAN X & MILLER AJ. 2012. Plant nitrogen assimilation and use efficiency. Annu Rev Plant Biol 63: 153-182.

YANG H, VON DER FECHT-BARTENBACH J, FRIML J, LOHMANN JU, NEUHÄUSER B & LUDEWIG U. 2015. Auxin-modulated root growth inhibition in *Arabidopsis thaliana* seedlings with ammonium as the sole nitrogen source. Funct Plant Biol 42: 239-251.

YONG JWH, NG YF, TAN SN & CHEW AYL. 2010. Effect of fertilizer application on photosynthesis and oil yield of *Jatropha curcas* L. Photosynthetica 48: 208-218.

ZHU J, HUI F, LI M, MA Y, YU H & JIANG W. 2015. Effect of different nitrogen concentrations on roots architecture and nitrogen use efficiency in potting tomato seedling. Transactions Chin Soc Agric Eng 31: 131-137.

ZHU Y, FAN X, XINCUN H, WU J & WANG T. 2014. Effect of different levels of nitrogen deficiency on switchgrass seedling growth. Crop J 2: 223-234.

How to cite
SANTOS LCN, GAION LA, PRADO RM, BARRETO RF & CARVALHO RF. 2020. Low auxin sensitivity of *diageotropica* tomato mutant alters nitrogen deficiency response. An Acad Bras Cienc 92: e20190254. DOI 10.1590/0001-3765202020190254.

Manuscript received on March 1, 2019;
accepted for publication on September 9, 2019

LUIZ C.N. DOS SANTOS[1]
https://orcid.org/0000-0001-6487-5517

LUCAS A. GAION[2]
https://orcid.org/0000-0003-4246-1975

RENATO DE MELLO PRADO[1]
https://orcid.org/0000-0003-1998-6343

RAFAEL F. BARRETO[1]
https://orcid.org/0000-0002-1170-5386

ROGÉRIO F. CARVALHO[3]
https://orcid.org/0000-0003-1270-7372

[1]Universidade Estadual Paulista (UNESP), Departamento de Solos e Adubos, Faculdade de Ciências Agrárias e Veterinária, Via de Acesso Prof. Paulo Donato Castellane, s/n, Zona Rural, 14884-900 Jaboticabal, SP, Brazil

[2]Universidade de Marília, Centro de Ciências Agrárias, Avenida Higino Muzzy Filho, 1001, Cidade Universitária, 17525-902 Marília, SP, Brazil

[3]Universidade Estadual Paulista (UNESP), Departamento de Biologia Aplicada à Agropecuária, Faculdade de Ciências Agrárias e Veterinária, Via de Acesso Prof. Paulo Donato Castellane, s/n, Zona Rural, 14884-900 Jaboticabal, SP, Brazil

Correspondence to: **Rogério Falleiros Carvalho**
E-mail: rogerio.f.carvalho@unesp.br

Author contributions

Conceived and designed the experiments: RMP, RFC; Performed the experiments: LCNS, RFB, LAG; Analyzed the data: LCNS, RFB, LAG; Drafted the manuscript: LCNS, LAG; Reviewed the manuscript and performed the final check: LAG, RMP, RFC.