proceedings

MDPI

Proceedings

Ectopic Lateral Root Branching in Fe-Deprived Maize Plants: Searching for the Genes Underpinning the Phenotype †

Yannis E. Ventouris, Sotiria-Theoklitia P. Protopappa, Aimilia-Eleni Nikolopoulou, Dimitris L. Bouranis and Styliani N. Chorianopoulou *

Plant Physiology and Morphology Laboratory, Crop Science Department, Agricultural University of Athens, Athens, Greece; yannisventouris@gmail.com (Y.E.V.); sotiriaprotopappa@gmail.com (S.-T.P.P.); mil1@aua.gr (A.-E.N.); bouranis@aua.gr (D.L.B.)

* Correspondence: s.chorianopoulou@aua.gr; Tel.: +30-2105294290

† Presented at the 1st International Electronic Conference on Plant Science, 1–15 December 2020; Available online: https://iecps2020.sciforum.net/.

Published: 1 December 2020

Abstract: Iron (Fe) is an essential element for plant growth and productivity, whilst human and animal diets rely on Fe from plant sources. Despite the large number of studies on plants' Fe deficiency responses, considerably less is known regarding the morphological and anatomical alterations the root system of plants undergoes, especially in the graminaceous plants following a chelation strategy to take up Fe^{3+} from the rhizosphere. A stress symptom observed in Fe-deprived maize plants is an ectopic lateral root branching occuring at the terminal 5 cm of the root. In order to understand this response, one-week-old maize seedlings were placed in containers with either full nutrient solution, or nutrient solution lacking a Fe source. Control and Fe-deprived plants were grown for another 14 days, and the trait of ectopic lateral root branching was observed both on roots that emerged before the onset of Fe deprivation, as well as on roots that emerged after the onset of the deprivation. Ongoing in silico analysis of a quantitative trait locus known to be related to this trait of maize grown under limited Fe, unveiled several genes coding for known and unknown proteins, as well as long intergenic non-coding RNAs.

Keywords: hydroponics; iron nutrition; lateral roots; root morphology; root phenomics; *Zea mays* L.

1. Introduction

In the soil, Fe is found in the form of Fe^{3+} (ferric iron) and Fe^{2+} (ferrous iron) and is highly dependent on the redox state of its environment. Organisms have developed specific mechanisms for Fe acquisition from the Fe-oxides (III): (i) protonation, (ii) chelation and (iii) reduction [1].

Eudicots and non-graminaceous monocots employ Strategy I to acquire iron from the soil. In this strategy, also known as reduction strategy, protons are pumped to the rhizosphere by activation of a plasma membrane H+-ATPase, which promotes the acidification of the soil solution and increases solubility of Fe^{3+} [2]. Subsequently, the ferric reductase enzyme, encoded by the Ferric Chelate Reductase gene, reduces Fe^{3+} to Fe^{2+} in the surface of the plasma membrane of root cells. After reduction, Fe is transported to the cytoplasm through Iron Regulated Transporters. Additionally, Strategy I plants were found to export an array of metabolites including organic acids, phenolics, flavonoids and flavins [3].

In Strategy II, also referred as chelation strategy, typical of the grass family (Poaceae), the roots release compounds called phytosiderophores. These molecules belong to the Mugineic Acid family (MAs) and can form stable complexes with Fe^{3+} in the rhizosphere. Deoxymugineic acid is the most abundant phytosiderophore and is exported by TOM1, the Transporter of Mugineic acid family phytosiderophores, in rice and barley [4]. The absorption of Fe^{3+}-phytosiderophore complexes is

performed by a Yellow Stripe/Yellow Stripe-Like transporter located at the plant root cells. It is well established that maize (*Zea mays* L.) follows this strategy for iron acquisition [5].

Iron is an essential element for plant growth and productivity, and it is necessary to understand the responses of plants to iron deficiency at both the physiological and morphological levels and reveal the molecular and genetic bases of these responses. Although there is a large number of studies available on plants' iron deficiency responses, satisfactory knowledge regarding the morphological and anatomical alterations in plant root systems is lacking, especially in the graminaceous plants. It seems that plants modify their root architecture by increased formation and branching of root hairs, root-tip swelling, and enhanced lateral root formation [6,7]. Especially for maize, a stress symptom observed in Fe-deprived plants was an ectopic lateral root branching at the terminal 5 cm of the root. In this study, control and Fe-deprived plants were grown for 3 weeks, and the trait of ectopic lateral root branching was observed both on roots that emerged before the onset of Fe deprivation (i.e., primary embryonic root, secondary embryonic roots), as well as on roots which developed after the onset of the deprivation (i.e., crown roots). Bioinformatic analysis of a quantitative trait locus known to be related to this trait of maize grown under limited Fe [6], resulted in a list of several genes coding for known and unknown proteins, with many transcription factors among them, as well as long intergenic non-coding RNAs.

2. Methods

2.1. Plant Growth Conditions and Treatments

Maize seeds (*Zea mays* L.) were placed on wet filter paper, in the dark for germination. Four days later, the most uniform plants were selected and maintained in a hydroponic batch culture for 3 days in well-aerated distilled water. On day 7 after sowing and for the next one week they were transferred in a hydroponic batch culture, and separated into two different treatments: in the first one, plants were set to grow in complete nutrient solution (C, 5 mM KNO_3, 1 mM KH_2PO_4, 2 mM $Mg(NO_3)_2$ $6H_2O$, 2.5 mM $CaSO_4$ $2H_2O$, 1 mM $MgSO_4$ $7H_2O$, 0.07 mM FeNaEDTA, 4 mM $Ca(NO_3)_2$ $4H_2O$, 0.9 μM $ZnCl_2$, 30 μM H_3BO_3, 0.9 μM $CuCl_2$, 0.5 μM MoO_3 85%, and 20 μM $MnCl_2$ $4H_2O$) while on the other, plants were subjected to iron deficient conditions (-Fe, 5 mM KNO_3, 1 mM KH_2PO_4, 2 mM $Mg(NO_3)_2$ $6H_2O$, 2.5 mM $CaSO_4$ $2H_2O$, 1 mM $MgSO_4$ $7H_2O$, 4 mM $Ca(NO_3)_2$ $4H_2O$, 0.9 μM $ZnCl_2$, 30 μM H_3BO_3, 0.9 μM $CuCl_2$, 0.5 μM MoO_3 85%, and 20 μM $MnCl_2$ $4H_2O$). All nutrient solutions were constantly aerated and replaced every 3 days, throughout the experiment. Growth conditions were 24/18 °C, relative humidity 40 %, 250 μmol photon m^{-2} s^{-1} and a 16-h photoperiod.

2.2. Samplings and Observations

Each experiment was repeated twice. Samplings (five replicates per sampling) took place both on day 7 prior to separation into the two nutrient solutions, as well as on days 14 and 21 after sowing. Root length, the percentage of root length covered with lateral roots, and the distance between the root tip and the point of lateral root emergence were measured in primary embryonic (PR), the seminal embryonic (SR), and crown (CR) roots on days 7, 14, 21 after sowing.

2.3. In Silico Analysis

Bioinformatic analysis was performed using the data from the Maize Genetics and Genomic Database (MGDB): https://www.maizegdb.org/ [8].

2.4. Statistical Analysis

In order to determine the significance of differences between samplings, the data were analyzed using the *t*-test variance analysis with two-tailed distribution and two-sample unequal variance in Microsoft Excel.

3. Results and Discussion

In Fe-deprived plants, the length of all root types examined was reduced compared to controls during the treatment. More specifically, the length of the primary root (PR) was reduced on day 21, whilst the respective lengths of the seminal (SR) as well as the crown (CR) roots were significantly decreased from day 14 onwards (Table 1). It is worth noting that on day 21, the roots of the Fe-deprived plants were almost half the length of these in control plants.

Table 1. Root length (cm, mean ± SE) of primary (PR), seminal (SR), and crown (CR) roots on days 7, 14, 21 after sowing. Asterisk (*) indicates statistically significant difference between -Fe treatment and the respective control at $p < 0.05$.

	Control			-Fe		
Days	**PR**	**SR**	**CR**	**PR**	**SR**	**CR**
7	11.23 ± 2.59	8.77 ± 1.84	0.00	11.23 ± 2.59	8.77 ± 1.84	0.00
14	33.27 ± 5.12	34.70 ± 4.63	15.27 ± 2.15	29.07 ± 4.71	23.17 ± 3.28 *	10.67 ± 1.25 *
21	60.33 ± 8.48	56.57 ± 6.91	38.67 ± 5.33	33.53 ± 5.79*	22.47 ± 4.35 *	14.47 ± 2.55 *

The observation of the root area covered with lateral roots revealed the presence of statistically significant differences between control and Fe-deprived roots both on days 14 and 21, in the case of CR, and on day 21 for PR and SR (Table 2). Fe deprivation resulted in greater coverage with lateral roots, which was above 90% in all root types on day 21.

Table 2. Percentage (%, mean ± SE) of root length of primary (PR), seminal (SR), and crown (CR) roots covered with lateral roots on days 7, 14, 21 after sowing. Asterisk (*) indicates statistically significant difference between -Fe treatment and the respective control at $p < 0.05$.

	Control			-Fe		
Days	**PR**	**SR**	**CR**	**PR**	**SR**	**CR**
7	40.36 ± 5.18	0.00	0.00	40.36 ± 5.18	0.00	0.00
14	80.46 ± 4.85	77.43 ± 3.26	44.32 ± 4.72	85.09 ± 4.92	83.74 ± 6.77	66.25 ± 5.39 *
21	86.52 ± 3.59	82.14 ± 4.28	77.67 ± 5.63	96.72 ± 3.27 *	93.62 ± 4.30 *	93.55 ± 5.66 *

The above-mentioned increase in the area covered with lateral roots under Fe-deprived conditions can be attributed to the corresponding decrease of the distance between the root tip and the point of lateral root initiation. As shown in Table 3, the presence of lateral roots was observed at a distance shorter than 5 cm from the root tip from day 14 onwards and at all maize root types. On day 21, emerging lateral roots were observed at a distance of 1 cm from the root tip.

Table 3. Distance (cm, mean ± SE) of the point of lateral root emergence and root tip in primary (PR), seminal (SR), and crown (CR) roots on days 7, 14, 21 after sowing. Asterisk (*) indicates statistically significant difference between -Fe treatment and the respective control at $p < 0.05$.

	Control			-Fe		
Days	**PR**	**SR**	**CR**	**PR**	**SR**	**CR**
7	6.70 ± 0.94	0.00	0.00	6.70 ± 0.94	0.00	0.00
14	6.50 ± 0.91	7.83 ± 0.77	8.50 ± 0.74	4.33 ± 0.57 *	3.77 ± 0.61 *	3.60 ± 0.44 *
21	8.20 ± 1.03	10.10 ± 1.21	7.77 ± 1.16	1.10 ± 0.28 *	1.03 ± 0.14 *	0.77 ± 0.18 *

The study of the quantitative trait loci (QTLs) associated with the formation of lateral roots at a distance shorter than 5 cm from the root tip under Fe deprivation (Branching at the Terminal 5 cm of Root, BTR), revealed 3 QTLs [6]. Among these, we have chosen to study the QTL located in chromosome 1, between the flanking markers "chrom7 - glb1", primarily due to the relatively high percentage of the explained phenotypic variance (20.9%) by this QTL. Interestingly, the same QTL is also responsible for the variation of the SPAD value of younger leaves. The genes found in chrom7 - glb1 are listed in Table 4. Among these 4 genes coding for long intergenic non-coding RNAs (lincRNAs), 6 genes coding for transcription factors, as well as 8 genes coding for unknown proteins, are present.

Table 4. List of the genes in the QTL located in chromosome 1, between the flanking markers "chrom7 - glb1" (V4 annotation of MGDB).

MGDB Gene ID	MGDB Gene/Protein Information
Zm00001d033374	TIDP3143, ubiquitin-conjugating enzyme
Zm00001d033375	cl27571_1, FMP27, GFWDK domain protein
Zm00001d022967	lincRNA
Zm00001d033377	9-cis-epoxycarotenoid dioxygenase (abscisic acid biosynthesis)
Zm00001d022968	lincRNA
Zm00001d033378	hb33, homeobox-transcription factor 33
Zm00001d033379	kip1, knotted interacting protein1 transcription factor
Zm00001d033380	tetratricopeptide-like helical domain superfamily protein
Zm00001d033382	-
Zm00001d033383	thic1, hydroxymethylpyrimidine phosphate synthase1 (thiamine biosynthesis)
Zm00001d033384	ribosomal protein L5 domain family
Zm00001d033385	UDP-D-galacturonate:1,4-a-poly-D-galacturonate 4-a-D-galacturonosyltransferase (homogalacturonan biosynthesis)
Zm00001d033386	zinc finger, RING/FYVE/PHD-type domain containing protein
Zm00001d033388	tetratricopeptide-like helical domain superfamily protein
Zm00001d033389	vq10, VQ motif-transcription factor10
Zm00001d022969	lincRNA
Zm00001d033390	basic-leucine zipper domain transcription factor
Zm00001d033391	-
Zm00001d033392	-
Zm00001d033393	-
Zm00001d033394	-
Zm00001d033395	-
Zm00001d022970	lincRNA
Zm00001d033396	grftf4, Growth-Regulating Factor (GRF) - transcription factor 4
Zm00001d033397	cl9255_1, CNNM transmembrane domain protein
Zm00001d033399	-
Zm00001d033400	metal-dependent protein hydrolase domain
Zm00001d033401	osca7, hyperosmolality-gated calcium-permeable channels7
Zm00001d033402	-
Zm00001d033403	tRNA
Zm00001d033404	hb110, homeobox-transcription factor 110
Zm00001d033405	TIDP3692, pyruvate kinase

Among the genes contained into this region of the maize genome, there are three genes related to hormonal biosynthesis and/or signaling. The gene Zm00001d033377 codes for an enzyme of the abscisic acid biosynthetic pathway, Zm00001d033375 codes for an enzyme related to ethylene response, whilst Zm00001d033379 and Zm00001d033396 code for two transcription factors related to gibberellin signaling (regulation of gibberellin biosynthesis and response to gibberellin respectively). Additionally, Zm00001d033375 is also related to cellular response to phosphate starvation. The gene Zm00001d033383 codes for an Fe-S cluster enzyme involved in thiamin biosynthesis. Future research is

required in order to reveal the underlying mechanisms by which the BTR phenotype occurs when plants experience Fe starvation as well as ~~of~~ how this phenotype improves Fe homeostasis in maize plants.

4. Conclusions

The trait of lateral root branching at the terminal 5 cm of the root in Fe-deprived maize plants was observed both in roots that emerged before the onset of Fe deprivation (i.e., the primary embryonic root and the secondary embryonic ones), as well as on roots that emerged after the onset of the deprivation (i.e., the crown roots). Bioinformatic analysis resulted in the identification of a region in maize genome chromosome underpinning this phenotype.

Author Contributions: S.N.C. and Y.E.V. conceived and designed the experiments; Y.E.V., S.-T.P.P. and A.-E.N. performed the experiments; S.N.C. and Y.E.V. analyzed the data; S.N.C., Y.E.V. and D.L.B. wrote the paper. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

PR	Primary embryonic roots
SR	Seminal embryonic roots
CR	Crown roots
BTR	Branching at the terminal 5 cm of root
lincRNA	long intergenic non-coding RNA
MGDB	Maize Genetics and Genomic Database
QTL	quantitative trait locus

References

1. Guerinot, M.L.; Yi, Y. Iron: Nutritious, noxious, and not readily available. *Plant Physiol.* **1994**, *104*, 815–820, doi:10.1104/pp.104.3.815.
2. Ramos, A.C.; Martins, M.A.; Okorokova-Façanha, A.L.; Olivares, F.L.; Okorokov, L.A.; Sepúlveda, N.; Feijó, J.Á; Façanha, A.R. Arbuscular mycorrhizal fungi induce differential activation of the plasma membrane and vacuolar H+ pumps in maize roots. *Mycorrhiza* **2009**, *19*, 69–80, doi:10.1007/s00572-008-0204-3.
3. Cesco, S.; Neumann, G.; Tomasi, N.; Pinton, R.; Weisskopf, L. Release of plant-borne flavonoids into the rhizosphere and their role in plant nutrition. *Plant Soil* **2010**, *329*, 1–25, doi:10.1007/s11104-009-0266-9.
4. Nozoye, T.; Nagasaka, S.; Kobayashi, T.; Takahashi, M.; Sato, Y.; Sato, Y.; Uozumi, N.; Nakanishi, H.; Nishizawa, N.K. Phytosiderophore efflux transporters are crucial for iron acquisition in graminaceous plants. *J. Biol. Chem.* **2011**, *286*, 5446–5454, doi:10.1074/jbc.M110.180026.
5. Darbani, B.; Briat, J.-F.; Holm, P.B.; Husted, S.; Noeparvar, S.; Borg, S. Dissecting plant iron homeostasis under short and long-term iron fluctuations. *Biotechnol. Adv.* **2013**, *31*, 1292–1307, doi:10.1016/j.biotechadv.2013.05.003.
6. Benke, A.; Urbany, C.; Marsian, J.; Shi, R.; von Wirén, N.; Stich, B. The genetic basis of natural variation for iron homeostasis in the maize IBM population. *BMC Plant Biol.* **2014**, *14*, 12, doi:10.1186/1471-2229-14-12.
7. Zanin, L.; Venuti, S.; Zamboni, A.; Varanini, Z.; Tomasi, N.; Pinton, R. Transcriptional and physiological analyses of Fe deficiency response in maize reveal the presence of Strategy I components and Fe/P interactions. *BMC Genom.* **2017**, *18*, 154, doi:10.1186/s12864-016-3478-4.

8. Portwood, J.L., II; Woodhouse, M.R.; Cannon, E.K.; Gardiner, J.M.; Harper, L.C.; Schaeffer, M.L.; Walsh, J.R.; Sen, T.Z.; Cho, K.T.; Schott, D.A.; et al. MaizeGDB 2018: The maize multi-genome genetics and genomics database. *Nucleic Acids Res.* **2019**, *47*, D1146–D1154, doi:10.1093/nar/gky1046.