UNIVERSIDAD MICHOACANA DE SAN NICOLÁS DE HIDALGO

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"Participación del represor SOLITARY-ROOT/INDOLE-3ACETIC ACID 14 (SLR1/IAA14) y de la sacarosa en la respuesta a Cr(VI), y su relación con la identidad celular y la respuesta auxínica en la raíz de *Arabidopsis thaliana* L."

Tesis que presenta

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Para obtener el grado de

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Fuentes de inspiración y fortaleza.

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RESUMEN

El desarrollo post-embrionario de las plantas es flexible y en la raíz le permite la adaptación a condiciones adversas, como la distribución heterogénea de nutrientes y la presencia de compuestos tóxicos. El cromo (VI) [Cr(VI)] inhibe el crecimiento de la raíz primaria y promueve la formación de raíces laterales y adventicias en *Arabidopsis thaliana*, procesos que son dependientes de la señalización por auxinas y en particular del represor SOLITARY ROOT/INDOLE-3 ACETIC ACID 14 (SLR1/IAA14). Para profundizar en el conocimiento sobre los mecanismos celulares y genéticos que modulan los programas de desarrollo en la raíz en respuesta al estrés por Cr(VI), en este trabajo se analizó la participación de SLR1/IAA14 y la disponibilidad de una fuente de carbono en dichos procesos. A través de un sistema de exposición eventual de la raíz al Cr(VI) se logró conocer la secuencia de eventos celulares y moleculares implicados en la inhibición de la raíz primaria durante la percepción de Cr(VI). Es notorio que el tamaño y longevidad de las células de la capa lateral de la raíz son alterados en respuesta a la percepción de Cr(VI), posiblemente como una respuesta adaptativa. Por otra parte, se evidenció que SLR1/IAA14 y el suministro de sacarosa son factores importantes para la reconfiguración de la arquitectura de la raíz y la tolerancia al Cr(VI), modificando la homeostasis de auxinas y la actividad del nicho de células iniciales en el meristemo. En este proceso, SLR1/IAA14 controla la transición a un programa determinado de la raíz primaria y estimula la formación de raíces adventicias, mientras que la sacarosa mantiene la actividad proliferativa del meristemo aún en concentraciones de Cr(VI) altamente inhibitorias para el crecimiento. En conjunto, los resultados establecen una relación directa entre la disponibilidad de carbono, la actividad meristemática y la tolerancia de *Arabidopsis* a un compuesto de gran importancia ambiental, el Cr(VI).

Palabras clave: *Arabidopsis*, cromo (VI), auxinas, sacarosa, raíz.

ABSTRACT

The post-embryonic root development in plants is flexible and allows adaptation to adverse conditions such as the heterogeneous distribution of nutrients or the exposure to toxic compounds. In *Arabidopsis thaliana*, chromium (VI) [Cr(VI] inhibits primary root growth and promotes lateral and adventitious root formation in a process that involves auxin signaling. SLR1/IAA14 encodes a stable repressor protein involved in auxin response and its gain-of-function increases the tolerance of primary roots to Cr (VI). To gain further insight in to the cellular and genetic mechanisms that underlie the root development programs involved in response to Cr(VI) stress, the roles of SLR1/IAA14 and carbon availability were analyzed. An experimental system, in which *Arabidopsis* roots are exposed to the eventual Cr(VI) presence, allowed the sequence of cellular and molecular events in the inhibition of primary root, to be revealed. On the one hand, the size and longevity of cells of the lateral root cap are altered in response to Cr(VI), triggering a change in the overall structure of root; meanwhile, SLR1/IAA14 and sucrose act as mediators of root development in response to Cr(VI), modifying auxin homeostasis and the activity of initial cells in the meristem. In this process, SLR1/IAA14 regulates the transition to a determinate root growth program and a more complex root system is formed due to adventitious root formation, in which sucrose protects meristematic cells from Cr(VI) toxicity. The data establish a link between carbon availability, initial cells functioning and root tolerance to a compound with increasing environmental and functional such as Cr(VI).

Keywords: *Arabidopsis*, chromium (VI), auxin, sucrose, root.

1. INTRODUCCIÓN

El crecimiento y desarrollo vegetal involucran la integración de señales ambientales y endógenas, las cuales junto con el programa genético intrínseco determinan la morfología y fisiología de la planta. Una característica del desarrollo post-embrionario de las plantas es la plasticidad, susceptible a diversas modificaciones causadas por interacciones con su ambiente biótico y abiótico (Forde y Lorenzo 2001).

El sistema radical en dicotiledóneas se caracteriza por ser pivotante, es decir tiene una raíz principal o primaria derivada del embrión, a partir de la cual se forman raíces laterales que aumentan su capacidad de absorción y anclaje; eventualmente se pueden formar raíces a partir de los tejidos vegetativos que reciben el nombre de adventicias (Bellini et al. 2014). El crecimiento y desarrollo post-embrionario de la raíz y del follaje son posibles por la presencia de células que conservan su capacidad de división, las cuales se concentran en áreas restringidas formando meristemos (Scheres et al. 2002). La raíz es un órgano heterotrófico que necesita los carbohidratos sintetizados por las hojas, las que a su vez necesitan de los nutrientes captados por la raíz. De esta manera, los nutrientes y la disponibilidad de energía son factores clave que controlan el crecimiento, lo que aunado a la naturaleza sésil de las plantas, optimizan su adaptación al ambiente (Lilley et al. 2012).

Entre los reguladores endógenos se encuentran las hormonas vegetales, de las cuales las auxinas (principalmente el ácido indol-3-acético, AIA) participan en varios procesos, entre ellos la regulación de la arquitectura de la raíz (Teale et al. 2006) y en particular la actividad del meristemo radical (Benkova y Hejatko 2009). Otro factor endógeno es la sacarosa, la cual contribuye al crecimiento y desarrollo, ya que representa la forma principal en la que el carbono que se ha asimilado en la fotosíntesis es transportado a los distintos órganos; además, se ha reportado que éste y otros carbohidratos pueden participar en la señalización para modular algunos procesos en el crecimiento y desarrollo vegetal (Ruan 2014).

Durante el crecimiento y desarrollo del sistema radical en el suelo se obtienen nutrientes minerales y agua, no obstante, se pueden presentar condiciones adversas como concentraciones elevadas de algunos nutrientes, incluyendo: el hierro (Fe), zinc (Zn) y cobre (Cu), o de elementos no esenciales como el cromo (Cr) o plomo (Pb); en ambas situaciones se pueden presentar efectos tóxicos. En concentraciones sub-

letales de estos elementos, se genera una respuesta en el sistema radical, que consiste en la inhibición del crecimiento de la raíz primaria por agotamiento de su capacidad meristemática y la formación de raíces laterales o adventicias. Esta respuesta incrementa la superficie de exploración de la raíz en el suelo y la posibilidad de crecer hacia áreas con condiciones más favorables o bien como una estrategia para tolerar el estrés (Potters et al. 2009).

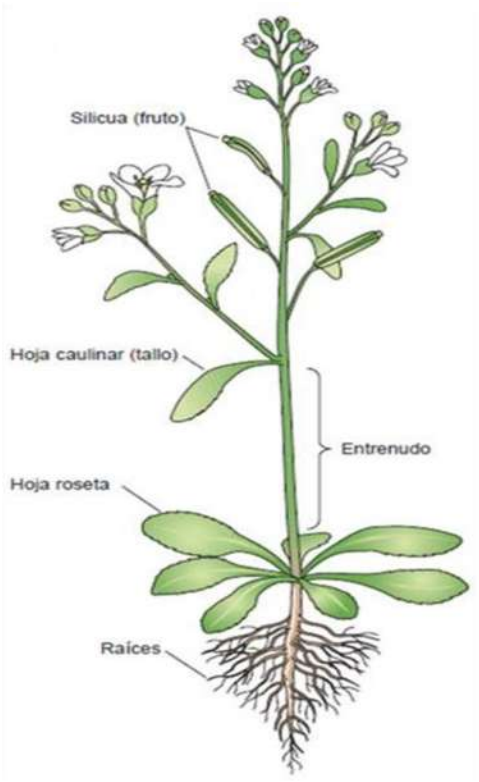
El Cromo (Cr) es un metal de transición, cuyas formas más estables y abundantes son la trivalente Cr(III) y la hexavalente Cr(VI). La contaminación por Cr(VI) afecta el desarrollo vegetal, repercutiendo severamente en la productividad de los cultivos (Shanker et al. 2005). En nuestro grupo de trabajo hemos analizado el efecto del Cr(VI) en *Arabidopsis thaliana*, caracterizado por la inhibición en el crecimiento de la raíz primaria e inducción del desarrollo de raíces laterales y adventicias. El análisis de los procesos moleculares implicados en estos programas de morfogénesis ha revelado la participación de genes de respuesta a auxinas (López-Bucio et al. 2014; Martínez-Trujillo et al. 2014). La mutación con ganancia de función en el gen *slr1/iaa14* genera que la proteína represora IAA14 sea más estable, lo cual evita su degradación en la señalización de auxinas, impidiendo la formación de raíces laterales (Fukaki et al. 2002); y en presencia de Cr(VI) muestra menor inhibición en el crecimiento de la raíz primaria (López-Bucio et al. 2015). Para profundizar en el conocimiento de los mecanismos celulares y genéticos que subyacen a los programas de desarrollo en la raíz en respuesta al estrés por Cr(VI), en este trabajo se analizó la participación de SLR1/IAA14 y el suministro de sacarosa en la tolerancia a Cr(VI), así como los cambios en la homeostasis de auxina y en la expresión de genes de identidad celular en el meristemo.

2. ANTECEDENTES

2.1. *Arabidopsis thaliana* (L) Heynh

Arabidopsis thaliana (L) Heynh, es una angiosperma de la familia Brassicaceae (Strasburger 2004), de tamaño pequeño (30 cm aproximadamente), con un ciclo de vida corto (6-8 semanas). *Arabidopsis* se autopoliniza, tiene una alta fecundidad y una producción de semilla abundante, lo cual la hace de fácil manejo y una excelente opción para estudios *in vitro* (Fig. 1). Adicionalmente, la secuenciación del genoma completo de *Arabidopsis* (que consta de 135 Mpb), ha hecho extensivo su uso como modelo de estudio y durante las dos últimas décadas ha permitido la creación de poblaciones de semillas mutagenizadas, así como una amplia colección de líneas transgénicas (Bennetzen 2001). Además, hay disponibilidad de mutantes insercionales afectadas en genes específicos, lo que permite analizar su participación en diferentes rutas de señalización en los programas de crecimiento y desarrollo (Orlando et al. 2009).

a)



b)

Figura 1. *Arabidopsis thaliana*. a) Dibujo de una planta adulta de *Arabidopsis* que muestra varios de sus órganos y su raíz pivotante (Modificado de Taiz y Zeiger 2006). b) Fotografía de las flores (Cortesía de Martínez-Trujillo).

2.2. Desarrollo embrionario

La embriogénesis se lleva a cabo en el óvulo fecundado y el resultado es un embrión latente e inmaduro dentro de la testa (formando la semilla). Durante la embriogénesis, se establece la polaridad vástago-raíz mediante patrones de crecimiento que determinan el eje apical-basal, en los que se forman los meristemos del tallo y raíz. En *Arabidopsis*, el desarrollo embrionario sigue un patrón constante de división celular (Mayer et al. 1991) en el que se establece la arquitectura básica de la planta mediante la integración de factores fisiológicos y genéticos que influencian este desarrollo. Esta etapa de la planta está compuesta por una serie de divisiones celulares (16 estados embrionarios) que conducen al cigoto a formar un organismo completo.

2.3. Procesos de morfogénesis

Una de las principales características del desarrollo vegetal es su plasticidad, ya que la mayor parte del desarrollo no se produce en el embrión sino después de la germinación, en respuesta a las necesidades nutricionales y a los cambios en el medio ambiente (Gray 2004).

El crecimiento en plantas es indeterminado debido a la actividad de los meristemos, un grupo de células con alta capacidad proliferativa, capaces de producir todos los tejidos y órganos (Stepanova et al. 2008). Los meristemos apicales de tallo y raíz se forman durante la embriogénesis y se consideran esenciales para el aumento en tamaño, ya que producen células que posteriormente crecen y se diferencian, por lo que también representan centros de integración de información para el funcionamiento y adaptación ante el agobio ambiental (Doerner 2003; Lastdrager et al. 2014).

2.4. La arquitectura del sistema radical

La raíz es un órgano especializado en la absorción de agua y nutrientes que debido a que estos no están distribuidos uniformemente en el suelo, la conformación espacial del sistema radical es crucial para el uso óptimo de los recursos disponibles. La arquitectura del sistema radical (ASR), comprende la longitud, número y posición de sus componentes: raíz primaria, raíces laterales,

raíces adventicias y pelos radicales.

La versatilidad para ajustar la ASR es un aspecto importante del rendimiento de la planta y su productividad ante condiciones ambientales fluctuantes como luz, agua y nutrientes (Smith y De Smet 2012; Mudgil et al. 2016). En *Arabidopsis*, el sistema radical presenta una organización bastante simple, ya que se encuentra formado por una raíz primaria, de la cual emergen las raíces laterales y estas últimas también se ramifican en un proceso repetitivo.

2.4.1. La raíz primaria

La formación de la raíz primaria ocurre desde la embriogénesis, cuando el embrión se polariza para establecer los meristemos que darán origen al follaje en el extremo superior y a la raíz en el extremo inferior (Mansfield y Briarty 1991). Una vez que el embrión madura y posteriormente, cuando la semilla germina y la plántula emerge, el crecimiento continuo de la raíz primaria ocurre por eventos coordinados de división, elongación y diferenciación celular, regulados en los meristemos (Scheres et al. 2002). El meristemo de la raíz contiene un grupo de células iniciales (células madre) que rodean al centro quiescente (QC, por sus siglas en inglés), con escasa actividad mitótica y que mantiene el estado indiferenciado de las células iniciales (van den Berg et al. 1997; Scheres 2007). El mantenimiento de las células iniciales asegura el aporte continuo de células nuevas en el meristemo y subyace el crecimiento indeterminado de la raíz (Sena et al. 2009).

La división de las células iniciales genera filas de una sola célula que se extienden a lo largo del eje longitudinal y forman capas de tejido de distinta naturaleza. En consecuencia, cada tejido tiene un linaje celular especifico (van den Berg et al. 1995). Las nuevas células producidas por las células iniciales progresan a través de tres fases de desarrollo en su camino a la madurez: i) en la zona meristemática se dividen para generar un suministro constante de células, ii) en la zona de elongación las células pierden la habilidad para dividirse e incrementan su longitud, y iii) en la zona de diferenciación las células adquieren sus funciones y características especializadas (Fig. 2a). Así, a partir de las células iniciales, se producen los tejidos del sistema vascular, la endodermis, el córtex, la epidermis, la capa lateral de la raíz y la columela (Fig. 2b) (Ishikawa y Evans 1995).

En la raíz de *Arabidopsis*, el tejido vascular está organizado dentro de un cilindro central o estela, el cual contiene al xilema (que transporta agua y nutrientes) y al floema (que transporta fotosintatos), interpuestos con células del procámbium no diferenciadas y una capa de periciclo circundante; mientras que los tejidos exteriores de la raíz exhiben simetría radial, la estela es bilateralmente simétrica (Fig. 2c) (Dolan et al. 1993; Petricka et al. 2012).

Las capas celulares del córtex y endodermis provienen de las células iniciales del córtex/endodermis mediante un proceso regulado por los factores de transcripción SHR y SCR (Fig. 2b-c) (Scheres et al. 1995; Di Laurenzio et al. 1996). Debido a que en el ápice de la raíz primaria se encuentra el meristemo, la capa lateral de la raíz y la columela forman una capa protectora que se desprende continuamente conforme la raíz crece y penetra el suelo; junto con la epidermis, estos tejidos constituyen la superficie exterior de la raíz, originados a partir de un grupo de células iniciales que experimenta una expansión celular rápida (Dolan et al. 1993; Petricka et al. 2012).

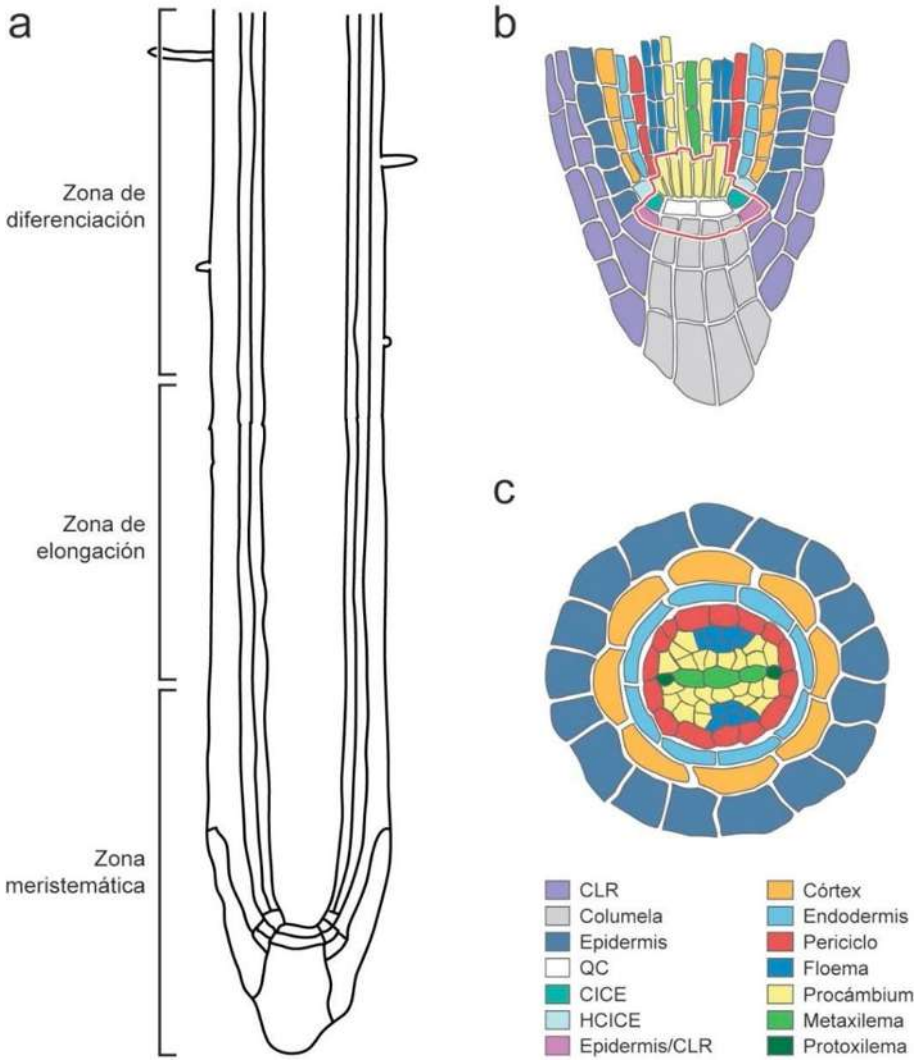


Figura 2. La raíz primaria de *Arabidopsis.* a) Las distintas zonas del desarrollo en la raíz primaria. La división celular ocurre en la zona meristemática, la expansión y elongación celular ocurre en la zona de elongación y la diferenciación celular (indicada por la formación de pelos radicales) ocurre en la zona de diferenciación. b) Organización del meristemo apical de la raíz. Los diferentes tipos de células se acomodan en filas celulares a través del eje longitudinal de la raíz. La región delimitada con rojo es el nicho de células iniciales. c) Organización radial de la raíz. El diagrama muestra una sección transversal de la raíz. Un eje central de xilema es flanqueado por dos haces de floema. CLR, capa lateral de la raíz; QC, centro quiescente; CICE, células iniciales del córtex y la endodermis, HCICE, hijas de células iniciales del córtex y la endodermis; Epidermis/CLR, células iniciales de la capa lateral de la raíz y la epidermis. Modificado de Petricka et al. (2012).

2.4.2. Raíces laterales

Además de la raíz primaria, el número y longitud de las raíces laterales representan otra característica dominante del sistema radical. Aunque el completo desarrollo de la raíces laterales es estructuralmente similar a las raíces primarias, la formación de una raíz lateral es un evento post-embrionario (De Smet 2012; Péret et al. 2012; Petricka et al. 2012). El paso inicial en el desarrollo de la raíz lateral tiene lugar en el meristemo basal de la raíz parental, donde las células del periciclo en los polos del xilema son "preparadas", es decir, adquieren la capacidad para formar una raíz lateral (De Smet et al. 2012).

Las raíces laterales se forman en la zona de diferenciación a partir de células del periciclo posicionadas adyacentemente a los polos del xilema; un subgrupo de esas células (llamadas células fuente) se estimula para dividirse y forma un primordio de raíz lateral (De Smet et al. 2012). A partir de la primera división celular en las células del periciclo hasta el primordio de la raíz lateral emergente se han identificado siete etapas que corresponden a diferentes pasos en la adquisición de la identidad celular y la organización del tejido (Malamy y Benfey 1997).

El primer evento morfológico relacionado con la iniciación de un primordio ocurre cuando dos células fuente dentro de la misma fila (en el periciclo) experimentan divisiones transversales asimétricas (Laskowski et al. 1995; Casimiro et al. 2003). Una serie idéntica de divisiones mitóticas ocurre en ambas células, formando una fila de aproximadamente 8 a 10 células "cortas". Después de un periodo de expansión radial ocurre una división longitudinal en las células centrales, originando un primordio con dos capas, lo cual está definido como etapa II. Durante la etapa III, la capa externa se divide longitudinalmente para formar una tercera capa. Una vez más, algunas células en los extremos de la segunda capa no se dividen, lo cual ocasiona que la estructura comience a tener forma de domo. Posteriormente, la capa interna se divide originando un primordio de cuatro capas que penetra el tejido endodérmico de la raíz parental donde se está formando el primordio durante la etapa IV. En las tres etapas siguientes, las divisiones transversales y longitudinales continúan y el primordio atraviesa los tejidos del córtex (etapa V) y la epidermis (etapa VI) hasta que emerge de la raíz lateral durante la etapa VII (Fig. 3) (Malamy y Benfey 1997; Casimiro et al. 2003).

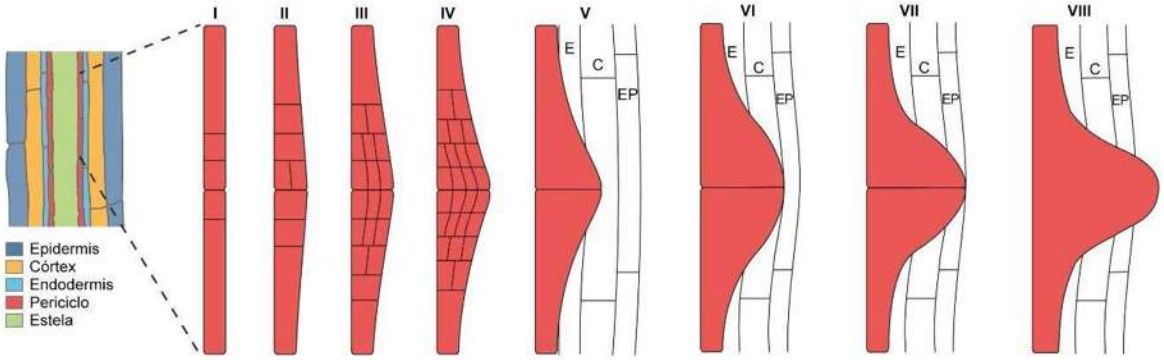


Figura 3. Desarrollo de la raíz lateral de *Arabidopsis*. Esquema de un segmento en la zona de diferenciación de la raíz primaria a través del eje longitudinal. Las raíces laterales se inician en la capa celular del pericliclo (rojo), cuando divisiones celulares transversales asimétricas ocurren se origina un primordio de raíz lateral (etapa I), el cual experimentará una serie de divisiones periclinales, avanzando por 6 etapas de desarrollo posteriores hasta su emergencia como una raíz lateral madura (etapa VIII). E, epidermis; C, córtex; E, endodermis. Adaptado de Casimiro et al. (2003) y Petricka et al. (2012).

El último paso en la formación de la raíz lateral es la emergencia desde el interior de la raíz primaria, gracias a la actividad de su propio meristemo, lo cual implica un incremento en la síntesis de auxina (Celenza et al. 1995). El desarrollo tardío de las raíces laterales es similar a las raíces primarias compartiendo la mayoría de los procesos de desarrollo, aunque las raíces laterales exhiben características únicas, como una respuesta gravitrópica alterada (Giehl et al. 2014).

2.4.3. Raíces adventicias

Las raíces adventicias, a diferencia de la raíz primaria y laterales, se forman de tejidos aéreos como tallos y hojas, y su origen está vinculado con eventos de agobio ambiental o daño (Bellini et al. 2014; Steffens y Rasmussen 2016). Por lo anterior, dichas estructuras son importantes para la supervivencia bajo estrés biótico y abiótico, incluyendo la exposición a metales pesados y/o deficiencia de nutrientes (Steffens 2014). Las raíces adventicias han evolucionado para ayudar a las plantas a tolerar una variedad de condiciones estresantes, y aunque poseen la misma función que las raíces laterales, están sujetas a mecanismos regulatorios diferentes (Bellini et al. 2014).

El desarrollo de raíces adventicias se divide en tres fases fisiológicas sucesivas pero interdependientes: la fase de inducción, que conduce a la activación del periciclo, la fase de iniciación, durante la cual divisiones celulares llevan a la formación del meristemo de la raíz; la fase de maduración, la cual corresponde al crecimiento interno del primordio, y finalmente la emergencia de la raíz que conduce a una nueva ramificación (Steffens y Rasmussen 2016).

2.5. Los nutrientes minerales

Las plantas necesitan una variedad amplia de elementos químicos que actúan como componentes estructurales o funcionales entre ellos, el carbono (C) que proviene del dióxido de carbono atmosférico, el hidrógeno (H) y oxígeno (O) del agua, así como nutrientes minerales presentes en el suelo. Un elemento esencial se define como aquel que es un componente intrínseco de la estructura o metabolismo del

organismo y cuya ausencia causa anormalidades en el crecimiento, desarrollo o reproducción (Epstein y Bloom 2005; Taiz y Zeiger 2006).

Los elementos esenciales se clasifican como macronutrientes o micronutrientes, de acuerdo a su concentración en los tejidos de la planta. Entre los macronutrientes se incluye al nitrógeno (N), fósforo (P), potasio (K), calcio (Ca), magnesio (Mg) y azufre (S); y entre los micronutrientes al boro (B), cloro (Cl), cobalto (Co), cobre (Cu), hierro (Fe), manganeso (Mn), molibdeno (Mo) y zinc (Zn) (Timilsena et al. 2015).

2.6. El carbono y la sacarosa

Las plantas se caracterizan por realizar la fotosíntesis, proceso que utiliza la energía luminosa para convertir el CO_2 en carbono orgánico en las hojas. La sacarosa es un disacárido formado por fructosa y glucosa, producto final de la fotosíntesis; es precursor de numerosos metabolitos y polímeros celulares, tales como almidón, celulosa, calosa y proteínas (Ruan 2014). Por otra parte, la sacarosa es considerada una molécula señal para la activación de los meristemos influenciando así diversos aspectos del crecimiento y desarrollo vegetal (Sairanen et al. 2012).

La sacarosa se produce en el citosol de las hojas maduras y es el azúcar predominante que se transporta a través del floema a los tejidos consumidores, como las raíces (Ruan 2014). El proceso implica dos diferentes rutas: simplástica y apoplástica. En la ruta simplástica, el transporte de sacarosa inicia con su carga a través de proyecciones de la membrana plasmática denominados plasmodesmos, que conectan una célula con otra, mientras que la ruta apoplástica transporta sacarosa a través del espacio extracelular entre las paredes celulares de las células. La ruta del apoplasto requiere transportadores dependientes de ATPasas, localizadas en la membrana plasmática de las células del floema. En *Arabidopsis*, el gen *SUC2* es uno de los seis transportadores de sacarosa específicos de floema que participan en la ruta apoplástica (Gottwald et al. 2000).

Una vez en los tejidos consumidores, la sacarosa se hidroliza y genera los monosacáridos, glucosa y fructosa, lo que puede ocurrir por enzimas específicas de la pared celular denominadas invertasas. De este modo, se provee de moléculas para el metabolismo y producción de energía, además de reducir la concentración de sacarosa en los sitios destino, lo que facilita su translocación en el floema (Ruan 2014).

La disponibilidad de carbohidratos regula directamente la expresión de genes y la síntesis de proteínas, aspectos vinculados con la síntesis de ATP, el metabolismo y en su papel como segundos mensajeros en rutas de transducción de señales (Eveland y Jackson 2012; Hammond y White 2008).

Los eucariontes entre ellos las plantas, poseen dos importantes redes regulatorias que responden a cambios en el estado nutricional y energético. Las proteínas cinasas denominadas sucrose non-fermenting related kinase 1 (SnRK1) y la cinasa blanco de rapamicina (TOR), son reguladores centrales que integran el crecimiento y desarrollo con el estado de energía y disponibilidad de carbono orgánico en las plantas. TOR es regulada por la disponibilidad intracelular de sacarosa y se activa cuando la disponibilidad de nutrientes es óptima, lo cual resulta en la estimulación del metabolismo anabólico, mientras que bajo escasez de nutrientes o energía, se activa SnRK1 para promover la removilización de los nutrientes (Dobrenel et al. 2016). Estos sistemas se modulan por el estatus de sacarosa, permaneciendo activos durante todo el ciclo de vida, particularmente bajo situaciones de estrés (Baena-González y Sheen 2008).

2.7. Reguladores del crecimiento vegetal o fitohormonas

Las hormonas vegetales o fitohormonas también son llamadas reguladores del crecimiento vegetal, debido a su característica principal de estimular la organogénesis. También controlan respuestas de defensa, estrés, aspectos del metabolismo, reproducción y envejecimiento, es decir, virtualmente cada aspecto en el ciclo de vida de las plantas está bajo el control hormonal en algún grado (Gray 2004).

Se consideran fitohormonas canónicas a las auxinas, citocininas, brasinoesteroides, giberelinas, ácido abscísico, etileno, ácido jasmónico y ácido salicílico, ya que sus mecanismos de síntesis, transporte, percepción y señalización son bien conocidos (Gray 2004). Cabe mencionar que la actividad de cada hormona vegetal se determina por su disponibilidad, la cual se controla a nivel de su metabolismo y distribución, además de la eficiencia en su percepción y transducción de señales, por lo tanto, las alteraciones en alguno de estos eventos tienen un impacto sobre la función hormonal (Vanstraelen y Benková 2012). En el

contexto de entender los factores que influyen sobre el crecimiento y desarrollo de la raíz, las auxinas juegan un papel primordial, por lo que a continuación se describen los mecanismos de señalización de estas fitohormonas.

2.7.1. Las auxinas en el crecimiento y desarrollo

Las auxinas son fitohormonas cuyo nombre deriva de la palabra griega “auxein” que significa crecer. Las auxinas son indispensables para el crecimiento y desarrollo en las plantas, ya que actúan en procesos como la división y expansión celular, regulan las respuestas a la luz y gravedad, la arquitectura general de la raíz y del follaje, la formación de los órganos y el desarrollo vascular (Teale et al. 2006). Las auxinas se sintetizan en tejidos jóvenes y se transportan a tejidos específicos, donde activan cascadas de señalización que modifican el crecimiento y desarrollo (Benjamins y Scheres 2008). La auxina endógena más abundante es el ácido indol-3-acético (AIA), que participa en la mayoría de los procesos en los que las auxinas han sido involucradas: en el desarrollo de la planta y las respuestas al medio ambiente. Además del AIA, otros tres compuestos con actividad auxínica son sintetizados naturalmente por las plantas: el ácido indol-3-butírico (IBA), ácido 4-cloroindol-3-acético (4-Cl- AIA) y ácido fenilacético (PAA) (Sauer et al. 2013).

2.7.2. El transporte de auxinas

Las auxinas se transportan a toda la planta a través del floema y se redistribuyen por un sofisticado sistema de transporte célula a célula, donde las proteínas PINFORMED (PIN) que funcionan como transportadores de eflujo, desempeñan un papel importante (Vieten et al. 2007). Las proteínas PIN ayudan a establecer un gradiente de auxinas dependiente del tejido (Petrášek et al. 2006) y su expresión se ve influenciada por señales internas y externas (Abel y Theologis 2010).

2.7.3. Percepción y señalización por auxinas

Las auxinas regulan virtualmente cada aspecto del crecimiento y desarrollo de las plantas actuando mediante la unión a uno de los 6 receptores: la proteína F-box TRANSPORT INHIBITOR RESPONSE1 (TIR1) y proteínas relacionadas AUXIN SIGNALING F-BOX PROTEIN 1, 2, 3, 4, 5 (AFB1–5) son determinantes para la

especificidad del sustrato. La percepción de auxinas ocurre a través de la interacción de esta hormona con los receptores TIR1/AFBs que participan como subunidad del complejo de ubiquitin ligasa SCF $^{TIR1/AFBs}$ (Calderón-Villalobos et al. 2010).

Los FACTORES DE RESPUESTA A AUXINA (ARF) son un grupo de factores de transcripción que regulan a los genes de respuesta a auxinas mediante su unión a los promotores, ya sea activando o reprimiendo la expresión de genes (Dharmasiri et al. 2005). A altos niveles de auxinas, ésta actúa como un estabilizador entre los complejos co-receptores $SCF^{TIR1/AFBs}$ y los represores AUXIN/INDOLE-3 ACETIC ACID (Aux/IAA), lo que permite la ubiquitinación de estos últimos, y su posterior degradación vía el proteosoma 26S (Hayashi 2012). Por lo tanto, los ARFs quedan libres para unirse a los genes que contienen elementos de respuesta a auxinas (TGTCTC) en sus promotores para activar o reprimir la transcripción (Fig. 4). El gran número de genes Aux/IAA (29 en *Arabidopsis*) y ARF (23 en *Arabidopsis*) indica que la respuesta a auxinas es muy compleja, dependiendo tanto de sus niveles endógenos como de la especificidad y la fuerza de la interacción entre diferentes grupos de proteínas incluyendo TIR1-AFBs-Aux/IAA y Aux/IAA-ARF (Villalobos et al. 2012).

2.7.3.1. Las proteínas Aux/IAA

Los genes *Aux/IAA* son inducidos rápidamente por auxinas y codifican para proteínas nucleares de 25 a 35 kD, las cuales tienen tiempos cortos de vida media, lo que asegura que estas proteínas no puedan ser acumuladas en altos niveles (Abel y Theologis 1996). En *Arabidopsis* se conocen 29 genes pertenecientes a esta familia. La mayoría de las proteínas Aux/IAA muestran cuatro dominios conservados en su estructura (I, II, III, y IV). Estos dominios contribuyen con superficies discretas que permiten distintas interacciones proteína-proteína. El dominio I (DI) facilita la interacción con TOPLESS (TPL), un co-represor de la transcripción (Szemenyei et al. 2008). El dominio II (DII) contiene una secuencia especifica de aminoácidos y es mejor conocido como motivo degron. El DII es requerido para la inestabilidad característica de las proteínas Aux/IAA, ya que dirige el punto de partida para su degradación y para el ensamble al complejo co-receptor TIR1/ Aux/IAA (Calderón-Villalobos et al. 2012). Los dominios III (DIII) y IV (DIV) median la homo y heterodimerización, incluyendo

interacciones con proteínas ARF (Overvoorde et al. 2005). Dado que la síntesis de muchas proteínas Aux / IAA es inducida rápidamente por auxina, la señalización de auxina experimenta ciclos de regulación negativa por retroalimentación (Dreher et al. 2006).

Las proteínas Aux/IAA participan en la percepción de auxinas formando parte de un complejo co-receptor junto con proteínas TIR1/AFB 1-5. Las diferentes combinaciones de TIR1/AFBs y proteínas Aux/IAA forman complejos co-receptores con distintas propiedades de sensibilidad a las auxinas. Esto probablemente contribuye a la versatilidad de las auxinas como moléculas de señalización en el crecimiento y desarrollo de la planta (Calderón-Villalobos et al.2012). Mutaciones en varias proteínas Aux/IAA en las cuales se estabiliza el DII, ocasiona que la unión a auxina sea suprimida y por lo tanto la repuesta a dicha hormona sea alterada (Dharmasiri et al. 2005, Calderón Villalobos et al. 2012).

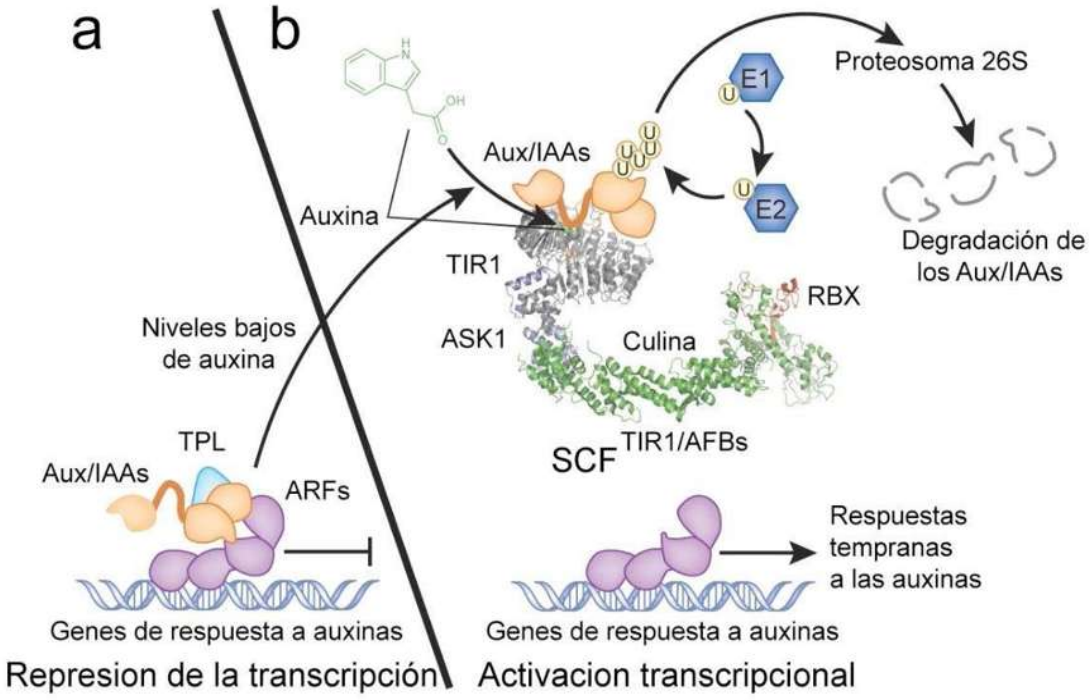


Figura 4. La ruta de señalización de auxinas. Los complejos SCF $^{TIR1/AFBs}$ se unen a la auxina y etiquetan a los represores Aux/IAA para su degradación. a) Niveles celulares bajos de auxinas: la transcripción de genes de respuesta a auxinas se encuentra reprimida por los represores Aux/IAAs. b) Niveles celulares de auxinas altos: la auxina se une a TIR1, lo cual permite la interacción de este último con los Aux/IAAs para promover la ubiquitinación y subsecuente degradación de dichos represores, permitiendo que los factores de transcripción ARFs promuevan la transcripción de genes. Modificado de Santner et al. (2009).

2.7.4. Las auxinas en la regulación del desarrollo de la raíz

Las auxinas están involucradas en cada aspecto del desarrollo de la raíz, desde la adquisición del destino celular, la iniciación del meristemo, la emergencia y la elongación (Bellini et al. 2014). El establecimiento de gradientes de auxina y su distribución regulan procesos como la división y expansión celular (Sabatini et al. 1999). Las proteínas PIN son las encargadas de establecer una distribución localizada de auxinas a través del eflujo de esta hormona en los diferentes tejidos. La localización de estas proteínas varía con respecto a cada tejido, por ejemplo: PIN1 participa en el movimiento de auxina en la parte central de la raíz, PIN2 establece un flujo hacia arriba en las regiones laterales de la raíz, PIN3 y PIN7 redistribuyen la auxina en la región de la columela y PIN4 mueve la auxina hacia el centro quiescente en el meristemo (Michniewicz et al. 2007) (Fig. 5).

La acción en conjunto de los genes *PIN* restringe el domino de expresión de los genes *PLETHORA* (*PLT*), los cuales son determinantes para la especificación de las células iniciales en el meristemo (Galinha et al. 2007). Los genes *PLT* codifican factores de transcripción, entre los cuales *PLT1* y *PLT2* alcanzan su máxima expresión en el meristemo de la raíz (Galinha et al. 2007). Es importante destacar que los niveles más altos de expresión de los genes *PLT* contribuyen con la estructura del nicho de células madre y su actividad proliferativa, mientras que una disminución en su expresión causa la diferenciación celular y la pérdida del meristemo (Aida et al. 2004; Galinha et al. 2007).

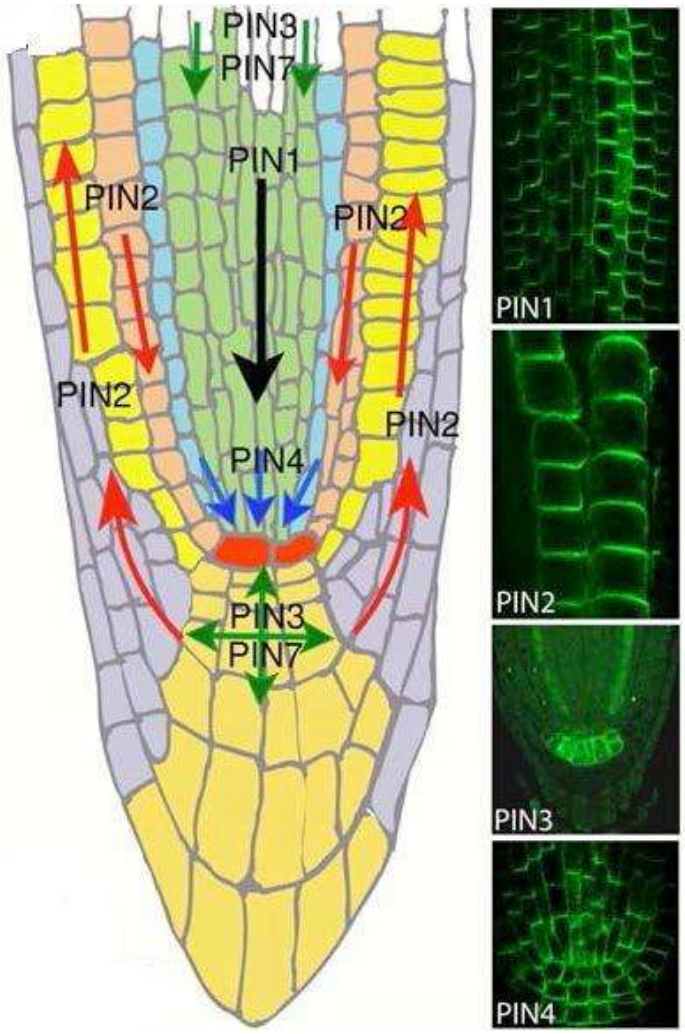


Figura 5. Localización de los transportadores PIN en la raíz de *Arabidopsis*. En el diagrama de la izquierda, las flechas indican la dirección del flujo de auxina conducida por las proteínas PIN. En la parte derecha se presenta la localización celular de las proteínas PIN utilizando la técnica de inmunolocalización. Michniewicz et al. (2007).

2.7.5. Las auxinas en la formación de raíces laterales

Además de su participación en la formación y establecimiento de la raíz primaria, las auxinas son requeridas para formación de raíces laterales y adventicias (Bellini et al. 2014). La distribución y gradientes de auxinas impactan en la formación de las raíces laterales a través de los diferentes módulos de respuesta a auxinas-AUX/IAA-ARF. Por ejemplo, en la iniciación de raíces laterales la degradación del represor SLR1/IAA14 permite la acción de los factores de transcripción ARF7 y ARF19 (Fukaki et al. 2002). De esta manera ARF7 y ARF19 promueven la expresión de genes necesarios para la división celular asimétrica en las células del periciclo, lo que da lugar a la iniciación de raíces laterales. Consecuentemente varias mutantes en los genes *AUX/IAA* con ganancia de función o mutantes en los genes *ARF* con pérdida de función, muestran alteraciones en la iniciación o desarrollo de raices laterales (Fukaki et al. 2002; Okushima et al. 2007).

Otras mutantes con fenotipos alterados en sus raíces son monopteros (*mp*/*arf5*) y bodenlos (*bdl*/*iaa12),* con raíces laterales colocadas irregularmente (De Smet et al. 2010); *tir1 afb2 afb3,* presenta defectos en el desarrollo de células del periciclo y en la morfología del primordio de la raíz lateral (Dubrovsky et al. 2011).

Lo anterior demuestra que desde la preparación de las células del periciclo hasta la emergencia del primordio de la raíz, los cambios en la distribución y gradientes de auxinas acompañan estos procesos (Malamy y Benfey 1997).

2.8. El cromo (Cr)

El cromo (Cr) es un metal de transición localizado en el grupo VI-B de la tabla periódica, y es el séptimo elemento de mayor abundancia en la corteza terrestre. El Cr se encuentra en rocas, suelo y emisiones volcánicas (McGrath y Smith 1990). Los usos del Cr y sus compuestos en la industria, incluyen aleaciones con acero inoxidable y procesos químicos industriales, el curtido de pieles, producción de pigmentos y la galvanoplastia. Estas aplicaciones incrementan su concentración en el ambiente por lo que se ha convertido en un contaminante importante del suelo, aire y agua (Armienta-Hernández y Rodríguez-Castillo 1995).

Las formas más estables y abundantes de Cr son la trivalente Cr(III) y la hexavalente Cr(VI), los otros estados de oxidación son inestables y de vida corta en los sistemas biológicos (Shupack 1991). El Cr(VI) se asocia al oxígeno para formar los oxianiones hidrocromato ($HCrO_4^-$), cromato (CrO_4^{2-}) o dicromato ($Cr_2O_7^{2-}$) (Shanker et al. 2005). En presencia de materia orgánica, el Cr(VI) es reducido a Cr(III); esta transformación es más rápida en suelos ácidos (McGrath y Smith 1990).

2.8.1. Efectos del Cr en los sistemas biológicos

Los efectos biológicos del Cr dependen de su estado de oxidación y localización celular. El Cr(VI) es considerado la forma más tóxica, mientras que el Cr(III) es relativamente inocuo debido a su insolubilidad y subsecuente incapacidad para cruzar las membranas celulares (Katz y Salem 1993). La reducción del Cr(VI) a Cr(III) se ha descrito en sistemas biológicos, involucrando la formación intermedia de Cr(V) (Kawanishi et al. 1986); estos compuestos reaccionan con H_2O_2 generando cantidades significativas de radicales $OH\cdot$, sin generación asociada de O_2. Los radicales $OH\cdot$ pueden producir alteraciones en el DNA, siendo el daño oxidativo el responsable de los efectos genotóxicos causados por el Cr(VI) (Shi y Dalal 1990). Por otro lado, el Cr(III) puede ejercer efectos tóxicos por su interacción con los grupos fosfato en el DNA (formando aductos Cr-DNA), a grupos carboxilo y sulfhidrilo en proteínas y por competencia con el transporte de hierro (Fe) (Cervantes y Campos-García 2007). El Cr también afecta la disponibilidad de sulfato (SO_4^{2-}) al competir por los sitios de transporte, e intervenir con su asimilación a nivel intracelular, alterando la síntesis de aminoácidos, y proteínas, entre ellas las del sistema de defensa. La alteración en la síntesis proteica por efecto del Cr es la mayor causa de inhibición del crecimiento en levaduras (Holland y Avery 2011).

2.8.2. Efectos del Cr en los sistemas vegetales

La toxicidad del Cr en los sistemas vegetales está estrechamente relacionada con alteraciones en la disponibilidad de nutrientes, como fosfato (PO_4^{3-}) y hierro (Fe) (López-Bucio et al. 2014; Martínez-Trujillo et al. 2014). Debido a que el Cr es un elemento no esencial para las células, no se ha reportado un mecanismo específico para su transporte, por lo que se ha sugerido que éste puede entrar a las células vegetales

utilizando transportadores de aniones esenciales como SO_4^{2-} y PO_4^{3-} (Shanker et al. 2005), interfiriendo con la captación, translocación y acumulación de varios nutrientes (Gardea-Torresdey et al. 2005).

El Cr induce estrés oxidativo que desencadena una peroxidación de lípidos, lo cual daña las membranas celulares, incluyendo las encargadas de la fotosíntesis, causando una disminución en el crecimiento (Hayat et al. 2012). El Cr se transporta desde la raíz hasta la parte aérea a través del xilema, acumulándose principalmente en las raíces y en cantidades menores en el follaje y órganos reproductivos (Shanker et al. 2005).

2.8.3. Efectos del Cr(VI) en *Arabidopsis*

El Cr(VI), en concentraciones subletales, afecta diversos procesos del desarrollo vegetal como la germinación, el desarrollo post-embrionario de la raíz y el follaje, con una reducción en biomasa e inhibición del crecimiento (Ortiz-Castro et al. 2007). Estos efectos deletéreos ocurren por la interferencia del Cr(VI) con la captación o metabolismo de nutrientes esenciales como S, P y Fe, que al ser suplementados en el medio, restablecen el crecimiento y confieren protección contra los efectos negativos del Cr(VI) (López-Bucio et al. 2014; Martínez-Trujillo et al. 2014).

La raíz es el principal órgano afectado por el Cr(VI), y aunque en concentraciones bajas (K_2CrO_4 40µM) puede tener un efecto promotor del crecimiento, a concentraciones más altas (100-140 µM), afecta drásticamente su crecimiento y desarrollo (Martínez-Trujillo et al. 2014; López-Bucio et al. 2015). Las concentraciones inhibitorias de Cr(VI) impactan drásticamente la arquitectura de la raíz, inhibiendo el crecimiento de la raíz primaria y promoviendo la emergencia de raíces laterales y adventicias, además de la presencia de pelos radicales muy cerca del ápice de la raíz primaria (Martínez-Trujillo et al. 2014; López-Bucio et al. 2015). Aunado a lo anterior, la expresión global de los genes en *Arabidopsis* es afectada por el suministro de 100 µM de K_2CrO_4; alterando la expresión de genes relacionados con la respuesta hormonal, la homeostasis del Fe, P, y el metabolismo de especies reactivas de oxígeno (López-Bucio et al. 2014; Martínez-Trujillo et al. 2014). Estas adaptaciones en el desarrollo de la raíz forman parte de una respuesta adaptativa al estrés por Cr(VI), reprogramando el metabolismo y la arquitectura de la raíz para sobrevivir (López-Bucio et al. 2015).

3. JUSTIFICACIÓN

El Cr(VI) afecta el crecimiento y la productividad vegetal e interfiere con la captación y metabolismo de nutrientes. En *Arabidopsis,* la mutación del gen *slr1/iaa14* incrementa la tolerancia de la raíz primaria al Cr(VI) y permite a la planta crecer bajo condiciones sub-letales de K_2CrO_4. Por otro lado, la disponibilidad de una fuente de carbono actúa como un estímulo nutricional importante para los programas de crecimiento y desarrollo. Hasta la fecha, se desconoce si el suministro de azúcares podría conferir alguna tolerancia al Cr(VI), mediante la posible interacción con la homeostasis de auxinas y la actividad de proliferación celular en el meristemo. Por lo que, avanzar en el entendimiento de los mecanismos moleculares de la respuesta al estrés por Cr(VI) podría ser una contribución de gran relevancia en el campo de la biología del desarrollo.

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4. HIPÓTESIS

Las respuestas del sistema radical de *Arabidopsis* al estrés generado por el Cr(VI) mediadas por el represor INDOLE ACETIC ACID14/ SOLITARY ROOT1, interactúan con una vía de señalización activada por la disponibilidad de carbono, manteniendo la homeostasis de auxinas y la identidad celular del meristemo en la raíz primaria.

5. OBJETIVOS

5.1 Objetivo general

Caracterizar la participación del represor SOLITARY ROOT/ INDOLE ACETIC (SLR1/IAA14) y de la sacarosa en la respuesta a Cr(VI) y su relación con la identidad celular y la respuesta auxínica en la raíz de *Arabidopsis thaliana* L.

5.2 Objetivos particulares

a) Analizar el efecto del suministro de sacarosa en la tolerancia a Cr(VI) en la raíz de *Arabidopsis* y su impacto en la distribución de auxinas y la expresión de genes de identidad celular en el meristemo.

b) Caracterizar el efecto del represor SLR1/IAA14 en el crecimiento y desarrollo de la raíz de *Arabidopsis* a la exposición eventual al Cr(VI) y sobre la expresión de genes relacionados con la actividad del nicho de células iniciales.

c) Analizar la participación de la capa lateral de la raíz como un posible sitio de percepción del Cr(VI) y su relación con la expresión de SLR1/IAA14.

6. RESULTADOS

Los principales resultados obtenidos durante la realización de este proyecto de tesis se presentan en los siguientes capítulos:

6.1. Capítulo 1

“Sucrose protects *Arabidopsis* roots from chromium toxicity influencing the auxin–plethora signaling pathway and improving meristematic cell activity”. (Publicado en la revista Journal of Plant Growth Regulation).

6.2. Capítulo 2

"Temporal root responses in *Arabidopsis thaliana* L. to chromate reveal structural and regulatory mechanisms involving the SOLITARY ROOT/IAA14 repressor for maintenance of identity meristem genes". (Sometido para su publicación en la revista Plant Growth Regulation).

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Sucrose Protects *Arabidopsis* Roots from Chromium Toxicity Influencing the Auxin–Plethora Signaling Pathway and Improving Meristematic Cell Activity

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Abstract Plants adapt to challenging growth conditions, such as the scarcity of nutrients or exposure to toxic metals, via changes in root system architecture. Chromium (Cr) is a non-essential element that when supplied in sublethal concentrations inhibits primary root growth through decreasing meristematic activity and affects photosynthesis. Here, we show that sucrose reverses the inhibitory effects of Cr(VI) on plant growth and development. Sucrose supplementation reactivated primary root growth under repressing Cr(VI) concentrations by restoring cell division and auxin distribution at the root meristem, keeping stem cell niche functioning. Analysis of the growth of *Arabidopsis* wild-type and mutant seedlings defective in auxin transport or signaling further revealed a critical role of auxin in mediating the effects of sucrose to protect plants from Cr(VI) toxicity. The results suggest that sucrose acts as a regulator in the maintenance of root meristem activity to overcome the stress generated by sublethal Cr(VI) concentrations.

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Keywords *Arabidopsis thaliana* · Chromium · Sucrose · Auxin · Root growth · Cell division

Introduction

Plants take up mineral nutrients and water from soil via the root system, whereas carbon is obtained by leaves from atmospheric CO_2 and sunlight energy (Johnson 2016). The photosynthesis process enables plants to synthesize energetically rich molecules such as sucrose, which are necessary for growth and developmental transitions during their life cycle (Ruan 2014). The sessile nature of plants, coupled with an ever changing environment, imply that diverse regulatory mechanisms must be activated for survival; these responses depend upon metabolic and/or gene expression adjustments (Atkinson and Urwin 2012).

The apical growth of the root relies on a group of stem cells with intense mitotic activity located at the root tip, which generate different tissues such as the stele, pericycle, endodermis, cortex, epidermis, lateral root cap, and the columella (Aichinger and others 2012). At the meristematic region, a strict balance between cell division and differentiation is controlled by the quiescent center (QC), which is composed of a few less mitotically active cells that act as an organizing center. The *PLETHORA* genes (*PLT1* and its paralog *PLT2*), encode transcription factors necessary for maintenance of both QC and stem cell identity, whose expression increases at the QC and is regulated by auxin, the main hormone controlling root growth (Aida and others 2004; Aichinger and others 2012).

The root is a heterotrophic organ, thus a continuous sucrose supply is required for its functioning (Gottwald and others 2000). In addition to its role as an energetic compound, sucrose metabolism provides a gateway for cell

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signaling as demonstrated by its promoting effect on the transition from the vegetative to the flowering phase and for the configuration of root architecture (MacGregor and others 2008; Yang and others 2013; Yu and others 2013). Indeed, the root meristematic activity is induced by photosynthesis and the shoot–root transport of metabolites, or by supplementation of sucrose or glucose, which act via the TOR kinase pathway to coordinate auxin response with the nutritional status to drive mitosis (Xiong and others 2013).

In *Arabidopsis thaliana*, sucrose transport depends on the families of the so-called SUCROSE CARRIERS (SUC), also known as SUCROSE TRANSPORTERS (SUT), and the SWEET (SUGAR WILL EVENTUALLY BE EXPORTED TRANSPORTERS) family; the SUC/SUT proteins facilitate the uptake of sucrose by the cells in symport with protons (Lalonde and others 2004), the SWEETs are uniporters that facilitate the efflux of sucrose to the apoplast (Chen and others 2012). The combined action of these families of transporters determines the flux of sucrose between organs and tissues according to the needs of the plant (Zakhartsev and others 2016). Among these proteins, SUC2 plays a critical role in sucrose translocation from the apoplast to phloem cells (Gottwald and others 2000).

Indole-3-acetic acid (IAA) is the main auxin present in plant tissues. This regulator is synthesized at stem and root tips and in young leaves, and is distributed throughout the plant via two physiologically and spatially separated transport pathways: (i) through the phloem by the mass flow mechanism (Muday and DeLong 2001; Davies 2004), and (ii) in a cell-to-cell manner through transmembrane proteins of the PIN-FORMED (PIN) family (Gälweiler and others 1998; Müller and others 1998; Krecek and others 2009). Within the root tip, auxins are redistributed by the different PIN proteins establishing an auxin gradient that guides organogenesis (Michniewicz and others 2007). The combined transport involving the influx carriers AUXIN RESISTANT1/LIKE AUX1 (AUX1/LAX) establishes an auxin distribution pattern with a maximum in the QC and adjacent stem cells, as well as in columella initials (Sabatini and others 1999; Péret and others 2012).

In *A. thaliana*, auxin binds to specific receptors from the TRANSPORT INHIBITOR RESPONSE1 (TIR1)/AUXIN SIGNALING F-BOX (AFB1-5) family (Dharmasiri and others 2005; Kepinski and Leysler 2005). These receptors are associated with SKP1-CULLIN1-F-BOX (SCF) complexes, responsible for ubiquitinizing proteins for degradation by the proteasome (Parry and others 2009). Activation of auxin response genes relies on AUXIN RESPONSE FACTORS (ARFs), whose activity is inhibited by AUXIN/INDOLE-3-ACETIC ACID (AUX/IAA) co-repressor proteins (Liscum and Reed 2002). In addition to binding the receptors, auxin can simultaneously target the AUX/IAA repressors allowing their ubiquitination and subsequent degradation, thereby releasing the ARFs to activate transcription of auxin response genes (Li and others 2016).

Chromium (Cr) is a non-essential element for plants that increases in abundance in the soil due to human industrial activities. This element can be found mainly in two oxidation states: Cr(III) and Cr(VI), the latter being the most mobile in ecosystems and therefore represents a major plant stressing factor (Shupack 1991). Cr(VI) accumulation limits agricultural activities because it inhibits germination, decreases root and shoot growth, and causes leaf yellowing, thus affecting photosynthesis (Shanker and others 2005). In the cytoplasm, Cr toxicity is mainly related to reduction of Cr(VI) to Cr(III) by both enzymatic and non-enzymatic reactions, through intermediate unstable Cr(V) and Cr(IV) states, forming reactive oxygen species (Cervantes and others 2001).

Because Cr is a non-essential element, plants apparently lack specific mechanisms for its uptake, but it has been suggested that the Cr(VI) transport in plants involves the carriers of sulfate and phosphate (Shanker and others 2005; López-Bucio and others 2014). The auxin pathway appears to be a critical target of Cr(VI) toxicity in roots, because both auxin transport and signaling are blocked by sublethal metal concentrations (Martínez-Trujillo and others 2014; López-Bucio and others 2014). Concomitantly, a nutritional deficiency syndrome is activated, involving low iron and low phosphate rescue systems because adequate supply of these nutrients not only protects plants from Cr(VI) deleterious effects but also restores normal growth and development (López-Bucio and others 2015).

Considering that sucrose is the main photosynthesis product, whose availability decreases upon Cr(VI) exposure, we hypothesized that managing endogenous sugar levels would help plants to better tolerate Cr(VI) stress. Here, we show that sucrose supply on the growth medium normalizes root growth and plant development under sublethal Cr(VI) concentrations, which correlates with restoration of the expression of genes involved in the cell cycle, auxin distribution and transport, stem cell niche, and sucrose transport. Moreover, we demonstrate the critical role of auxin signaling and transport in such sucrose-mediated amelioration of stress management.

Materials and Methods

Biological Material

A. thaliana ecotype Col-0 (wild-type; WT) and the transgenic lines: *DR5::GFP* (Ulmasov and others 1997), *CycB1::uidA* (Colón-Carmona and others 1999), *AtSUC2::GFP* (Imlau and others 1999), *PIN7pro::PIN7::GFP* (Blilou and others 2005), and *PLT1::GFP* (Aida and others 2004), and the *A.*

thaliana mutants *aux1-7* (Pickett and others 1990), *axr1-3* (Lincoln and others 1990), *tir1afb2afb3* (Parry and others 2009), and *iaa14/slr1* (Fukaki and others 2002) were used in this research.

Plant Growth Conditions

Seeds were superficially disinfected by treatment with 95% (v/v) ethanol for 10 min and a commercial 6% chloride solution for 5 min. Seeds were then washed three times with sterile distilled water and kept in darkness at 4 °C for 48 h to synchronize germination. Seeds were sown in Petri dishes with 0.5× MS medium (Murashige and Skoog 1962), pH 5.7, supplemented with phytagar 1% (w/v) (PhytoTechnology Laboratories), and different sucrose concentrations. Plates were placed vertically in a plant growth chamber (Percival AR-95 L) with a photoperiod of 16 h of light/8 h darkness, 300 µmol m^{-2} s^{-1} of light intensity, and a temperature of 22 °C.

Sucrose and Cr(VI) Treatments

To evaluate the effect of sucrose and Cr(VI) on primary root growth and gene expression, seeds of WT *A. thaliana* as well as transgenic and mutant lines were germinated and grown in MS 0.5× media supplemented with 100 µM potassium chromate (K_2CrO_4, Sigma) or without chromate. The media were supplemented with sucrose (Bioxon) at different concentrations: 17.5 mM (0.6%), considered as a basal concentration in culture medium, 35 mM (1.2%), 70 mM (2.4%), and 140 mM (4.8%). Plants were grown for 10 days and then growth and developmental parameters were analyzed.

Analysis of *uidA* Expression

For histochemical analysis of *uidA* expression, 10 days after germination (dag) *Arabidopsis* plants were stained and incubated 8 h at 37 °C in a phosphate buffer (0.5 mg mL^{-1} of 5-bromo-4-chloro-3-indolyl-β-D-glucuronide in 100 mM sodium phosphate, pH 7). Plants were cleared using a 0.24 N HCl-20% methanol (v/v) solution and incubated 1 h at 60 °C. The solution was replaced by 7% NaOH (w/v)-60% ethanol (v/v) and incubated 20 min at room temperature. Plant hydration was performed by treatments with decreasing ethanol solutions: 40, 20, and 10% (v/v), 30 min each; plants were fixed in glass slides covered with 50% glycerol (v/v).

Propidium Iodide Staining and GFP Detection

For fluorescent staining with propidium iodide, plants were mounted on microscope slides and treated with a 1.0 mg mL^{-1} solution of propidium iodide (PI). Each sample was analyzed separately for propidium iodide (with a 568-nm wavelength argon laser for excitation, and an emission window of 585–610 nm) and GFP (488 nm excitation/505–550 nm emission), using a confocal microscope (Olympus FV1000), after which the two micrographs were merged to produce a final image. Fifteen independent seedlings were analyzed per line, and treatment representative images were selected for the figures.

Statistical Analysis

Averages and confidence intervals were calculated using the Excel Microsoft Office 2010 program. Analysis of variance (ANOVA) and Tukey significance tests were performed using the SPSS 19.0 for Windows statistical software ($p < 0.05$).

Results

Sucrose Protects *Arabidopsis* Roots from Cr(VI) Toxicity

To assess the possible role of the root energetic status in plant tolerance to Cr(VI), WT *A. thaliana* seeds were germinated and grown for 10 days in MS 0.5× media supplemented with 100 µM Cr(VI) and increasing sucrose concentrations. As expected, Cr(VI) strongly repressed root growth, whereas supplementation of 70 and 140 mM sucrose reactivated root growth by 60 and 100%, respectively (Fig. 1).

Root growth is determined by the activity of the root apical meristem, thus we evaluated the mitotic activity of the primary root meristem in transgenic *CycB1::uidA A.*

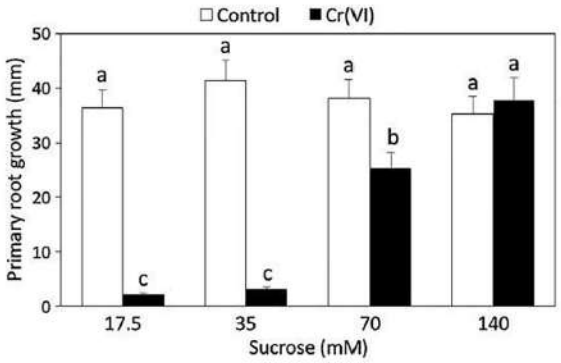

Fig. 1 Effect of Cr(VI) and sucrose on *Arabidopsis thaliana* primary root growth. WT (Col-0) seedlings were germinated and grown for 10 days in media with increasing sucrose concentrations, supplied or not with 100 µM Cr(VI). The confidence interval for alpha = 0.05 is shown; different letters indicate statistical differences with the Tukey test ($p < 0.05$, $n = 30$)

thaliana seedlings, which express a mitotic cyclin at the G2-to-M phase transitions of the cell cycle. GUS expression was observed as blue dots in the meristematic zone of plants grown in media without Cr(VI) and supplied with different sucrose concentrations (Fig. 2a–d). However, under 100 µM Cr(VI) treatment and basal sucrose concentration (17.5 mM), GUS expression was lost and the root tip became thicker and wider, consistent with toxicity symptoms (Fig. 2e). Noteworthy, sucrose supplementation restored *CycB1::uidA* expression and normalized root tip structure in a dose-dependent manner (Fig. 2f–h). These data indicate the important role of the local energetic status for metal tolerance and normal root growth under Cr(VI) toxicity.

Sucrose Improves Auxin–Plethora Gene Expression in *Arabidopsis* Root Meristems Exposed to Cr(VI)

The longitudinal and radial organization of the root is under the control of a signaling network mediated by the hormone auxin and the PLETHORA (PLT) transcriptional factors acting downstream (Benková and Hejátko 2009). The auxin distribution pattern was analyzed in *A. thaliana* transgenic seedlings expressing *DR5::GFP*, which is induced by auxin. The auxin distribution in primary root meristems without Cr(VI) was clearly located at the quiescent center and columella initial cells (Fig. 3a). Indeed, Cr(VI) decreased auxin-inducible gene expression as well as the levels of *PIN7pro::PIN7::GFP* and *PLT1::GFP* proteins (Fig. 3b, f, j), whereas 140 mM sucrose restored their expression (Fig. 3d, h, l). These data indicate that the auxin–PLT network at the root meristem is a direct target of Cr(VI) and is modulated by the plant energetic status.

Long Distance Sugar Transport is Blocked by Cr(VI)

Long distance sucrose transport from the shoot to the root is critical for root meristem functioning (Xiong and others 2013). To determine how sucrose transport in primary roots is affected by Cr(VI), the expression of the sucrose transporter SUC2 was investigated in *Arabidopsis SUC2::GFP* transgenic seedlings. In media lacking Cr(VI), supplementation of sucrose repressed *SUC2::GFP* expression in the primary root in a dose-dependent manner (Fig. 4a–d). Cr(VI) strongly inhibited *SUC2::GFP* expression (Fig. 4e) together with changing the structure of the root meristem, whereas sucrose alleviated root growth and normalized sucrose transporter expression (Fig. 4g, h).

Auxin Signaling Mediates the Effects of Sucrose to Protect Roots from Cr(VI) Toxicity

To determine the role of auxin signaling during sucrose restoration of root growth in seedlings exposed to Cr(VI), a genetic analysis comparing *A. thaliana* primary root growth in WT and mutant lines *aux1-7, axr1-3, slr-1*, and *tir1afb2afb3* in response to Cr(VI) with or without sucrose was performed. The results showed that 100 µM Cr(VI) inhibited the growth of the primary root in most genotypes tested; this effect was more noticeable in the WT, with approximately 90% inhibition of growth, and was weaker in

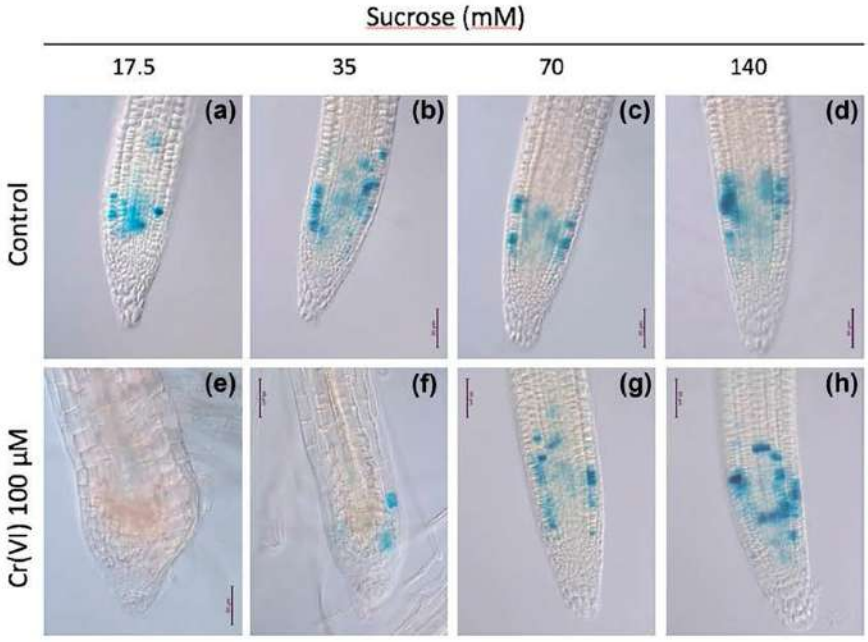

Fig. 2 Effect of Cr(VI) and sucrose on the expression of the *CycB1::uidA* marker in *Arabidopsis thaliana*. Seeds of the *CycB1::uidA* transgenic line were germinated and grown for 10 days in media with increasing sucrose concentrations, supplied or not with 100 µM Cr(VI). Images are representative individuals from 15 plants analyzed. Bar = 100 µm. The experiment was repeated three times with similar results

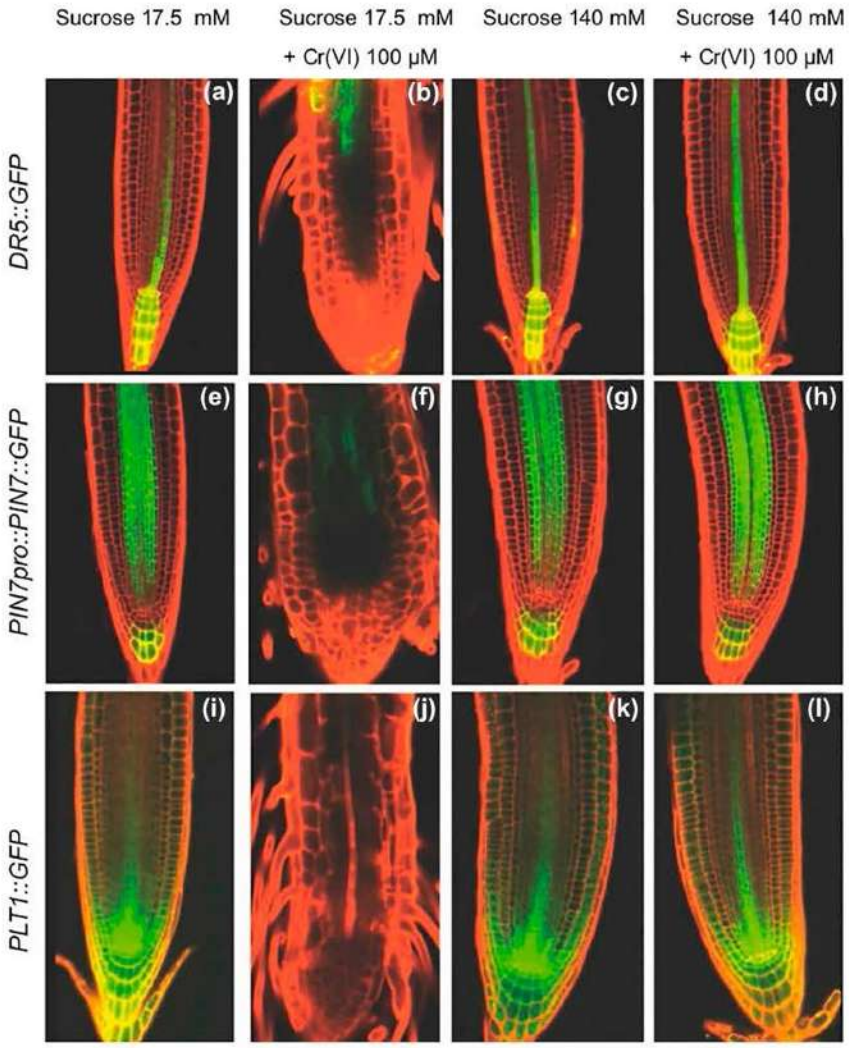

Fig. 3 Effect of Cr(VI) and sucrose on the expression of the *DR5::GFP, PIN7pro::PIN7::GFP, and PLT1::GFP* markers in *Arabidopsis thaliana*. Seeds of the different transgenic lines were germinated and grown for 10 days in the media with sucrose 17.5 mM (control) or 140 mM (high sucrose), supplied or not with 100 µM Cr(VI). Images are representative individuals from 10 plants analyzed for each transgenic line. The experiment was repeated three times with similar results

slr-1 (approximately 50%), and other auxin-related mutants (Fig. 5). Interestingly, the effects of sucrose restoring root growth under Cr(VI) supply were observed for *slr-1* and *axr1–3* mutants, but absent in the *aux1–7* and *tir1afb2afb* mutants (Fig. 5). These data imply a critical role of auxin transport and response for sucrose-mediated tolerance to Cr(VI).

Discussion

The post-embryonic growth of the root depends upon the activity of the meristem, which can be affected by metal exposure or low nutrient availability (Potters and others 2007). Our results showed that sucrose supplementation promotes cell division, reactivating primary root growth in seedlings exposed to a sublethal Cr(VI) concentration; concomitantly, this metal strongly affected the expression of the sucrose transporter SUC2 in roots, indicating that an impaired availability of photosynthates influences cell division at the root meristem. These results are in agreement with those reported by Gottwald and others (2000), who tested the phenotypes of three knockout mutants in the *AtSUC2* gene, generating plants that were unable to export sucrose from leaves to the root. In these plants, root growth was interrupted at an early stage when grown on media lacking sucrose, however, normal root growth was observed upon sugar supplementation.

In addition to *SUC2, SUC1*, and *SUT4*, and the *SWEET11,12,13* genes expressed in roots, the encoded

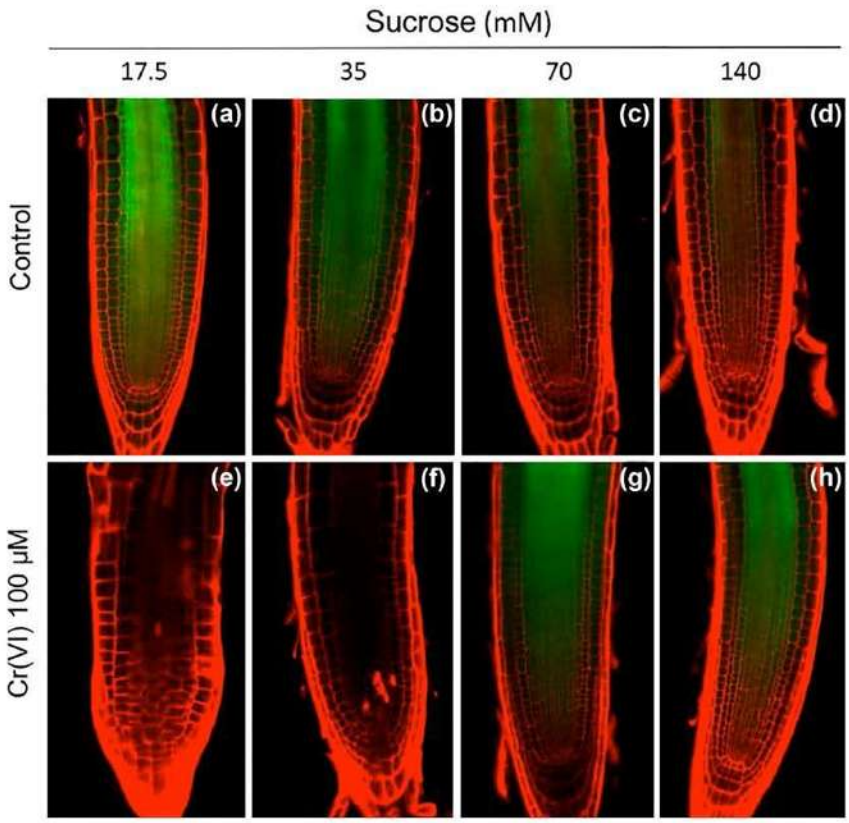

Fig. 4 Effect of Cr(VI) and sucrose on the expression of *SUC2::GFP* in *Arabidopsis thaliana* seedlings. Seeds of the *SUC2::GFP* transgenic line were germinated and grown for 10 days in media with increasing sucrose concentrations, supplied or not with 100 µM Cr(VI). Images are representative individuals from 10 plants analyzed. The experiment was repeated three times with similar results

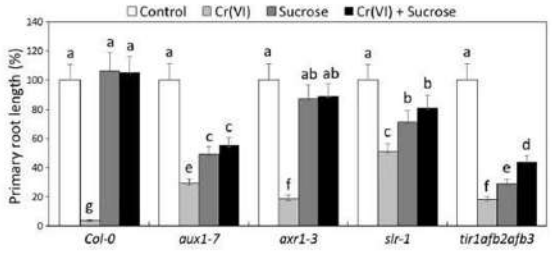

Fig. 5 Effect of Cr(VI) and sucrose on primary root growth of *Arabidopsis* WT and auxin-related mutants. Seeds of the different genotypes were germinated and grown for 10 days in with sucrose 17.5 mM (control) or 140 mM (high sucrose), supplied or not with 100 µM Cr(VI). The confidence interval for alpha = 0.05 is shown; different letters indicate statistical differences with the Tukey test ($p < 0.05$, $n = 30$)

proteins are considered the main transporters that facilitate the sucrose influx/efflux across cell membranes (Zimmermann and others 2004; Zakhartsev and others 2016); besides, the participation of other not well-characterized proton-independent transporters might be involved in the sucrose uptake by roots (Chaudhuri and others 2008). It remains to be elucidated whether genes other than *SUC2*, encoding sucrose transporters, are also inhibited by sublethal Cr(VI) concentrations and if their expression is reactivated by exogenous sucrose, which may point to sugar transport as a checkpoint for plant adaptation to xenotoxic stress.

A possible mechanism by which sucrose reactivates the mitotic process might be related to the activity of the TARGET OF RAPAMYCIN (TOR) kinase, in a pathway that concertedly integrates the energy, nutrients, growth factors, and stress status to cell proliferation in the *Arabidopsis* root meristem (Xiong and Sheen 2011; Xiong and others 2013). Therefore, it is possible that local availability of sucrose (or its glucose derivative) in the meristem may be sensed as a triggering factor eliciting nutritional and stress responses in plant roots. Our data show that sucrose supplementation may help plants to cope with sublethal concentrations of non-essential elements, such as Cr(VI), which may lead to potential agricultural applications.

DR5, *PIN7*, and *PLT1* expressions in primary roots of plants grown in media supplemented with Cr(VI), directly correlated meristem functioning with an auxin–PLT pathway. The results obtained on primary root growth and meristem cell division further suggest a connection between

sucrose and auxin–PLT pathway for root resistance to Cr(VI) and possibly to other metals, as reported for the scarcity of some nutrients. For instance, sucrose supplementation increased auxin response under low iron availability conditions (Lin and others 2015).

The PIN family of auxin transporters controls auxin distribution to regulate cell division and expansion in the primary root. Moreover, the PIN proteins have an important role in the formation and maintenance of the meristem by directly regulating the *PLETHORA* (*PLT*) genes, which are major determinants for root stem cell specification (Blilou and others 2005). Decreased expression of *PLT1::GFP* and *PIN7pro::PIN7::GFP* following root exposure to Cr(VI) was correlated with the loss of both cell division and normal auxin distribution within the meristem; consistently, available data show that the *Arabidopsis* double mutant *plt1plt2* develops a short primary root, has decreased meristematic activity, and shows disorganized columella cell layers (Aida and others 2004). The primary root growth of auxin transport *aux1–7* and auxin receptor *tir1afb2afb* mutants, which both failed to normalize root growth in response to Cr(VI) following sucrose supply, supports the notion that auxin signaling acts downstream of a sugar pathway to maintain cell proliferating activity in the root meristem.

Auxin is not only the canonical regulator of root morphogenesis: recent data point to its critical role in stress adaptation; for example, during iron limitation *aux1–7* and *pin1 Arabidopsis* mutants had an altered expression of genes involved in iron uptake (Lin and others 2015). Thus, we cannot exclude the possibility that the auxin signaling pathway could be part of a nutritional mechanism linking iron, phosphate, and Cr(VI) root responses as revealed by studies on global gene expression analyses and root growth restoration, where Cr(VI) affects the homeostasis of iron and phosphate in *Arabidopsis* (Martínez-Trujillo and others 2014; López-Bucio and others 2014).

The participation of other hormones besides auxin in mediating the inhibitory effect of Cr(VI) on the primary root growth cannot be ruled out, since changes in expression of genes involved in the ethylene and jasmonic acid signaling pathways are elicited by Cr(VI) sublethal concentrations in *Arabidopsis* (Martínez-Trujillo and others 2014). It is possible that the canonical ethylene signaling pathway involving ETR1, EIN2, CTR1, and EIN3 could mediate primary root growth inhibition or lateral root formation in response to chromate as these genes play a role in auxin–ethylene crosstalk for the control of root organogenesis (Muday and others 2012). It is also tempting to speculate that chromate could induce cell death at the meristem or activate cell differentiation programs that trigger meristem exhaustion as that occurs in primary roots of *Arabidopsis* experiencing low phosphate availability (López-Bucio and others 2002; Sánchez-Calderón and others 2005).

Although Cr(VI) toxicity has been associated with the production of reactive oxygen species (Stohs and Bagchi 1995), and our group has demonstrated the induction of genes encoding ROS-detoxifying enzymes possibly related to an adaptive mechanism (Martínez-Trujillo and others 2014), it remains to be tested if sucrose or any of its derived sugars directly decreases the ROS levels at the root meristem and their relationships with other cellular processes remains as an open question.

Plants adjust their growth and development in response to environmental conditions through adjusting phytohormone responses; in addition to its role as an energetic molecule, sucrose regulates plant physiological programs such as root foraging for nutrients (López-Arredondo and others 2014) and also controls hypocotyl elongation (Lilley and others 2012). Our results suggest that sucrose is a critical regulator which maintains the meristem activity of the primary root acting likely via the auxin–PLT pathway, and confers resistance to sublethal concentrations of a non-essential element, chromium. It remains to be determined if this protective effect may also occur during root exposure to other potentially toxic ions such as aluminum or heavy metals that strongly repress root growth and plant productivity in many regions around the world.

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References

Aichinger E, Kornet N, Friedrich T, Laux T et al (2012) Plant stem cell niches. Annu Rev Plant Biol 63:615–636

Aida M, Beis D, Heidstra R, Willemsen V, Blilou I, et al (2004) The PLETHORA genes mediate patterning of the *Arabidopsis* root stem cell niche. Cell 119:109–120

Atkinson NJ, Urwin PE (2012) The interaction of plant biotic and abiotic stresses: from genes to the field. J Exp Bot 63:3523–3543

Benková E, Hejatko J (2009) Hormone interactions at the root apical meristem. Plant Mol Biol 69:383–396

Blilou I, Xu J, Wildwater M, Willemsen V, Paponov I, Friml J, Heidstra R, Aida M, Palme K, Scheres B (2005) The PIN auxin efflux facilitator network controls growth and patterning in *Arabidopsis* roots. Nature 433:39–44

Cervantes C, Campos-García J, Devars S, Gutiérrez-Corona F, Tavera HL, Torres-Guzmán JC, Moreno-Sánchez R (2001) Interactions of chromium with microorganisms and plants. FEMS Microbiol Rev 25:335–347

Chaudhuri B, Hörmann F, Lalonde S, Brady SM, Orlando DA, Benfey P, Frommer WB (2008) Protonophore-and pH-insensitive glucose and sucrose accumulation detected by FRET nanosensors in *Arabidopsis* root tips. Plant J 56:948–962

Chen LQ, Qu XQ, Hou BH, Sosso D, Osorio S, Fernie AR, Frommer WB (2012) Sucrose efflux mediated by SWEET proteins as a key step for phloem transport. Science 335:207–211

Colón-Carmona A, Yuo R, Haimovitch-Gal T, Doerner P (1999) Spatiotemporal analysis of mitotic activity with a labile cyclin-GUS fusion protein. Plant J 20:503–508
Davies PJ (2004) Plant hormones: biosynthesis, signal transduction, action! Kluwer Academic Publishers, Dordrecht
Dharmasiri N, Dharmasiri S, Estelle M (2005) The F-box protein TIR1 is an auxin receptor. Nature 435:441–445
Fukaki H, Tameda S, Masuda H, Tasaka M (2002) Lateral root formation is blocked by a gain-of-function mutation in the *SOLITARY-ROOT/IAA14* gene of *Arabidopsis*. Plant J 29:153–168
Gälweiler L, Guan C, Müller A, Wisman E, Mendgen K, Yephremov A, Palme K (1998) Regulation of polar auxin transport by AtPIN1 in *Arabidopsis* vascular tissue. Science 282:2226–2230
Gottwald JR, Krysan PJ, Young JC, Evert RF, Sussman MR (2000) Genetic evidence for the in planta role of phloem-specific plasma membrane sucrose transporters. Proc Natl Acad Sci USA 7:13979–13984
Imlau A, Truernit E, Sauer N (1999) Cell-to-cell and long-distance trafficking of the green fluorescent protein in the phloem and symplastic unloading of the protein into sink tissues. Plant Cell 11:309–322
Johnson MP (2016) Photosynthesis. Essays Biochem 60:255–273
Kepinski S, Leyser O (2005) The *Arabidopsis* F-box protein TIR1 is an auxin receptor. Nature 435:446–451
Krecek P, Skupa P, Libus J, Naramoto S, Tejos R, Friml J, Zazímalová E (2009) The PIN-FORMED (PIN) protein family of auxin transporters. Genome Biol 10(249):1–11
Lalonde S, Wipf D, Frommer WB (2004) Transport mechanisms for organic forms of carbon and nitrogen between source and sink. Annu Rev Plant Biol 55:341–372
Li SB, Xie ZZ, Hu CG, Zhang JZ (2016) A review of auxin response factors (ARFs) in plants. Front Plant Sci 7(47):1–6
Lilley JLS, Gee CW, Sairanen I, Ljung K, Nemhauser JL (2012) An endogenous carbon-sensing pathway triggers increased auxin flux and hypocotyl elongation. Plant Physiol 160:2261–2270
Lin XY, Ye YQ, Fan SK, Jin CW, Shao Jian S, Zheng SJS (2015) Increased sucrose accumulation regulates iron-deficiency responses by promoting auxin signalling in *Arabidopsis* plants. Plant Physiol 170:907–920
Lincoln C, Britton JH, Estelle M (1990) Growth and development of the *axr1* mutants of *Arabidopsis*. Plant Cell 2:1071–1080
Liscum E, Reed JW (2002) Genetics of Aux/IAA and ARF action in plant growth and development. Plant Mol Biol 49:387–400
López-Arredondo DL, Leyva-González MA, González-Morales SI, López-Bucio J, Herrera-Estrella L (2014) Phosphate nutrition: improving low-phosphate tolerance in crops. Annu Rev Plant Biol 65:95–123
López-Bucio J, Hernández-Abreu E, Sánchez-Calderón L, Nieto-Jacobo MF, Simpson J, Herrera-Estrella L (2002) Phosphorus availability alters root architecture and causes changes in hormone sensitivity in the *Arabidopsis* root system. Plant Physiol 129:244–256
López-Bucio J, Hernández-Madrigal F, Cervantes C, Ortiz-Castro R, Carreón-Abud Y, Martínez-Trujillo M (2014) Phosphate relieves chromium toxicity in *Arabidopsis thaliana* plants by interfering with chromate uptake. Biometals 27:363–370
López-Bucio J, Ortiz-Castro R, Ruiz-Herrera LF, Vargas-Juárez C, Hernández-Madrigal F, Carreón-Abud Y, Martínez-Trujillo M (2015) Chromate induces adventitious root formation via auxin signalling and SOLITARY ROOT/IAA14 gene function in *Arabidopsis thaliana*. Biometals 28:353–365
MacGregor DR, Deak KI, Ingram PA, Malamy JE (2008) Root system architecture in *Arabidopsis* grown in culture is regulated by sucrose uptake in the aerial tissues. The Plant Cell 20:2643–2660
Martínez-Trujillo M, Méndez-Bravo A, Ortiz-Castro R, Hernández-Madrigal F, Ibarra-Laclette E, Ruiz-Herrera LF, Long TA, Cervantes C, Herrera-Estrella L, López-Bucio J (2014) Chromate alters root system architecture and activates expression of genes involved in iron homeostasis and signalling in *Arabidopsis thaliana*. Plant Mol Biol 86:35–50
Michniewicz M, Brewer PB, Friml J (2007) Polar auxin transport and asymmetric auxin distribution open Access. The *Arabidopsis* Book 5:e0108. doi:10.1199/tab.0108 2007.
Muday GK, DeLong A (2001) Polar auxin transport: controlling where and how much. Trends Plant Sci 6:535–542
Muday GK, Rahman A, Binder BM (2012) Auxin and ethylene: collaborators or competitors? Trends Plant Sci 17:181–195
Müller A, Guan C, Gälweiler L, Tänzler P, Huijser P, Marchant A, Parry G, Bennett M, Wisman E, Palme K (1998) *AtPIN2* defines a locus of *Arabidopsis* for root gravitropism control. EMBO J 17:6903–6911
Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol Plant 15:473–497
Parry G, Calderon-Villalobos LI, Priggeb M, Peretc B, Dharmasiria S, Itohd H, Lechnera E, Grayd E, Bennett WM, Estelle M M (2009) Complex regulation of the TIR1/AFB family of auxin receptors. Proc Natl Acad Sci USA 106:22540–22545
Péret B, Swarup K, Ferguson A, Seth M, Yang Y, Dhondt S, James N, Casimiro I, Perry P, Syed A, Yang H, Reemmer J, Venison E, Howells C, Perez-Amador MA, Yun J, Alonso J, Beemste TS, Laplaze L, Murphy A, Bennett MJ, Nielsen E, Swarup R (2012) AUX/LAX genes encode a family of auxin influx transporters that perform distinct functions during *Arabidopsis* development. Plant Cell 24:2874–2885
Pickett FB, Wilson AK, Estelle M (1990) The *aux1* mutation of *Arabidopsis* confers both auxin and ethylene resistance. Plant Physiol 94:1462–1466
Potters G, Pasternak TP, Guisez Y, Palme KJ, Jansen AK (2007) Stress-induced morphogenic responses: growing out of trouble? Trends Plant Sci 12:98–105
Ruan YL (2014) Sucrose metabolism: gateway to diverse carbon use and sugar signalling. Annu Rev Plant Biol 65:33–67
Sabatini S, Beis D, Wolkenfelt H, Murfett J, Guilfoyle T, Malamy J, Benfey P, Leyser O, Bechtold N, Weisbeek P, Scheres B (1999) An auxin-dependent distal organizer of pattern and polarity in the *Arabidopsis* root. Cell 99:463–472
Sánchez-Calderón L, López-Bucio J, Chacón-López A, Cruz-Ramírez A, Nieto-Jacobo F, Dubrovsky JG, Herrera-Estrella L (2005) Phosphate starvation induces a determinate developmental program in the roots of *Arabidopsis thaliana*. Plant Cell Physiol 46:174–178
Shanker AK, Cervantes C, Loza-Tavera H, Avudainayagam S (2005) Chromium toxicity in plants. Environ Int 31:739–775
Shupack SI (1991) The chemistry of chromium and some resulting analytical problems. Environ Health Perspect 92:7–11
Stohs SJ, Bagchi D (1995) Mechanisms in the toxicity of metal ions. Free Radical Biol Med 18:321–336
Ulmasov T, Murfett J, Hagen G, Guilfoyle TJ (1997) Aux/IAA proteins repress expression of reporter genes containing natural and highly active synthetic auxin elements. Plant Cell 7:288–299
Xiong Y, Sheen J (2012) Rapamycin and glucose-target of rapamycin (TOR) protein signalling in plants. J Biol Chem 287:2836–2842
Xiong Y, McCormack M, Li L, Hall Q, Xiang C, Sheen Jen J (2013) Glc-TOR signalling leads transcriptome reprogramming and meristem activation. Nature 496:181–186
Yang L, Xu M, Koo Y, He J, Poethig RS (2013) Sugar promotes vegetative phase change in *Arabidopsis thaliana* by repressing the expression of *MIR156A* and *MIR156C*. eLife 2:e00260. doi:10.7554/eLife.00260
Yu S, Cao L, Zhou CM, Zhang TQ, Lian H, Sun Y, Wu J, Huang J, Wang G, Wang JW (2013) Sugar is an endogenous cue for

juvenile-to-adult phase transition in plants. eLife 2:e00269. doi:10.7554/eLife.00269

Zakhartsev M, Medvedeva I, Orlov Y, Akberdin I, Krebs O, Schulze WX (2016) Metabolic model of central carbon and energy metabolisms of growing *Arabidopsis thaliana* in relation to sucrose translocation. BMC Plant Biol 16:262. doi: 10.1186/s12870-016-0868-3

Zimmermann P, Hirsch-Hoffman M, Hennig L, Gruissem W (2004) GENEVESTIGATOR: *Arabidopsis* microarray database and analysis toolbox. Plant Physiol 136:2621–2632

Plant Growth Regulation

Temporal root responses in Arabidopsis thaliana L. to chromate reveal structural and regulatory mechanisms involving the SOLITARY ROOT/IAA14 repressor for maintenance of identity meristem genes

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Temporal root responses in *Arabidopsis thaliana* L. to chromate reveal structural and regulatory mechanisms involving the SOLITARY ROOT/IAA14 repressor for maintenance of identity meristem genes.

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ABSTRACT

The *Arabidopsis* root system is modified in response to stress generated by high concentrations of non-essential ions such as chromate [Cr(VI)]. In this work, distribution of auxin and its transporters PIN1 and PIN7, as well as expression of genes that maintain identity of the root meristem, were analyzed in *Arabidopsis thaliana* wild type (WT) seedlings and in the mutant affected in the *SOLITARY ROOT* (*SLR1/IAA14*) locus, required for root response to Cr(VI). We show that primary root inhibition, auxin transporter levels, and expression of meristem identity genes were maintained in the *slr-1* mutants but not in WT plants in response to Cr(VI) in a time and concentration-dependent manner. Noteworthy, the outermost single cell layer of the lateral root cap, which normally dies and tends to peel off, remains viable and increases in size following exposure of WT plants to Cr(VI) but not in *slr-1* mutants. Our results suggest that: 1) the primary root tip senses Cr(VI), 2) the external lateral root cap may play a protective role during Cr(VI) exposure, and 3) Cr(VI) impacts cell division in root meristems via auxin redistribution and SLR1/IAA14 function, influencing expression of root meristem genes.

Keywords: Chromate, root growth, meristem identity, auxin.

INTRODUCTION

Post-embryonic root development enables plant adaptation to environmental stress via its important role in water and nutrient acquisition. Its plasticity allows plants in their natural ecosystems to grow and adapt to fluctuating availability of essential and non-essential elements (Epstein and Bloom 2004; Ruiz-Herrera et al. 2015). In *Arabidopsis thaliana*, the root system consists of the primary root, derived from the embryo and lateral roots that are formed from the pericycle during post-embryonic development; eventually, *adventitious* roots can be formed from hypocotyls. The growth and spatial distribution of the primary root, lateral roots, and adventitious roots, determine the overall root architecture (Bellini et al. 2014).

Root growth is supported by cell division at the meristem, where groups of cells with high mitotic activity proliferate and then elongate (Benková and Hejatko 2009). In *Arabidopsis,* the root apical meristem (RAM) comprises a group of stem cells that surround four less mitotically active cells in the quiescent center (QC), which maintains the undifferentiated state and high proliferative rate of meristem cells (van den Berg et al. 1997). For stem cell niche specification, the PLETHORA (PLT) (Aida et al. 2004; Blilou et al. 2005; Galinha et al. 2007), SHORT ROOT (SHR) (Benfey et al. 1993) and SCARECROW (SCR) (Sabatini et al. 2003) transcription factors, influence auxin transport and signalling. Because plant cells do not migrate, cell division patterns regulate production of clonally related cells that when differentiated establish the tissues that comprise the primary root such as root cap, epidermis, cortex, endodermis, pericycle, xylem and phloem (Arnaud et al. 2010; Dolan et al. 1993; Scheres et al. 2002; Willemsen et al. 2008).

The root cap forms a dome of cells at the very root tip covering the meristem, and is organized in the columella cell layers and lateral root cap (Dolan et al. 1993). This structure has a central role in sensing environmental factors such as gravity, soil moisture, and availability of nutrients, as well as in protecting roots during soil exploration or exposure to adverse growth factors (Arnaud et al. 2010).

Auxin plays a critical role in balancing cell division, elongation and differentiation (Benková et al. 2003; Friml 2003; Vanneste and Friml 2009; Perrot-Rechenmann 2010; Lee et al. 2013). It is mainly synthesized at the shoot apex, root tip and

emerging lateral roots, and can be distributed to other tissues via the phloem (Muday and DeLong 2001), or directionally cell-to-cell by PIN-FORMED (PIN) proteins, whose asymmetric location in cell membranes determines the output and intercellular auxin flow (Blilou et al. 2005; Vieten et al. 2007; Petrášek et al. 2009). These combined transport systems allow auxin gradients to be formed, which coordinate growth and patterning (Péret et al. 2012; Sabatini et al. 1999).

Auxin signalling involves mainly three components: the TRANSPORT INHIBITOR RESPONSE1 (TIR1)/AUXIN SIGNALLING F-BOX (AFB1-5) receptors, the AUXIN RESPONSE FACTORS (ARFs) and the AUXIN/INDOLE-3-ACETIC ACID REPRESSORS (AUX/IAAs) (Dharmasiri et al. 2005; Kepinski and Leyser 2005). The receptors are associated with an ubiquitin ligase complex, integrated by SKP1, Cullin and an F-box protein (SCF) (Gray et al. 1999; Dharmasiri et al. 2005). At low auxin levels, AUX/IAA repressors bind to ARFs to form a co-repressor complex, preventing transcriptional activation of auxin response genes. When auxin levels increase, it acts as a molecular “glue” between AUX/IAA repressors and the SCF/TIR1 co-receptor, allowing the ubiquitination of the repressors for degradation by the proteasome (Gray et al. 1999; Dharmasiri et al. 2005; Kepinski and Leyser 2005; Perrot-Rechenmann 2010; Ruiz-Rosquete et al. 2012), thus releasing the ARFs, which can now activate auxin responsive genes (Reed 2001; Mockaitis and Estelle 2008). Auxin also controls lateral roots initiation; in this process, the SLR1/IAA14 protein represses AUXIN RESPONSE FACTORS 7 (ARF7) and 19 (ARF19), both involved in promotion of cell division in pericycle cells (Fukaki et al. 2002; Weijers et al. 2005; Muto et al. 2007).

Chromium (Cr) is a transition metal located in group VI-B of the periodic table, and its ions occur naturally in the environment (Shanker et al. 2005). Their levels increase due to anthropogenic activities, such as leather tanning and chrome-plating, making this element derivatives common contaminants of water and soils (Kotaś and Stasicka 2000). The most stable and common Cr forms are the trivalent Cr(III) and the hexavalent Cr(VI) species; this latter is usually associated with oxygen as chromate (CrO_4^{2-}) or dichromate ($Cr_2O_7^{2-}$) ions (McGrath and Smith 1990). Cr(VI) is considered the most dangerous form due to its mobility in biological systems, and because once into the cell it generates Cr(III), which is highly toxic and may cause deleterious effects

such as lipid peroxidation, protein modifications and DNA damage (Holland and Avery 2011; Viti et al. 2014).

Plants are affected by Cr(VI) during germination, post-embryonic root growth, and plant phase transitions; for this reason, Cr(VI) and its compounds represent an increasing risk for agriculture (Shanker et al. 2005; Hayat et al. 2012). The *Arabidopsis* root system has been used to clarify the molecular events underlying plant adaptation to Cr(VI) exposure, which include inhibition of primary root growth and induction of lateral and/or adventitious roots; also, this architectural reconfiguration is believed to redirect root growth for avoiding stress damage and/or exposure (Martínez-Trujillo et al. 2014). The SLR1/IAA14 auxin response protein influences primary root adaptation to Cr(VI), since its gain-of-function role renders plants more resistant to Cr(VI) supplementation, which occurs via its direct effect on auxin biosynthesis and distribution within root tips (López-Bucio et al. 2015). However, the molecular and temporal events following Cr(VI) perception remain unknown. Here, we report the establishment of a growth system allowing *Arabidopsis* seedlings to respond to Cr(VI) gradients by supplying increasing Cr(VI) concentrations to some distance from the root tip. Comparison of growth of WT and *slr-1* mutants, as well as molecular and genetic analyses, not only confirmed the critical role played by SLR1/IAA14 in mediating root architecture reconfiguration in response to chromate, but also revealed novel structural and regulatory roles of the lateral root cap and meristem for Cr(VI) tolerance.

MATERIALS AND METHODS

Plant material

The *Arabidopsis thaliana* L. WT (ecotype Col-0) and the gain-of-function mutant *slr-1* (Fukaki et al. 2002) were used as the genetic backgrounds. The following transgenic *Arabidopsis* lines with WT genetic background were used: *CycB1pro::uidA* (Colón-Carmona et al. 1999), *DR5pro::GFP* (Ottenschläger et al. 2003), *PIN1pro::PIN1::GFP* (Benková et al. 2003) *PIN7pro::PIN7::GFP* (Blilou et al. 2005), *PLT1pro::GFP, PLT2pro::CFP* (Aida et al. 2004, Galinha et al. 2007), *SHRpro::SHR::GFP* (Sabatini et al. 1999), *SCRpro::SCR::GFP* (Sabatini et al. 2003). The different genetic markers mentioned above were transferred to *slr-1* mutants via outcrossing.

Growth conditions

Seeds of the different *Arabidopsis* lines were surface disinfected with 95% (v/v) ethanol for 5 min and 20% (v/v) bleach for 7 min. After five washes in distilled water, seeds were germinated and grown on agar plates containing 0.2X MS medium (Murashige and Skoog 1962). The MS medium and agar were purchased from PhytoTechnology. Petri dishes were placed vertically at 65° angle to allow the root growth on the agar surface and the aerial growth over the medium. Plants were placed in a growth chamber (Percival AR-95L) with a photoperiod 16 h light/8 h darkness, with light intensity 300 mmol m^{-2} s^{-1}, and 22°C temperature.

Experimental procedure for gradual exposure to Cr(VI)

Arabidopsis seeds of the different lines were germinated in 0.2X MS medium and allowed to grow for 4 days after germination. Then, a cut in the agar gel was made with a scalpel in either horizontal or vertical directions with respect to the primary root growth axis and 100 µM $K_2CrO_4^{2-}$ was added (33 µL) in the space left by the scalpel (at approximately 2.5 cm from the plants) with the help of a micropipette. In control conditions, a water volume equal to the Cr(VI) solution was added. Cr(VI) diffusion was determined by visual analysis and by changes in pH as determined with an indicator paper. In the horizontal system, Cr(VI) first came into contact with the tip of the primary root, while in the vertical system Cr(VI) came into contact simultaneously with the entire root system and the foliage. The Petri dishes were placed in the Percival chamber and incubated for 6 more days to compare growth responses under both conditions.

Root measurements

The *Arabidopsis* root system and the RAM integrity of the primary root were first analyzed with a stereoscopic microscope (Leica MZ6). Primary root length was determined using a ruler. RAM size and length of the lateral root cap cells were determined using semi-permanent preparations and an Axiostar Zeiss Plus Carl Zeiss microscope; images were captured using a Sony Cybershot DSC-S75 digital camera

and processed with Zeiss Axio Vision 4AC software (Carl Zeiss).

Histochemical analysis

For histochemical analysis of *CycB1pro::uidA* activity, *Arabidopsis* seedlings were incubated overnight at 37 ºC in a GUS reaction buffer (0.5 mg mL^{-1}) of 5-bromo-4-chloro-3-indolyl-β-D-glucuronide in 100 mM sodium phosphate, pH 7). Stained plants were cleared and fixed with 0.24N HCl in 20% (v/v) methanol and incubated for 60 min at 62°C. The solution was substituted by 7% (w/v) NaOH in 60% (v/v) ethanol for 20 min at room temperature. Plants were dehydrated with ethanol treatments at 40%, 20%, and 10% (v/v) for a 24-h period each and fixed in 50% (v/v) glycerol. Processed roots were placed on glass slides and sealed with commercial nail varnish. At least 15 transgenic plants were analyzed and a representative plant was chosen for each Cr(VI) treatment and imaged using Nomarski optics on a Leica DMR microscope.

Confocal microscopy

Transgenic seedlings expressing GFP (green fluorescent protein) were mounted on microscope slides into a 10 mg ml^{-1} solution of propidium iodide (PI) and fluorescent signals from roots were detected in a confocal laser scanning microscope (Olympus, FV1000). For PI detection, wavelengths employed had an excitation line of 568 nm with an emission window of 585–610 nm; GFP was excited with a 488 nm line and the emission detected at 505–550 nm.

Statistical analysis

For all experiments, overall data were statistically analyzed using the SPSS19 program for Windows. Univariate and multivariate analyses with Tukey's post-hoc test were used for testing the differences in growth and root development responses.

RESULTS

The effect of Cr(VI) on the *Arabidopsis* primary root growth depends upon the zone where it is first perceived

To understand the sequence of events underlying the root response to Cr(VI), *Arabidopsis* WT and *slr-1* mutant seeds were germinated and grown for 4 days in medium without Cr(VI); then Cr(VI) was added either in horizontal or vertical directions as described in materials and methods. Plants were allowed to grow for 10 days after treatments and primary root growth measurement was performed.

In horizontal exposition to Cr(VI), primary roots of WT plants were inhibited over 60% with respect to controls without Cr(VI), while primary root growth of *slr-1* seedlings was not inhibited at all (Fig. 1A). It should be noted that shoots of both WT and *slr-1* plants did not show chlorosis symptoms (Fig. 1B). On the other hand, in vertical Cr(VI) application, there was greater inhibition of primary root growth both in WT and *slr-1* plants (over 80% and 97%, respectively). Besides, strong growth inhibition and chlorosis symptoms in shoots, as well as increased lateral root formation, typified the WT response under this condition, while *slr-1* mutants that failed to form lateral roots were more sensitive to the toxic effects of Cr(VI) (Fig. 1A, 1B). The distribution of Cr(VI) into the growth medium of plants could be confirmed through visual analysis of pH changes; in the horizontal application diffused Cr(VI) reaches first the tip of the primary root (Supplementary Fig. S1), while in vertical application diffused Cr(VI) arrives simultaneously to leaves, hypocotyl and the whole primary root.

From these results, two aspects can be highlighted: i) plants adapt better to Cr(VI) exposure when the primary root tip first perceives the oxyanion, and ii) the magnitude of primary root growth inhibition depends on the ability of plants to produce lateral roots, a process mediated by SLR1/IAA14. Being the horizontal exposure to Cr(VI) the system allowing better growth both in WT and *slr-1* plants, subsequent analyses were done in that way.

The effects of Cr(VI) on the mitotic activity and RAM performance is influenced by SLR1/IAA14

Growth of WT *Arabidopsis* primary roots is indeterminate due to the activity of the RAM, where a balance between cell division and differentiation is necessary. To

determine the participation of SLR1/IAA14 protein in cell proliferation in response to Cr(VI), WT and *slr-1* plants harboring the *CycB1pro::uidA* marker, which is detected only in mitotic cells, were exposed to Cr(VI) in the horizontal application system, and gene expression investigated daily during 6 days after treatment. β-glucuronidase (GUS) reporter gene expression in WT seedlings did not change during the first three days after Cr(VI) application, but by days 3 and 4 expression increased and correlated with more robust root tips, and by day 6 it drastically diminished (Fig. 2). In contrast, root tips of the *slr-1* mutants exposed to Cr(VI) showed increased GUS expression with time (Fig. 2). At the end of the experiment, WT seedlings showed a 30% decrease in the length of the meristem while *slr-1* plants had a 20% increase (Fig. 2B). Surprisingly, the width of the root, measured at the meristem, increased both in the WT and *slr-1* mutants by 45% and 35%, respectively, as a response to Cr(VI) treatment (Fig. 2C). Thus, it can be concluded that the mitotic activity and the RAM size in the primary root are affected differentially by Cr(VI) in WT plants respect to *slr-1* mutants.

The size and surveillance of lateral root cap cells increase in response to Cr(VI)

In *Arabidopsis*, the root cap is comprised of four cell layers. To restrict growth of the root cap, the generation of new cells has to be compensated via disposal of the outermost layer, which experiences programmed cell death and degradation (Kumpf and Nowak 2015). The observation that Cr(VI) application increased root thickness (Fig. 2C), could be explained either by increased isodiametric growth of cells, by the formation of additional cell layers or both. To test these possibilities, detailed cellular analysis of lateral root cap cells was performed in *Arabidopsis* primary roots treated or not with Cr(VI), and stained with propidium iodide as a vital marker. In WT seedlings, cells of the outermost lateral root cap layer that in normal conditions are small and totally stained red by propidium iodide, in Cr(VI) increased in length, and remained alive for prolonged periods (Fig. 3A and B). In *slr-1* mutants, root meristem cells attained a normal morphology and increase in length of the lateral root cap cells was less evident that in the WT (Fig. 3A). These data show that the protective growth of the external cell layer of the root cap is compromised by the gain-of-function in SLR1/IAA14 and that this may be an adaptive trait to tolerate toxic levels of Cr(VI).

Cr(VI) influences distribution of auxin and the level of auxin transporters in *Arabidopsis* primary roots

Cr(VI) negatively regulates auxin transport and response in the primary root tip, and concomitantly, another auxin maximum is established at the site of adventitious roots initiation that drives its organogenesis (López-Bucio et al. 2015). To determine the temporal sequence of events elicited by Cr(VI) in *Arabidopsis* primary root and to establish the specific role of SLR1/IAA14 in this program, distribution of auxin and auxin transporters PIN1 and PIN7 were analyzed via mobilizing the *DR5pro::GFP*, *PIN1pro::PIN1::GFP* and *PIN7pro::PIN7::GFP* constructs to *slr-1* mutants, and comparing their expression levels with WT plants in a time course experiment. Cr(VI) treatment modulated auxin distribution in the primary root of WT plants by increasing auxin responsiveness in root tips by day 3 (Fig. 4d); this correlates with growth of the outermost lateral root cap layer (arrow), or decreasing it at later times. Such changes are associated with the loss of the meristem and the advancement of differentiation, since root hairs are formed close to the root tip (Fig 4f). In contrast, in *slr-1* mutants expression of *DR5pro::GFP* was increased by Cr(VI) in a time-dependent manner, as revealed by the green (GFP) or yellow (GFP plus propidium iodide red staining) in root tips (Fig 4 g, l), while no differentiation signs were evident at the end of the experiment. In a similar manner, the levels of auxin transporters PIN1 and PIN7 in primary roots were differentially modulated by Cr(VI) in WT plants and *slr-1* mutants (Fig. 5a-p). These results show that SLR1/IAA14 modifies the response to Cr(VI) orchestrating auxin response and distribution at the root tip.

Cr(VI) inhibits expression of meristem identity genes in a SLR1/IAA14 dependent manner

To test whether SLR1/IAA14 could influence stem cell maintenance acting as a mediator during the response to Cr(VI), expression of meristem identity markers *PLT1pro::GFP, PLT2pro::CFP*, *SHRpro::SHR::GFP* and *SCRpro::SCR::GFP,* was analyzed in the primary root of WT and *slr-1* mutants. *PLT1* expression was lost at day 5 following Cr(VI) treatment in WT and *slr-1* plants, while *PLT2* expression was terminated in WT but not in *slr-1* plants (Fig. 6). *SCR* expression notably decreased at

day 4 and disappeared at day 5 both in WT and *slr-1* mutants. SHR expression decreased from day 4 and disappeared at day 5 in WT plants, while in *slr-1* mutants it was sustained during the experiment (Fig. 7). These results show that expression of maintenance and identity genes are altered by exposure to Cr(VI) with the SLR1/IAA14 mediation.

DISCUSSION

In *Arabidopsis*, the root response to Cr(VI) is plastic and enables adaptation to its toxicity via structural and regulatory mechanisms that are starting to be revealed. Previous reports showed growth-inhibitory and early differentiation effects of Cr(VI) on primary roots, which were tightly correlated with de novo organogenesis, since lateral and adventitious root are developed in plants exposed to toxic concentrations of chromate (López-Bucio et al. 2015). To increase understanding of cellular mechanisms and developmental pathways underlying the responses of the root system to stress generated by Cr(VI), root growth, cell division activity, and root meristem structure, were correlated with auxin response and meristem identity genes in *Arabidopsis* WT plants and *slr-1* mutants; this was performed by using an *in vitro* system in which gradual exposure to Cr(VI) of the primary root allows monitoring earlier and late morphogenetic responses.

The lower inhibition of root growth, and overall growth of WT seedlings in the horizontal exposure to Cr(VI) (where it first reaches the primary root tip), when compared to the vertical application (reaching to the foliage and the entire root system), suggests that the root cap, possibly the lateral root cap, functions as a key Cr(VI) sensor. In this way, some kind of signals would move to distant parts of the root (i.e. pericycle cells), to elicit lateral root development. This finding is in line with the role proposed for the root cap as a structure that senses and transmits environmental signals to distant tissues to increase or halt root growth rates through auxin signalling (Tsugeki and Fedoroff 1999). Lack of primary root growth inhibition of *slr-1* mutants in the horizontal application system, suggests a critical role of SLR1/IAA14 in Cr(VI) response to this sensing mechanism. Therefore, it is possible that root meristem consumption and the start of determinate primary root growth occur when SLR1/IAA14 is degraded in WT plants following Cr(VI) application. Since *slr-1* is a dominant, gain- of-function mutant, where SLR1/IAA14 cannot be degraded (Fukaki et al. 2002), thus it remains

active to block auxin signalling acting as a repressor, which interferes with Cr(VI) modulated auxin responses.

In WT plants, Cr(VI) treatment increased width of the primary root, which correlated with an increase in size of cells of the lateral root cap. This response suggests a dramatic physiological alteration in these cells, which normally die as new cap cells are being produced, which may be of great adaptive significance towards improving tolerance to toxic Cr(VI) levels in the environment. In consonance, the genetic ablation of root cap cells causes isodiametric growth of meristem cells, induces differentiation of the meristem and stimulates formation of lateral roots (Tsugeki and Fedoroff 1999). Repression of primary root growth elicited by Cr(VI) is tightly correlated with an auxin response, as cell division and lateral root cap structure remain less affected in *slr-1* mutants.

Maintenance of cell division and root growth in *slr-1* mutants may be related to conservation of the auxin distribution area in the primary root, which unlike WT plants in which the auxin maximum is lost, it causes meristem differentiation. In the present study, the gradual decrease in auxin distribution in the primary root of WT plants by effect of Cr(VI) was also correlated with decreased levels in PIN1 and PIN7 proteins, which is in agreement with the function of these auxin efflux transporters in establishing auxin gradients within the root tip (Perilli et al. 2012). In a similar way, in *Arabidopsis* WT plants exposed to chromate, PIN1 and PIN2 transporters lost their expression, and this correlates with the strong capability of plants to produce adventitious roots, which replace primary roots for growth (López-Bucio et al. 2015). Indeed, the persistence of normal auxin transporter distribution in *slr-1* plants exposed to Cr(VI) directly correlated with the maintained primary root growth, which supports the role of auxin distribution for cell proliferating activity and indeterminate growth (Stepanova et al. 2008). Thus, SLR1/IAA14 mediates the normal auxin redistribution in the primary root as a direct response to Cr(VI).

The longitudinal and radial organization of the root is under hormonal control, where mainly auxin and transcriptional factors regulate meristem activity (Blilou et al. 2005). Decrease in expression of *PLT1* and its paralog *PLT2* in the primary root of WT plants correlated with decrease in auxin distribution and expression of PIN transporters. This can be explained because PIN and PLT proteins are part of a feedback loop mechanism for root meristem maintenance in response to the auxin gradient, also termed auxin maximum at the very root tip (Aida et al. 2004; Blilou et al. 2005; Aichinger et al. 2012). Accordingly, in *slr-1* plants exposed to Cr(VI), where a normal auxin distribution already occurs, *PLT2* expression was maintained, which suggests that expression of *PLT2* is sufficient for maintaining growth in the primary root. These data are consistent with the proposed redundancy in the functions of PLT1 and PLT2 proteins in identity determination of the stem cells of roots (Benfey et al. 1993; Aida et al. 2004) and with the additive and dosage-dependent function of these genes (Galinha et al. 2007).

In a similar way as the *PTL* genes, *SHR* expression was also abated in the primary roots of WT plants, in agreement with its function as a key regulator of root growth and development (Benfey et al. 1993; Di Laurenzio et al. 1996). In contrast, in *slr-1* plants, *SHR* expression although at a lower level, was maintained 5 days after Cr(VI) exposure, which corresponds with the SHR protein function in maintaining indeterminate growth in the root (Lucas et al. 2011). Additionally, loss of SHR activity led to major changes in the root, which gives rise to the fibrous architecture of the root system, as a part of the root phenotype produced by Cr(VI) in WT plants (López-Bucio et al. 2015).

Reduction of *SCR* expression in WT plants is consistent with decreased mitotic activity in the meristem, since the SCR protein sustains stem cell and meristem activity and prevents cell differentiation by suppressing *ARR1* transcription, a cytokinin signalling element (Moubayidin et al. 2010). The paradox of the visually lost expression of *SCR* in *slr-1* plants where the primary root continues growing, deserves further research, and allow us to devise two possibilities: i) the participation of SLR1/IAA14 downstream of SCR modifies the equilibrium between cell division and differentiation in response to

Cr(VI), or ii) the sensitivity or threshold to Cr(VI) is different in the primary root with the absence of lateral roots due to the gain-of-function of SLR1/IAA14.

The participation of SLR1/AA14 in mediating root responses to Cr(VI) in this work, is supported by the report of Shani et al. (2017) who showed that stress pathways interact with the auxin gene regulatory network through transcription of Aux/IAA genes, functioning as hubs that integrate genetic and environmental information to achieve the appropriate developmental program. Besides, in several types of stress there is a generic response that involves lateral root formation as a direct consequence (Potters et al. 2007), which could be demonstrated here, since SLR1/IAA14 mediates root responses to the stress generated by Cr(VI).

Taken together, our results suggest that: i) the root cap senses Cr(VI), allowing a better response for adapting the root architecture according to challenging growth situations, ii) SLR1/IAA14 directly or indirectly participate in maintaining normal auxin distribution, mitotic activity and expression of meristem identity genes in the primary root exposed to Cr(VI), and iii) the sensitivity or threshold to maintain growth in the primary root is mediated by SLR1/IAA14 by increasing the tolerance to stress and lengthening the time for transition from indeterminate to determinate growth.

REFERENCES

Aichinger E, Kornet N, Friedrich T, Laux T (2012) Plant stem cell niches. Annual Review of Plant Biology 63:615-636.

Aida M, Beis D, Heidstra R, Willemsen V, Blilou I, Galinha C, Nussaume L, Noh YS, Amasino R, Scheres B (2004) The *PLETHORA* genes mediate patterning of the *Arabidopsis* root stem cell niche. Cell 119:109-120.

Arnaud C, Bonnot C, Desnos T, Nussaume L (2010) The root cap at the forefront. Critical Reviews in Biology 333:335-343.

Bellini C, Pacurar DI, Perrone I (2014) Adventitious roots and lateral roots: similarities and differences. Annual Review of Plant Biology 65:639-666.

Benfey PN, Linstead PJ, Roberts K, Schiefelbein JW, Hauser MT, Aeschbacher RA (1993) Root development in *Arabidopsis*: four mutants with dramatically altered root morphogenesis. Development 119:57-70.

Benková E, Hejatko J (2009) Hormone interactions at the root apical meristem. Plant Molecular Biology 69:383-396.

Benková E, Michniewicz M, Sauer M, Teichmann T, Seifertová D, Jürgens G, Friml J (2003) Local, efflux-dependent auxin gradients as a common module for plant organ formation. Cell 115:591-602.

Blilou I, Xu J, Wildwater M, Willemsen V, Paponov I, Friml J, Heidstra R, Aida M, Palme K, Scheres B (2005) The PIN auxin efflux facilitator network controls growth and patterning in *Arabidopsis* roots. Nature 433:39-44.

Colón-Carmona A, You R, Haimovitch-Gal T, Doerner P (1999) Spatio-temporal analysis of mitotic activity with a labile cyclin–GUS fusion protein. Plant Journal 20:503-508.

Dharmasiri N, Dharmasiri S, Estelle M (2005) The F-box protein TIR1 is an auxin receptor.

Nature 435:441-445.

Di Laurenzio L, Wysocka-Diller J, Malamy JE, Pysh L, Helariutta Y, Freshour G, . . . Benfey PN (1996) The SCARECROW gene regulates an asymmetric cell division that is essential for generating the radial organization of the *Arabidopsis* Root. Cell 86:423-433.

Dolan L, Janmaat K, Willemsen V, Linstead P, Poethig S, Roberts K, Scheres B (1993) Cellular organisation of the *Arabidopsis thaliana* root. Development 119:71-84.

Epstein E, Bloom AJ (2004) Mineral nutrition of plants: Principles and Perspectives. Second edition. Sinnauer editorial. USA. 400 pp

Friml J (2003) Auxin transport — shaping the plant. Current Opinion in Plant Biology 6:7-12. Fukaki H, Tameda S, Masuda H, Tasaka M (2002) Lateral root formation is blocked by a gain-of-function mutation in the *SOLITARY-ROOT/IAA14* gene of *Arabidopsis*. Plant Journal 29:153-168.

Galinha C, Hofhuis H, Luijten M, Willemsen V, Blilou I, Heidstra R, Scheres B (2007) PLETHORA proteins as dose-dependent master regulators of *Arabidopsis* root development. Nature 449:1053-1057.

Gray WM, del Pozo JC, Walker L, Hobbie L, Risseeuw E, Banks T, Crosby WL, Yang M, Ma H, Estelle M (1999) Identification of an SCF ubiquitin–ligase complex required for auxin response in *Arabidopsis thaliana*. Genes and Development 13:1678-1691.

Hayat S, Khalique G, Irfan M, Wani AS, Tripathi BN, Ahmad A (2012) Physiological changes induced by chromium stress in plants: an overview. Protoplasma 249:599-611.

Holland SL, Avery SV (2011) Chromate toxicity and the role of sulfur. Metallomics 3:1119- 1123.

Kepinski S, Leyser O (2005) The Arabidopsis F-box protein TIR1 is an auxin receptor. Nature 435:446-451.

Kotaś J, Stasicka Z (2000) Chromium occurrence in the environment and methods of its speciation. Environmental Pollution 107:263-283.

Kumpf RP, Nowack MK (2015) The root cap: a short story of life and death. Journal of Experimental Botany 66:5651-5662.

Lee Y, Lee WS, Kim SH (2013) Hormonal regulation of stem cell maintenance in roots. Journal of Experimental Botany 64:1153-1165.

López-Bucio J, Ortiz-Castro R, Ruíz-Herrera LF, Juárez CV, Hernández-Madrigal F, Carreón- Abud Y, Martínez-Trujillo M (2015) Chromate induces adventitious root formation via auxin signalling and *SOLITARY-ROOT/IAA14* gene function in *Arabidopsis thaliana*. BioMetals 28:353-365.

Lucas M, Swarup R, Paponov IA, Swarup K, Casimiro I, Lake D, Peret B, Zappala S, Mairhofer S, Whitword M, Wang J, Ljung K, Marchant A, Sandberg G, Holdsworth MJ, Palme K, Pridmore T, Mooney S, Bennett MJ (2011) SHORT-ROOT regulates primary, lateral, and adventitious root development in *Arabidopsis*. Plant Physiology 155:384-398.

Martínez-Trujillo M, Méndez-Bravo A, Ortiz-Castro R, Hernández-Madrigal F, Ibarra-Laclette E, Ruiz-Herrera LF, Long TA, Cervantes C, Herrera-Estrella L, López-Bucio J (2014) Chromate alters root system architecture and activates expression of genes involved in iron homeostasis and signaling in *Arabidopsis thaliana*. Plant Molecular Biology 86:35- 50.

McGrath SP, Smith S (1990) Chromium and nickel. In: Heavy Metals in Soils Ed Alloway BJ. Wiley, New York pp 125-150.

Mockaitis K, Estelle M (2008) Auxin Receptors and Plant Development: A New Signaling Paradigm. Annual Review of Cell and Developmental Biology 24:55-80.

Moubayidin L, Perilli S, Dello Ioio R, Di Mambro R, Costantino P, Sabatini S (2010) The rate of cell differentiation controls the arabidopsis root meristem growth phase. Current Biology 20:1138-1143.

Muday GK, DeLong A (2001) Polar auxin transport: controlling where and how much. Trends in Plant Science 6:535-542.

Murashige T, Skoog F (1962) A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia Plantarum 15:473-497.

Muto H, Watahiki MK, Nakamoto D, Kinjo M, Yamamoto KT (2007) Specificity and similarity of functions of the Aux/IAA genes in auxin signaling of Arabidopsis revealed by promoter- exchange experiments among *MSG2/IAA19, AXR2/IAA7*, and *SLR/IAA14*. Plant Physiology 144:187-196.

Ottenschläger I, Wolff P, Wolverton C, Bhalerao RP, Sandberg G, Ishikawa H, Evans M, Palme K (2003) Gravity-regulated differential auxin transport from columella to lateral root cap cells. Proc Natl Acad Sci USA 100:2987-2991.

Péret B, Swarup K, Ferguson A, Seth M, Yang Y, Dhondt S, James N, Casimiro I, Perry P, Syed A, Yang H, Reemmer J, Venison E, Howells C, Prerez-Amador MA, Yung J, Alonso J, Beemster GTS, Laplaze L, Murphy A, Bennett MJ,

Nielsen E, Swarup R (2012) *AUX/LAX* genes encode a family of auxin influx transporters that perform distinct functions during *Arabidopsis* development. Plant Cell 24:2874-2885.

Perilli S, Di Mambro R, Sabatini S (2012) Growth and development of the root apical meristem.

Curr Opin Plant Biol 15:17-23.

Perrot-Rechenmann C (2010) Cellular responses to auxin: division versus expansion. Cold Spring Harbor Perspectives in Biology 2:a001446.

Petrášek J, Friml J (2009) Auxin transport routes in plant development. Development 136:2675-2688.

Reed JW (2001) Roles and activities of Aux/IAA proteins in *Arabidopsis*. Trends in Plant Science 6:420-425.

Ruiz-Herrera LF, Shane MW, López-Bucio J (2015) Nutritional regulation of root development.

Wiley Interdisciplinary Reviews: Developmental Biology 4: 431-443.

Ruiz-Rosquete M, Barbez E, Kleine-Vehn J (2012) Cellular auxin homeostasis: gatekeeping is housekeeping. Molecular Plant 5:772-786

Sabatini S, Beis D, Wolkenfelt H, Murfett J, Guilfoyle T, Malamy J, Benfey P, Leyser O, Bechtold N, Weisbeek P, Scheres B (1999) An auxin-dependent distal organizer of pattern and polarity in the *Arabidopsis* root. Cell 99:463-472.

Sabatini S, Heidstra R, Wildwater M, Scheres B (2003) SCARECROW is involved in positioning the stem cell niche in the *Arabidopsis* root meristem. Genes and Development 17:354-358.

Scheres B, Benfey P, Dolan L (2002) Root Development. The Arabidopsis Book/ American Society of Plant Biologists 1:e0101.

Shanker AK, Cervantes C, Loza-Tavera H, Avudainayagam S (2005) Chromium toxicity in plants. Environ Int 31:739-753.

Stepanova AN, Robertson-Hoyt J, Yun J, Benavente LM, Xie DY, Doležal K, Schleret A, Jürgens G, Alonso JM (2008) *TAA1*-Mediated auxin biosynthesis is essential for hormone crosstalk and plant development. Cell 133:177-191.

Tsugeki R, Fedoroff NV (1999) Genetic ablation of root cap cells in *Arabidopsis*. Proc Natl Acad Sci USA 96: 12941-12946.

Van den Berg C, Willemsen V, Hendriks G, Weisbeek P, Scheres B (1997) Short-range control of cell differentiation in the *Arabidopsis* root meristem. Nature 390: 287-289.

Vanneste S, Friml J (2009) Auxin: A trigger for change in plant development. Cell 136:1005- 1016.

Vieten A, Sauer M, Brewer PB, Friml J (2007) Molecular and cellular aspects of auxin-transport-mediated development. Trends in Plant Science 12: 160-168.

Viti C, Marchi E, Decorosi F, Giovannetti L (2014) Molecular mechanisms of Cr(VI) resistance in bacteria and fungi. FEMS Microbiology Reviews 38:633-659.

Weijers D, Benková E, Jäger KE, Schlereth A, Hamann T, Kientz M, Wilmoth J, Reed JW, Jürgens G (2005) Developmental specificity of auxin response by pairs of ARF and Aux/IAA transcriptional regulators. EMBO Journal 24:1874-1885.

Willemsen V, Bauch M, Bennett T, Campilho A, Wolkenfelt H, Xu J, Haseloff J, Scheres B (2008) The NAC domain transcription factors FEZ and SOMBRERO control the orientation of cell division plane in *Arabidopsis* root stem cells. Developmental Cell 15:913-922.

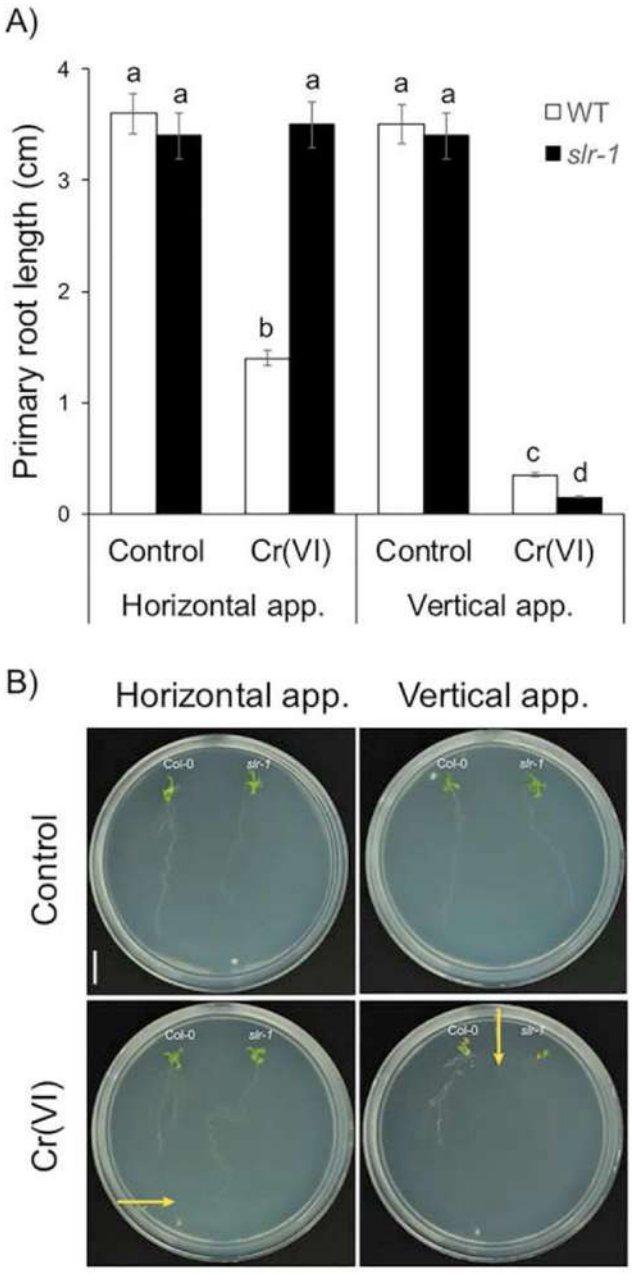


Figure 1. Growth of *Arabidopsis* WT and *slr-1* plants in medium with horizontal and vertical Cr(VI) applications. WT (ecotype Col-0) and mutant *slr-1* seeds were germinated in 0.2X MS medium. Six days after germination (dag), Cr(VI) was added in two directions (horizontal or vertical) with respect to the root growth axis. A) Primary root length of 12 dag plants; bars represent confidence interval from 15 plants; different letters indicate statistical differences for Tukey tests at P <0.05; the experiment was repeated twice with similar results. B) Representative 12 dag plants growing in the different media; bar = 1 cm; yellow arrows indicate the Cr(VI) applications in the medium. Note an opposite effect on the sensitivity of mutant *slr-1* to Cr(VI) in both systems.

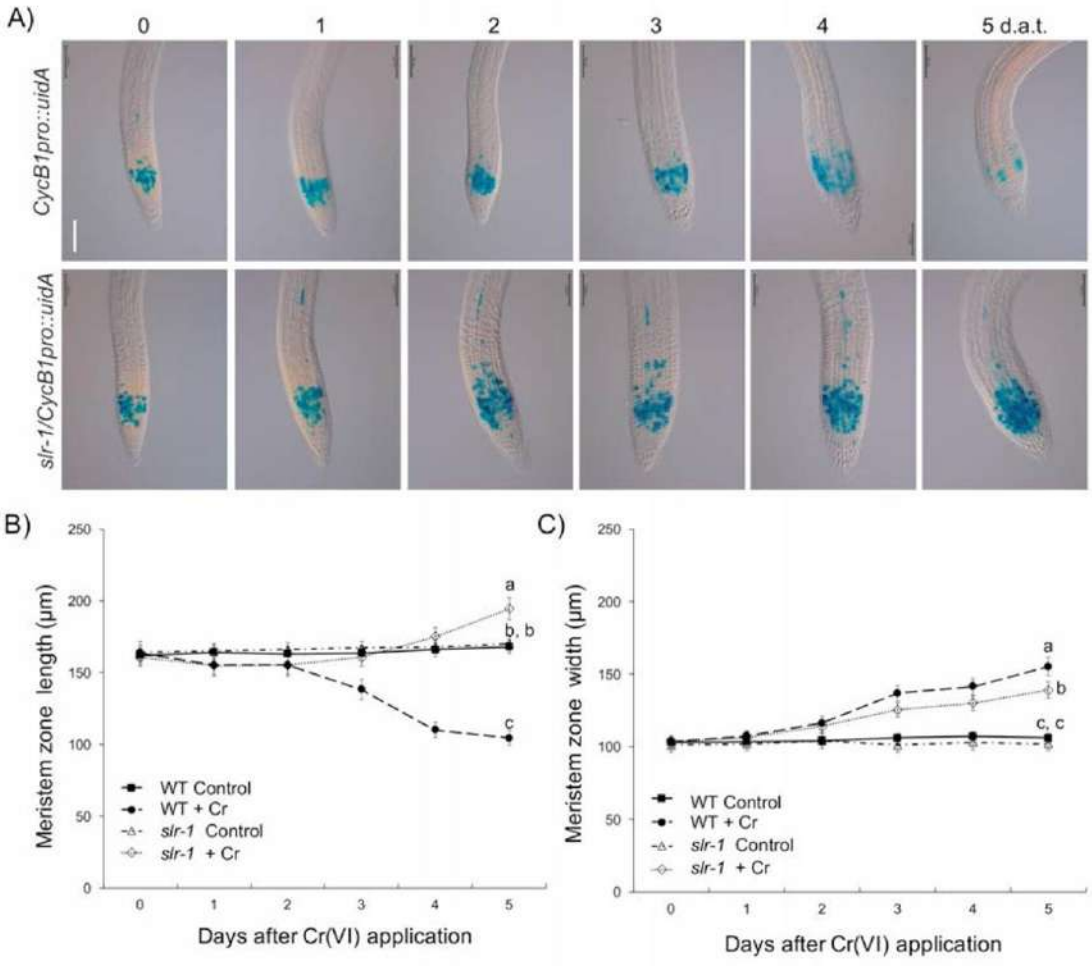


Figure 2. Mitotic activity and meristem size in the primary root of *Arabidopsis* WT and *slr-1* plants exposed to Cr(VI). WT and *slr-1 Arabidopsis* seeds harboring the *CycB1pro::uidA* gene were germinated in 0.2X MS medium and 4 days after germination plants were exposed to the horizontal Cr(VI) application. A) *CycB1pro::uidA* expression was analyzed daily for 6 days in WT and *slr-1* plants. Photographs show representative individuals from 15 cleared plants; bar = 150 µm; d.a.t. = days after treatment. Note an increase in *CycB1pro::uidA* expression in the first days of exposure in both WT and *slr-1*. In B) Meristem length, and C) Meristem width; bars represent confidence interval from 15 plants; different letters indicate statistical differences for Tukey tests at P <0.05. The experiment was repeated twice with similar results.

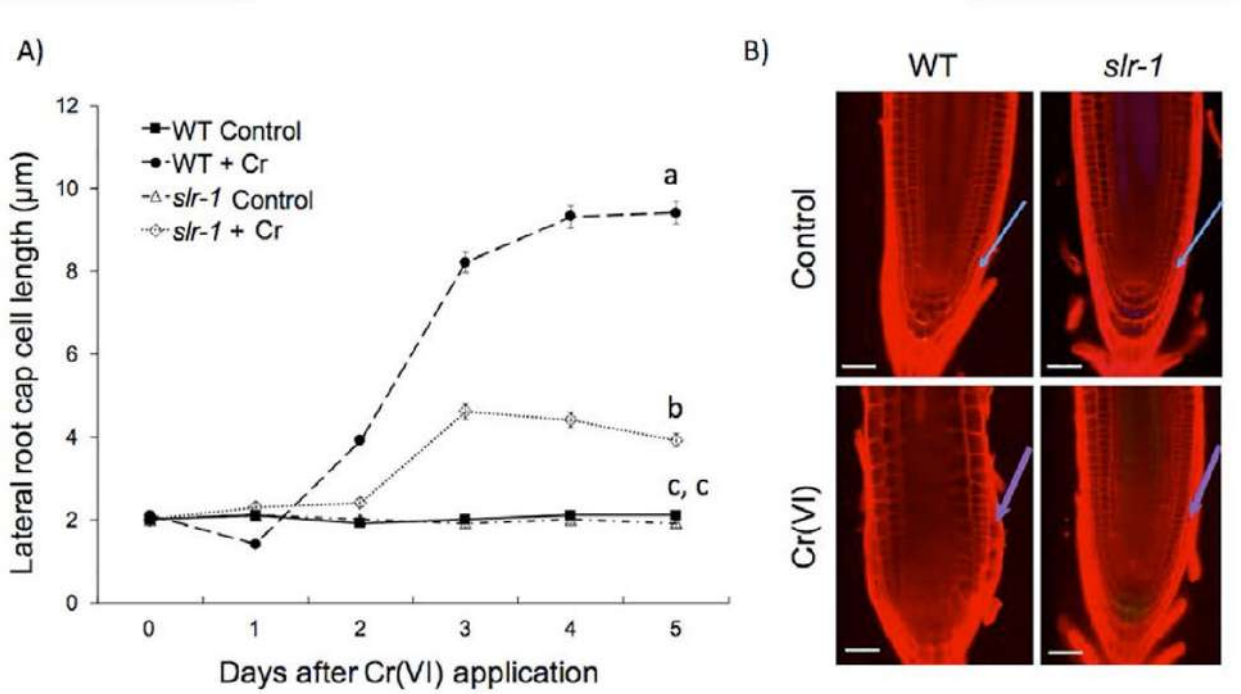


Figure 3. Effect of Cr(VI) on lateral root cap cells of the primary root in WT and *slr-1 Arabidopsis* plants. WT and mutant *slr-1* seeds were germinated in 0.2X MS medium and 4 days after germination plants were exposed to the horizontal Cr(VI) application for 6 days; day 0 is the time when Cr(VI) was applied. A) Kinetics of lateral root cap cells length; bars represent confidence interval from 15 plants; different letters indicate statistical differences for the Tukey test at P <0.05. B) Primary roots of plants at day 5; lateral root cap cells are indicated by arrows. Photographs are representative from 15 plants; bar = 50 µm. The experiment was repeated twice with similar results.

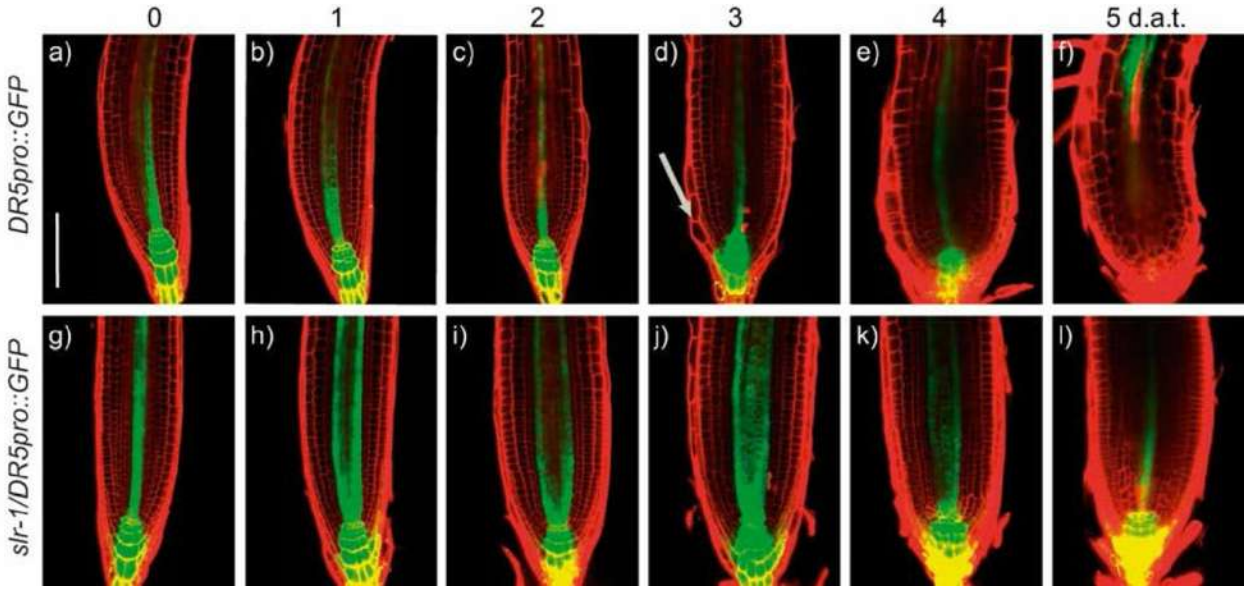


Figure 4. Effect of Cr(VI) on auxin distribution in the primary root in WT and *slr-1 Arabidopsis* plants. Seeds were germinated in 0.2X MS medium and 4 days after germination plants were exposed to the horizontal Cr(VI) application. Representative kinetic of the *DR5pro::GFP* expression pattern in WT (a-f) and *slr-1* plants (g-l). d.a.t. = days after treatment; day 0 is the time when Cr(VI) was applicated. Arrow indicates a lateral root cap cell. Plants were stained with propidium iodide and analyzed by confocal microscopy. Photographs are representative of 5 plants analyzed; bar = 200 µm.

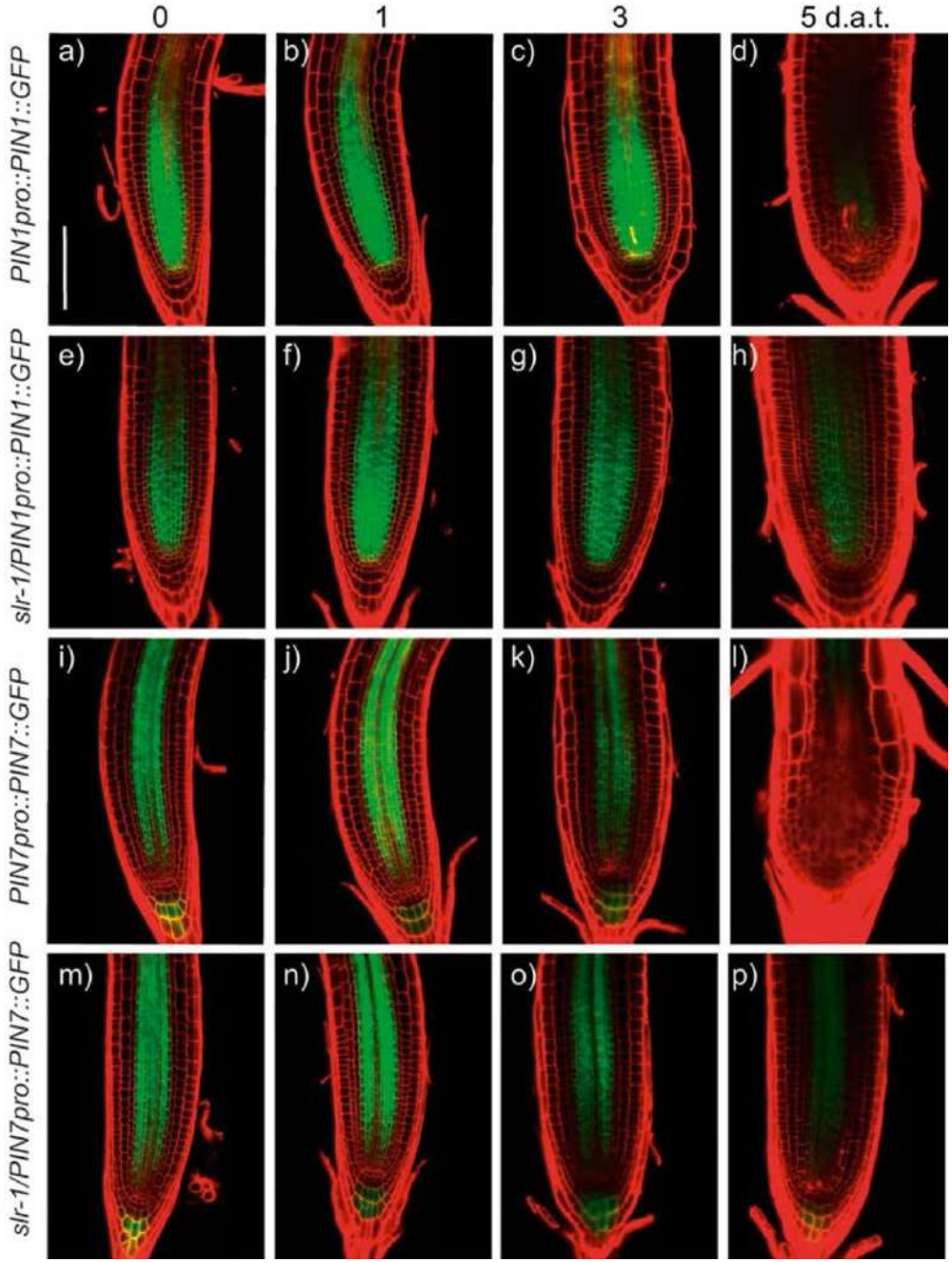


Figure 5. PIN1 and PIN7 expression in the primary root of WT and *slr-1* *Arabidopsis* plants in response to Cr(VI). Representative kinetics of *PIN1pro::PIN1::GFP* and *PIN7pro::PIN7:GFP* expression pattern in WT (a-f) and *slr-1* background (g-l). Seeds were germinated in 0.2X MS medium and 4 days after germination (d.a.g.) plants were exposed to the horizontal Cr(VI) application. Plants were stained with propidium iodide and analyzed by confocal microscopy. Photographs are representative of 5 plants analyzed; bar = 200 µm; d.a.t. = days after treatment. Note that in the PIN1 and PIN7, expression diminished more notably in WT than in *slr-*

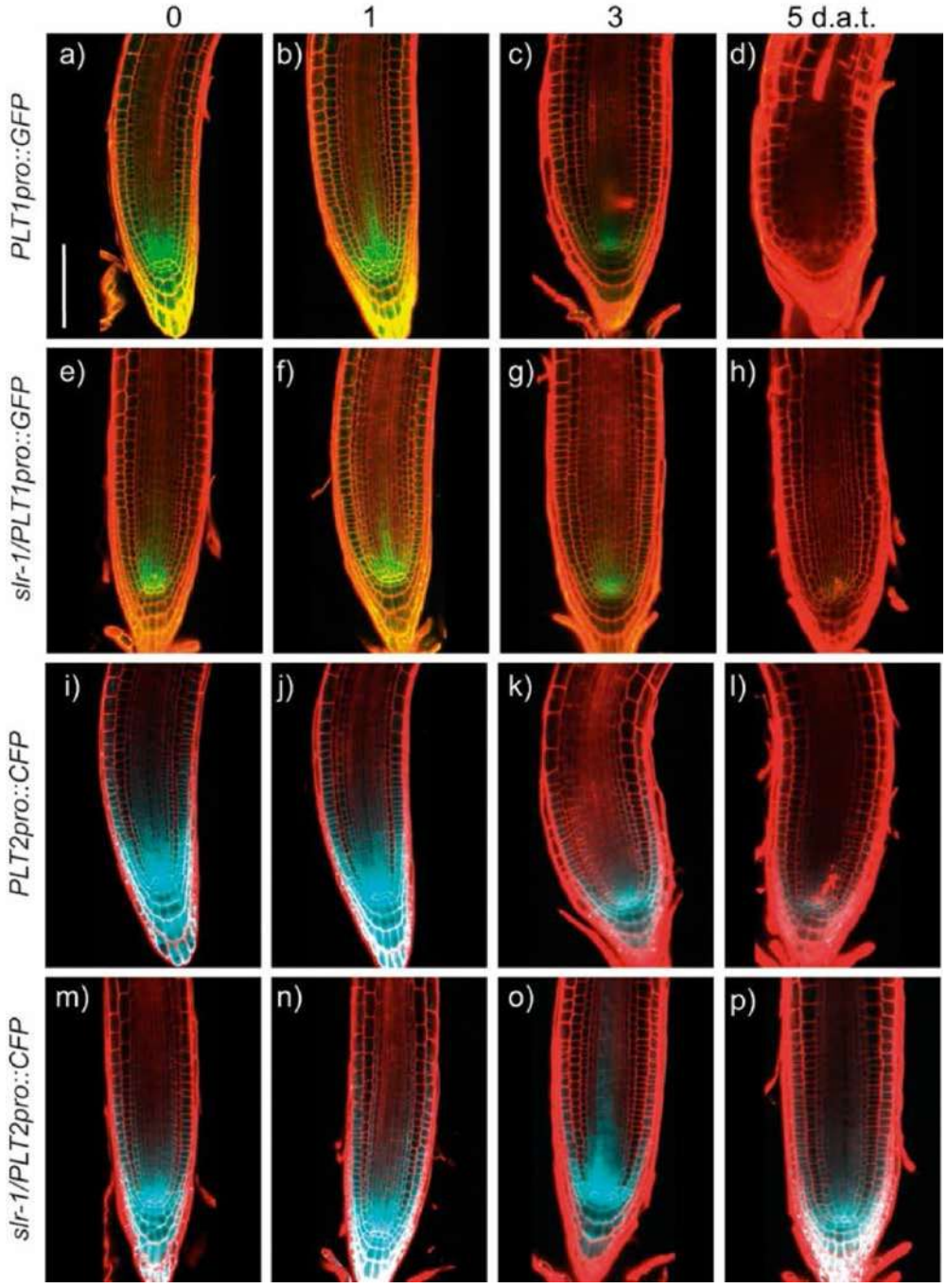


Figure 6. Expression of the *PLT1* and *PLT2* genes in the primary root of WT and *slr-1 Arabidopsis* plants in response to Cr(VI). Representative kinetics of *PLT1pro::GFP* and *PLT2pro::CFP* expression pattern in WT (a-f) and *slr-1* (g-l) backgrounds. Seeds were germinated in 0.2X MS medium and 4 days after germination plants were exposed to the horizontal Cr(VI) application. Plants were stained with propidium iodide and analyzed by confocal microscopy. Photographs are representative of 5 plants analyzed; bar = 200 µm; d.a.t. = days after treatment, day 0 is the time when Cr(VI) was applied. Note that *PLT2* expression diminished more notably in WT than in *slr-1*.

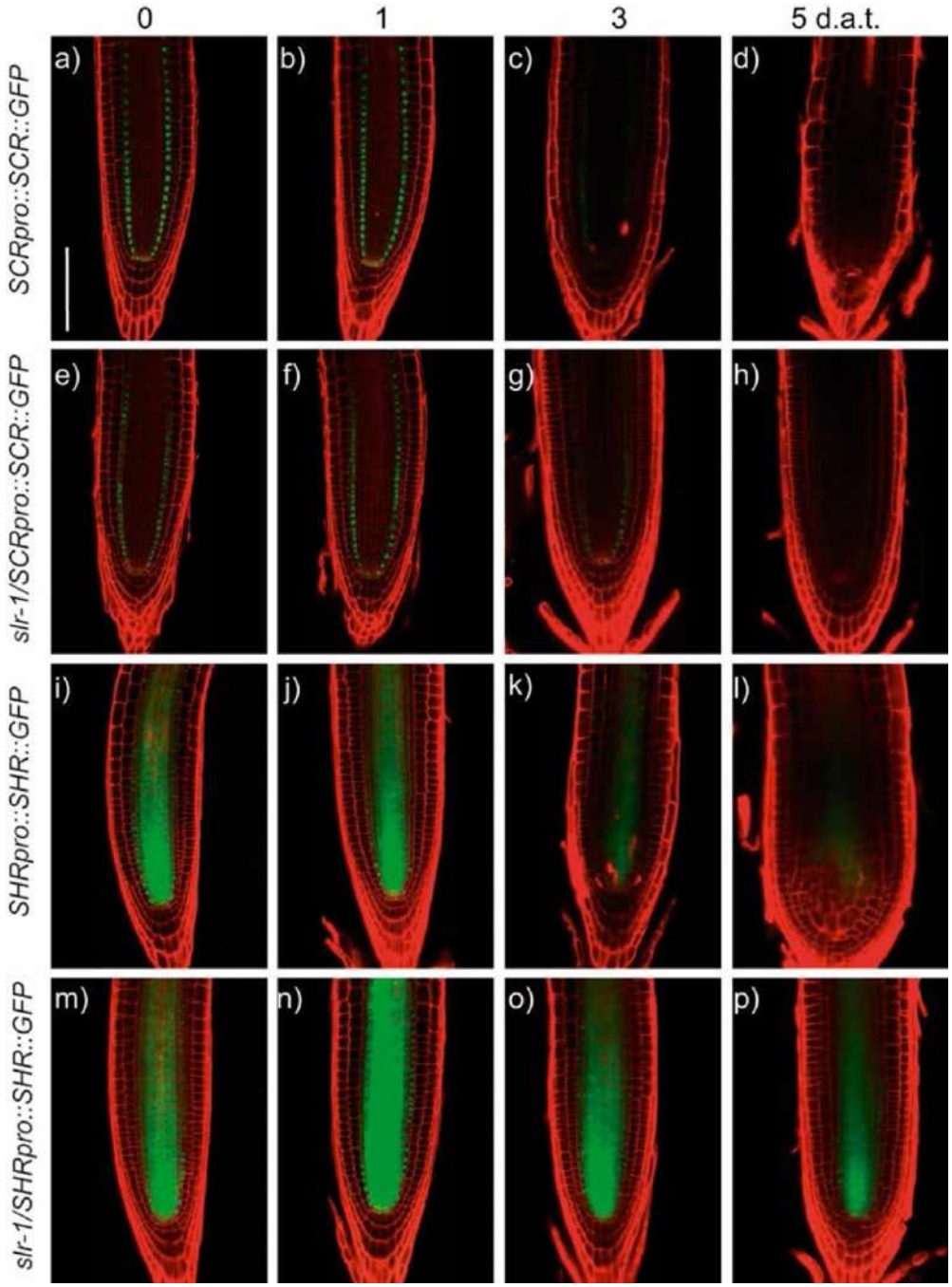
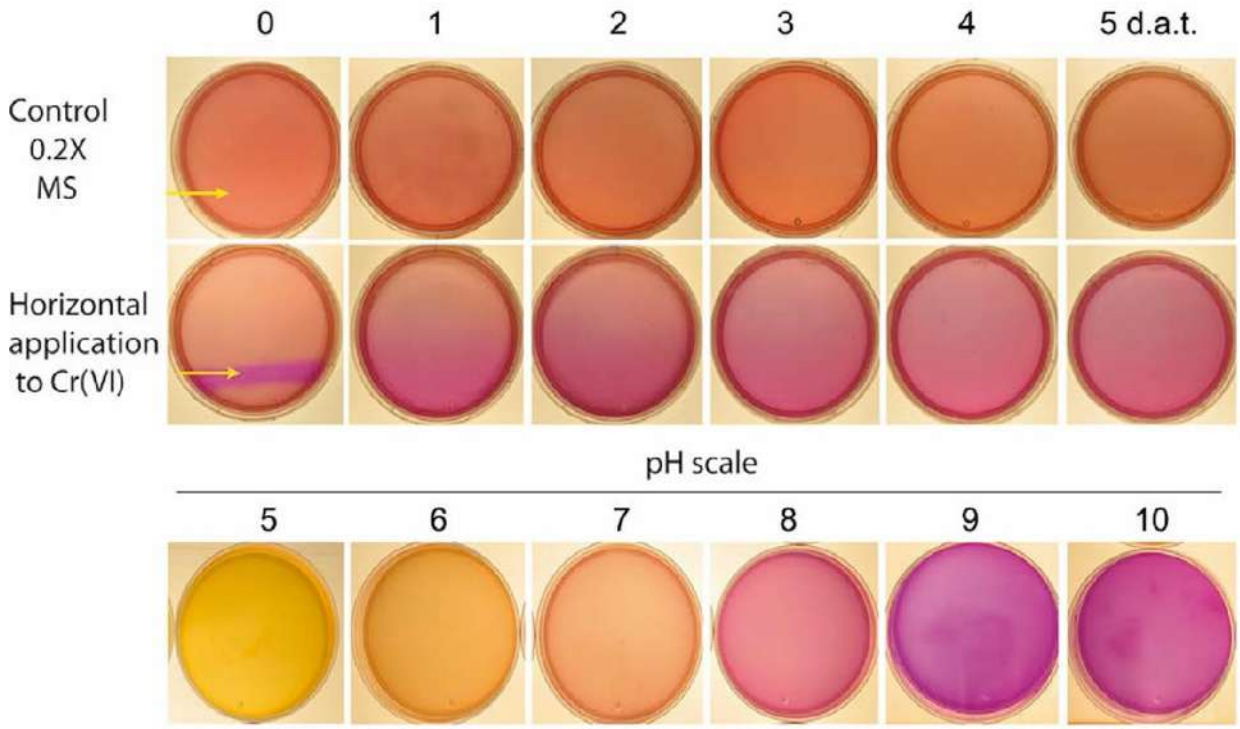


Figure 7. SHR and SCR expression in the primary root of WT and *slr-1* *Arabidopsis* plants in response to Cr(VI). Seeds were germinated in 0.2X MS medium and 4 days after germination plants were exposed to the horizontal Cr(VI) application. Representative kinetics of *SCRpro::SCR::GFP* and *SHRpro::SHR* expression pattern in WT (a-f) and *slr-1* (g-l) backgrounds. Plants were stained with propidium iodide and analyzed by confocal microscopy. Photographs are representative of 5 plants analyzed; bar = 200 µm. Note that SCR expression was more diminished in WT than in *slr-1*.



Supplementary Figure 1. Analysis of Cr(VI) diffusion in the medium by changes in pH. 0.2X MS medium were prepared with bromocresol purple as an indicator of pH changes. A) Growth 0.2X MS medium with and without application of Cr(VI) during 6 days; control and horizontal application of Cr(VI). B) pH scale in bromocresol purple, following the procedures described in materials and methods. D.a.t. = days after treatment, day 0 is the time when Cr(VI) was applied. Note that in Cr(VI) application, there is a gradual change in the pH of the medium, indicative of the formation of Cr(VI) gradients.

7. DISCUSIÓN GENERAL

En general, la respuesta al estrés involucra la inhibición del crecimiento para preservar las funciones vitales (Lastdrager et al. 2014). El estrés por Cr(VI) en *Arabidopsis* inhibe el crecimiento de la raíz primaria y genera una reprogramación en los patrones de desarrollo del sistema radical (López-Bucio et al. 2014). Los resultados obtenidos en este trabajo permitieron conocer la implicación de dos factores, el represor IAA14 y la sacarosa, en la mediación de las respuestas del sistema radical de *Arabidopsis* ante el estrés causado por Cr(VI).

7.1. La sacarosa mantiene el crecimiento de la raíz primaria ante el estrés del Cr(VI) en *Arabidopsis,* a través de un proceso mediado por la distribución de auxinas

En este trabajo, la toxicidad del Cr(VI) en *Arabidopsis* se correlacionó con una disminución de la expresión de genes relacionados con la distribución de auxinas, división celular e identidad de células iniciales, así como el efecto protector de la sacarosa en mantener los patrones de expresión de estos genes y por ende el mantenimiento del meristemo en presencia de Cr(VI).

Considerando que el Cr(VI) afecta la captación de nutrientes como P y Fe, generando un estrés nutricional (Shanker et al. 2005; López-Bucio et al. 2014; Martínez-Trujillo et al. 2014), las plantas se adaptan a estas condiciones mediante la activación de genes que incrementan la captación de nutrientes y modulan el crecimiento de la raíz (Eveland y Jackson 2012). Lo anterior explica la disminución en la expresión de *SUC2* en respuesta a Cr(VI), de manera similar a lo reportado por Lei et al. (2011) donde alteraciones en la expresión de *SUC2* inhiben la respuesta a la deficiencia de fosfato.

La deficiencia en la disponibilidad de sacarosa en la raíz por efecto del Cr(VI), causa modificaciones en los patrones de crecimiento y desarrollo del sistema radical, lo que sugiere la posible participación de rutas de respuesta a estrés dependientes de la concentración de carbohidratos en la planta. TOR y su contraparte SnRK1 forman parte de un complejo regulador maestro que integra información acerca del estado nutricional de las células, tejidos y de las condiciones externas del medio al metabolismo celular, para coordinar las respuestas en el desarrollo (Dobrenel et al.

2011; Dobrenel et al. 2016). Por lo que, la suplementación de sacarosa y su efecto en mejorar la tolerancia a Cr(VI) observada en nuestros resultados, sugieren que TOR puede promover el crecimiento a altas concentraciones de este azúcar en el medio. Lo anterior se apoya también en lo reportado por O'Hara y colaboradores (2013) referente a que la disponibilidad de carbohidratos se altera en respuesta a estrés, regulando el crecimiento y desarrollo de la planta, como la Trehalosa-6-fosfato (T6P), producto del metabolismo de sacarosa, que funciona como mensajero en la señalización del estado nutricional de la planta, con base en la disponibilidad de carbono.

El transporte de sacarosa, al igual que el de nutrientes y moléculas de señalización, posibilita el funcionamiento de los meristemos e influye en el crecimiento y desarrollo de la planta (Lastdrager et al. 2014). Debido a que los meristemos son poblaciones de células cuya actividad proliferativa necesita una provisión constante de carbohidratos para la obtención de energía, se requiere que su mantenimiento sea cuidadosamente coordinado con el estatus nutricional (Francis y Halford 2006). De manera que los resultados de este trabajo con respecto a la inhibición en la actividad mitótica por Cr(VI) y su reversión por sacarosa, aunado a la mediación de éste efecto por elementos implicados en la señalización y transporte de auxinas (PIN1, AUX1-7, TIR1), permite proponer que las redes moleculares que impulsan la división y expansión celular dependen en gran medida de la disponibilidad de carbohidratos.

Los cambios sobre la distribución de auxina en la raíz en respuesta a Cr(VI) y su restablecimiento por sacarosa, concuerda con los trabajos donde la homeostasis de auxina y su señalización provocan modificaciones en el desarrollo de la raíz dependientes de la concentración de carbohidratos (Mishra et al. 2009; Lilley et al. 2012; Sairanen et al. 2012). En esta acción concertada auxina-azúcar en el control de la división y elongación celular (Wang y Ruan 2013), la auxina promueve la proliferación celular, y a su vez, el metabolismo y transporte de auxinas es modulado por azúcares (Ljung 2013).

Los datos obtenidos demuestran que la suplementación de sacarosa promueve la expresión de genes que coordinan el ciclo celular (*CycB1*) y la identidad meristemática (*PLT1*), lo que es concordante con los reportes donde la sacarosa puede regular programas específicos del desarrollo y la transición entre éstos, mediante la expresión de genes que controlan el mantenimiento e identidad celular (Wu et al. 2005; Satoh-Nagasawa et al. 2006). Además, debido a que la auxina coordina la división celular y la expresión de los genes *PLT* en el meristemo, los cambios en el crecimiento de la raíz en respuesta a Cr(VI) dependientes de la concentración de sacarosa son también dependientes de la percepción y homeostasis de auxinas.

Considerados de manera conjunta, el encontrar que la sacarosa confiere tolerancia a las plantas de *Arabidopsis* al Cr(VI), abre nuevas perspectivas biotecnológicas, ya que el crecimiento de la raíz primaria es crucial para la sobrevivencia. La sacarosa mantuvo la distribución normal de auxina, y la identidad celular del meristemo, lo que sugiere que la sacarosa puede ser un regulador global para mantener el crecimiento de la raíz en concentraciones tóxicas de elementos no esenciales, en parte mediado por una vía de señalización hormonal.

7.2. SLR1/IAA14 mantiene el crecimiento de la raíz primaria, la distribución normal de auxina y la expresión de genes que confieren identidad al meristemo en condiciones sub-letales de Cr(VI)

La percepción de las señales ambientales para el crecimiento del sistema radical depende de la zona apical de la raíz primaria, como se ha demostrado para el gravitropismo (Blancaflor y Masson 2003; Morita y Tasaka 2004). La utilización de una mutante de ganancia de función (*slr-1/ iaa14*) (Fukaki et al. 2002), permitió caracterizar cómo esta condición influye en los mecanismos celulares y genéticos en la respuesta de la raíz primaria al estrés por Cr(VI), y cómo se correlacionan con la continuación de su crecimiento o su detención; es decir, con el cambio de un crecimiento indeterminado a uno determinado.

Las respuestas de *Arabidopsis* a la exposición horizontal de Cr(VI), demuestran que la percepción de este compuesto en la raíz por la zona apical permite una reprogramación del desarrollo del sistema radical con la emergencia de raíces laterales; además, la detención del crecimiento de la raíz primaria se correlacionó con el aumento de tamaño de las células en la capa lateral de la raíz (LRC, por sus siglas en ingles). Lo anterior apoya las propuestas de Barlow (2003) y Smith (2007), que consideran al extremo de la raíz, en particular las células LRC, como un componente clave en la percepción e integración de mecanismos relacionados en la respuesta al ambiente.

La continuación del crecimiento de la raíz primaria de la mutante *slr1/ iaa14* ante la exposición eventual al Cr(VI), a diferencia de las plantas silvestres, demostró la importancia del represor IAA14 en la respuesta al estrés, por lo que participando de manera directa o indirecta al impedir la formación de raíces laterales, regula la actividad meristemática inhibiendo el crecimiento de la raíz. Lo anterior se suma a los reportes acerca del papel de las proteínas Aux/IAAs como centros que integran información genética y ambiental para desencadenar una respuesta óptima en el desarrollo de la raíz y su adaptación al estrés, regulando de esta manera la actividad meristemática (Yuan y Huang 2016; Shani et al. 2017).

La respuesta diferencial a la exposición eventual al Cr(VI) entre las plantas silvestres y la mutante *slr1/iaa14* confirmaron lo establecido en reportes referente a que las señales locales dependientes de gradientes de auxina, como las proteínas PLETHORA (PLT) y SCARECROW (SCR)/SHORTROOT (SHR), son necesarias para el mantenimiento de la actividad meristemática (Sabatini et al. 2003; Aida et al. 2004). Estos resultados se correlacionan con los obtenidos previamente por López-Bucio y colaboradores (2015), referentes a que la distribución de auxina decrece al detenerse el crecimiento de la raíz primaria, mientras que se establece un patrón normal de auxina en las raíces adventicias formadas. De esta forma, la presencia de IAA14 y su función en la regulación negativa en la iniciación de raíces laterales no permite un cambio de la distribución de auxinas en plantas expuestas a Cr(VI), debido a la ausencia de ramificaciones, lo que genera una presencia constante de auxinas en la raíz primaria y su crecimiento indeterminado en esta condición de estrés.

La distribución de auxinas en la raíz depende de los transportadores PIN (Blilou et al. 2005; Dello Ioio et al. 2008); los gradientes de auxina formados son necesarios para mantener la división celular en el meristemo, lo que se correlaciona con los resultados obtenidos sobre la pérdida de PIN1 y PIN7 en la raíz primaria por exposición horizontal de Cr(VI) en plantas silvestres y con lo reportado para PIN1 y PIN2 en plantas silvestres de *Arabidopsis* germinadas y crecidas en medios suplementados con Cr(VI) (López-Bucio et al. 2015).

Es evidente que la participación de IAA14 al impedir el desarrollo de raíces laterales mantiene una distribución y transporte de auxina normal en la raíz primaria ante la exposición eventual al Cr(VI), sin embargo, no se descarta una participación más directa en el mantenimiento del nicho meristemático, como ocurre con otros represores de la señalización de auxina: tal es el caso de SHY2/IAA3 que es considerado clave en la diferenciación del meristemo coordinando la represión de los genes PIN (Dello Ioio et al. 2008).

Con lo mencionado anteriormente se puede concluir que, ante la exposición eventual de Cr(VI), la presencia de IAA14 y el impedimento en la iniciación de raíces laterales de manera indirecta o directa mantiene la distribución normal de auxina en la raíz primaria, lo que conlleva a la expresión de los genes de división celular y de identidad del meristemo, manteniendo el crecimiento indeterminado de la raíz primaria.

8. CONCLUSIÓN GENERAL

Las respuestas del crecimiento post-embrionario del sistema radical de *Arabidopsis* ante el estrés por Cr(VI) son modificadas por dos factores: el represor IAA14 y la sacarosa, los cuales permiten el mantenimiento de la distribución normal de auxina y sus transportadores en la raíz primaria, lo que permite la división celular y la expresión de genes de mantenimiento del meristemo, sugiriendo papeles reguladores para estos factores en las respuestas al estrés generado por Cr(VI).

9. REFERENCIAS

Abel S, Theologis A (1996) Early genes and auxin action Plant physiology 111:9-17

Abel S, Theologis A (2010) Odyssey of Auxin. Cold Spring Harbor Perspectives in Biology 2:a004572 doi:10.1101/cshperspect.a004572

Aida M, Beis D, Heidstra R, Willemsen V, Blilou I, Galinha C, Nussaume L, Noh YS, Amasino R, Scheres B (2004) The PLETHORA genes mediate patterning of the Arabidopsis root stem cell niche. Cell 119:109-120 doi:10.1016/j.cell.2004.09.018

Armienta-Hernández MA, Rodríguez-Castillo R (1995) Environmental exposure to chromium compounds in the valley of León, México. Environmental Health Perspectives 103:47-51

Baena-González E, Sheen J (2008) Convergent energy and stress signaling. Trends in Plant Science 13:474-482 doi:10.1016/j.tplants.2008.06.006

Barlow PW (2003) The Root Cap: Cell Dynamics, Cell Differentiation and Cap Function Journal of Plant Growth Regulation 21:261-286

Bellini C, Pacurar DI, Perrone I (2014) Adventitious Roots and Lateral Roots: Similarities and Differences. Annual Review of Plant Biology 65:639-666 doi:10.1146/annurev-arplant- 050213-035645

Benjamins R, Scheres B (2008) Auxin: The Looping Star in Plant Development. Annual Review of Plant Biology 59:443-465 doi:10.1146/annurev.arplant.58.032806.103805

Benkova E, Hejatko J (2009) Hormone Interactions at the Root Apical Meristem. Plant molecular biology 69:383-396 doi:10.1007/s11103-008-9393-6

Bennetzen JL (2001) Arabidopsis arrives. Nature genetics 27:3 doi:10.1038/83726

Blancaflor EB, Masson PH (2003) Plant gravitropism. Unraveling the ups and downs of a complex process. Plant physiology 133:1677-1690 doi:10.1104/pp.103.032169

Blilou I, Xu J, Wildwater M, Willemsen V, Paponov I, Friml J, Heidstra R, Aida M, Palme K, Scheres B (2005) The PIN auxin efflux facilitator network controls growth and patterning in Arabidopsis roots. Nature 433:39-44.

Calderon-Villalobos LI, Tan X, Zheng N, Estelle M (2010) Auxin perception--structural insights. Cold Spring Harb Perspect Biol 2:a005546 doi:10.1101/cshperspect.a005546

Casimiro I, Beeckman T, Graham N, Bhalerao R, Zhang H, Casero P, Sandberg G, Bennett MJ (2003) Dissecting Arabidopsis Lateral Root Development.Trends in Plant Science 8:165- 171 doi:10.1016/S1360-1385(03)00051-7

Celenza JL, Jr., Grisafi PL, Fink GR (1995) A pathway for lateral root formation in Arabidopsis thaliana Genes & development 9:2131-2142

Cervantes C, Campos-García J (2007) Reduction and Efflux of Chromate by Bacteria. In: Nies DH, Silver S (eds) Molecular Microbiology of Heavy Metals. Springer Berlin Heidelberg, Berlin, Heidelberg, pp 407-419. doi:10.1007/7171_2006_087

De Smet I (2012) Lateral root initiation: one step at a time New Phytologist 193:867-873 doi:10.1111/j.1469-8137.2011.03996.x

De Smet I, Lau S, Voß U, Vanneste S, Benjamins R, Rademacher EH, Schlereth A, De Rybel B, Vassileva V, Grunewald W, Naudts M, Levesque MP, Ehrismann JS, Inzé D, Luschnig C, Benfey PN, Weijers D, Van Montagu MCE, Bennett MJ,

Jürgens G, Beeckman T (2010) Bimodular auxin response controls organogenesis in <em>Arabidopsis</em&gt Proceedings of the National Academy of Sciences 107:2705

Dello Ioio R, Nakamura K, Moubayidin L, Perilli S, Taniguchi M, Morita MT, Aoyama T, Costantino P, Sabatini S (2008) A genetic framework for the control of cell division and differentiation in the root meristem Science 322:1380-1384 doi:10.1126/science.1164147

Dharmasiri N, Dharmasiri S, Estelle M (2005) The F-box protein TIR1 is an auxin receptor Nature 435:441 doi:10.1038/nature03543

Di Laurenzio L, Wysocka-Diller J, Malamy JE, Pysh L, Helariutta Y, Freshour G, Hahn MG, Feldmann KA, Benfey PN (1996) The SCARECROW Gene Regulates an Asymmetric Cell Division That Is Essential for Generating the Radial Organization of the Arabidopsis Root Cell 86:423-433 doi:https://doi.org/10.1016/S0092-8674(00)80115-4

Dobrenel T, Caldana C, Hanson J, Robaglia C, Vincentz M, Veit B, Meyer C (2016) TOR Signaling and Nutrient Sensing. Annual Review of Plant Biology 67:261-285

Dobrenel T, Marchive C, Sormani R, Moreau M, Mozzo M, Montane MH, Menand B, Robaglia C, Meyer C (2011) Regulation of plant growth and metabolism by the TOR kinase. Biochemical Society transactions 39:477-481 doi:10.1042/BST0390477

Doerner P (2003) Plant Meristems: A Merry-Go-Round of Signals. Review Current Biology 13:R368-R374 doi:10.1016/S0960-9822(03)00280-X

Dolan L, Janmaat K, Willemsen V, Linstead P, Poethig S, Roberts K, Scheres B (1993) Cellular organisation of the Arabidopsis thaliana root Development 119:71-84

Dreher KA, Brown J, Saw RE, Callis J (2006) The Arabidopsis Aux/IAA Protein Family Has Diversified in Degradation and Auxin Responsiveness The Plant cell 18:699-714 doi:10.1105/tpc.105.039172

Dubrovsky JG, Napsucialy-Mendivil S, Duclercq J, Cheng Y, Shishkova S, Ivanchenko MG, Friml J, Murphy AS, Benková E (2011) Auxin minimum defines a developmental window for lateral root initiation. New Phytologist 191:970-983 doi:10.1111/j.1469-8137.2011.03757.x

Eveland AL, Jackson DP (2012) Sugars, signalling, and plant development Journal of Experimental Botany 63:3367-3377 doi:10.1093/jxb/err379

Epstein E, Bloom AJ (2004) Mineral nutrition of plants: Principles and Perspectives. Second edition. Sinnauer editorial. USA. 400 pp

Forde B, Lorenzo H (2001) The Nutritional Control of Root Development. Plant and Soil 232:51– 68

Francis D, Halford NG (2006) Nutrient Sensing in Plant Meristems. Plant molecular biology 60:981-993 doi:10.1007/s11103-005-5749-3

Fukaki H, Tameda S, Masuda H, Tasaka M (2002) Lateral root formation is blocked by a gain- of-function mutation in the SOLITARY-ROOT/IAA14 gene of Arabidopsis. The Plant Journal 29:153-168 doi:10.1046/j.0960-7412.2001.01201.x

Galinha C, Hofhuis H, Luijten M, Willemsen V, Blilou I, Heidstra R, Scheres B (2007) PLETHORA proteins as dose-dependent master regulators of

Arabidopsis root development. Nature 449:1053-1057 doi:10.1038/nature06206
Gardea-Torresdey JL, de la Rosa G, Peralta-Videa JR, Montes M, Cruz-Jimenez G, Cano- Aguilera I (2005) Differential Uptake and Transport of Trivalent and Hexavalent Chromium by Tumbleweed (Salsola kali). Archives of Environmental Contamination and Toxicology 48:225-232 doi:10.1007/s00244-003-0162-x
Giehl RFH, Gruber BD, von Wirén N (2014) It's time to make changes: modulation of root system architecture by nutrient signals. Journal of Experimental Botany 65:769-778 doi:10.1093/jxb/ert421
Gottwald JR, Krysan PJ, Young JC, Evert RF, Sussman MR (2000) Genetic evidence for the in planta role of phloem-specific plasma membrane sucrose transporters. Proceedings of the National Academy of Sciences of the United States of America 97:13979-13984
Gray WM (2004) Hormonal Regulation of Plant Growth and Development. PLoS Biology 2:e311 doi:10.1371/journal.pbio.0020311
Hammond JP, White PJ (2008) Sucrose transport in the phloem: integrating root responses to phosphorus starvation. Journal of Experimental Botany 59:93-109 doi:10.1093/jxb/erm221
Hayashi K-i (2012) The Interaction and Integration of Auxin Signaling Components. Plant and Cell Physiology 53:965-975 doi:10.1093/pcp/pcs035
Hayat S, Khalique G, Irfan M, Wani AS, Tripathi BN, Ahmad A (2012) Physiological changes induced by chromium stress in plants: an overview Protoplasma 249:599-611 doi:10.1007/s00709-011-0331-0
Holland SL, Avery SV (2011) Chromate toxicity and the role of sulfur. Metallomics 3:1119-1123 doi:10.1039/c1mt00059d
Ishikawa H, Evans ML (1995) Specialized zones of development in roots. Plant physiology 109:725-727
Katz SA, Salem H (1993) The toxicology of chromium with respect to its chemical speciation: A review. Journal of Applied Toxicology 13:217-224 doi:10.1002/jat.2550130314
Kawanishi S, Inoue S, Sano S (1986) Mechanism of DNA cleavage induced by sodium cromate (VI) in the presence of hydrogen peroxide. J BiolChem 261: 5952-5958
Laskowski MJ, Williams ME, Nusbaum HC, Sussex IM (1995) Formation of lateral root meristems is a two-stage process. Development 121:3303
Lastdrager J, Hanson J, Smeekens S (2014) Sugar signals and the control of plant growth and development. Journal of Experimental Botany 65:799-807 doi:10.1093/jxb/ert474
Lei M, Liu Y, Zhang B, Zhao Y, Wang X, Zhou Y, Raghothama KG, Liu D (2011) Genetic and Genomic Evidence That Sucrose Is a Global Regulator of Plant Responses to Phosphate Starvation in Arabidopsis. Plant physiology 156:1116-1130 doi:10.1104/pp.110.171736
Lilley JL, Gee CW, Sairanen I, Ljung K, Nemhauser JL (2012) An endogenous carbon-sensing pathway triggers increased auxin flux and hypocotyl elongation. Plant physiology 160:2261-2270 doi:10.1104/pp.112.205575
Ljung K (2013) Auxin metabolism and homeostasis during plant development. Development 140:943
Lopez-Bucio J, Hernandez-Madrigal F, Cervantes C, Ortiz-Castro R, Carreon-Abud Y, Martínez-Trujillo M (2014) Phosphate relieves chromium toxicity in Arabidopsis

thaliana plants by interfering with chromate uptake. Biometals 27:363-370 doi:10.1007/s10534-014-9718-7

López-Bucio J, Ortiz-Castro R, Ruíz-Herrera LF, Juárez CV, Hernández-Madrigal F, Carreón- Abud Y, Martínez-Trujillo M (2015) Chromate induces adventitious root formation via auxin signalling and SOLITARY-ROOT/IAA14 gene function in *Arabidopsis thaliana*. BioMetals 28:353-365 doi:10.1007/s10534-015-9838-8

Malamy JE, Benfey PN (1997) Organization and cell differentiation in lateral roots of Arabidopsis thaliana. Development 124:33-44

Mansfield SG, Briarty LG (1991) Early embryogenesis in Arabidopsis thaliana. II. The developing embryo. Canadian Journal of Botany 69:461-476 doi:10.1139/b91-063

Martínez-Trujillo M, Méndez-Bravo A, Ortiz-Castro R, Hernández-Madrigal F, Ibarra-Laclette E, Ruiz-Herrera LF, Long TA, Cervantes C, Herrera-Estrella L, López-Bucio J (2014) Chromate alters root system architecture and activates expression of genes involved in iron homeostasis and signaling in Arabidopsis thaliana. Plant Molecular Biology 86:35- 50 doi:10.1007/s11103-014-0210-0

Mayer U, Ruiz RAT, Berleth T, Miséra S, Jürgens G (1991) Mutations affecting body organization in the Arabidopsis embryo. Nature 353:402 doi:10.1038/353402a0

McGrath SP, Smith S (1990) Chromium and nickel. In: Heavy Metals in Soils Ed Alloway BJ. Wiley, New York pp 125-150

Michniewicz M, Zago MK, Abas L, Weijers D, Schweighofer A, Meskiene I, Heisler MG, Ohno C, Zhang J, Huang F, Schwab R, Weigel D, Meyerowitz EM, Luschnig C, Offringa R, Friml J (2007) Antagonistic Regulation of PIN Phosphorylation by PP2A and PINOID Directs Auxin Flux. Cell 130:1044-1056 doi:10.1016/j.cell.2007.07.033

Mishra BS, Singh M, Aggrawal P, Laxmi A (2009) Glucose and auxin signaling interaction in controlling Arabidopsis thaliana seedlings root growth and development. PloS one 4:e4502 doi:10.1371/journal.pone.0004502

Morita MT, Tasaka M (2004) Gravity sensing and signaling. Current opinion in plant biology 7:712-718 doi:10.1016/j.pbi.2004.09.001

Mudgil Y, Karve A, Teixeira PJPL, Jiang K, Tunc-Ozdemir M, Jones AM (2016) Photosynthate Regulation of the Root System Architecture Mediated by the Heterotrimeric G Protein Complex in Arabidopsis. Frontiers in plant science 7:1255 doi:10.3389/fpls.2016.01255

O'Hara LE, Paul MJ, Wingler A (2013) How Do Sugars Regulate Plant Growth and Development? New Insight into the Role of Trehalose-6-Phosphate. Molecular Plant 6:261-274 doi:https://doi.org/10.1093/mp/sss120

Okushima Y, Fukaki H, Onoda M, Theologis A, Tasaka M (2007) ARF7 and ARF19 regulate lateral root formation via direct activation of LBD ／ ASL genes in Arabidopsis. Plant Cell 19: 118–130.

Orlando DA, Brady SM, Koch JD, Dinneny JR, Benfey PN (2009) Manipulating Large-Scale Arabidopsis Microarray Expression Data: Identifying Dominant Expression Patterns and Biological Process Enrichment. Methods in molecular biology (Clifton, NJ) 553:57- 77 doi:10.1007/978-1-60327-563-7_4

Ortiz-Castro R, Martínez-Trujillo M, López-Bucio J, Cervantes C, Dubrovsky J (2007) Effects of dichromate on growth and root system architecture of Arabidopsis thaliana seedlings. Plant Science 172: 684-691

Overvoorde PJ, Okushima Y, Alonso JM, Chan A, Chang C, Ecker JR, Hughes B, Liu A, Onodera C, Quach H, Smith A, Yu G, Theologis A (2005) Functional Genomic

Analysis of the AUXIN/INDOLE-3-ACETIC ACID Gene Family Members in Arabidopsis thaliana The Plant cell 17:3282-3300 doi:10.1105/tpc.105.036723
Péret B, Swarup K, Ferguson A, Seth M, Yang Y, Dhondt S, James N, Casimiro I, Perry P, Syed A, Yang H, Reemmer J, Venison E, Howells C, Perez-Amador MA, Yun J, Alonso J, Beemster GTS, Laplaze L, Murphy A, Bennett MJ, Nielsen E, Swarup R (2012) AUX/LAX Genes Encode a Family of Auxin Influx Transporters That Perform Distinct Functions during Arabidopsis Development. The Plant cell 24:2874-2885 doi:10.1105/tpc.112.097766
Petrášek J, Mravec J, Bouchard R, Blakeslee JJ, Abas M, Seifertová D, Wiśniewska J, Tadele Z, Kubeš M, Čovanová M, Dhonukshe P, Skůpa P, Benková E, Perry L, Křeček P, Lee OR, Fink GR, Geisler M, Murphy AS, Luschnig C, Zažímalová E, Friml J (2006) PIN Proteins Perform a Rate-Limiting Function in Cellular Auxin Efflux. Science 312:914
Petricka JJ, Winter CM, Benfey PN (2012) Control of Arabidopsis Root Development. Annual Review of Plant Biology 63:563-590 doi:10.1146/annurev-arplant-042811-105501
Potters G, Pasternak TP, Guisez Y, Jansen MAK (2009) Different stresses, similar morphogenic responses: integrating a plethora of pathways. Plant, Cell & Environment 32:158-169 doi:10.1111/j.1365-3040.2008.01908.x
Ruan Y-L (2014) Sucrose Metabolism: Gateway to Diverse Carbon Use and Sugar Signaling. Annual Review of Plant Biology 65:33-67 doi:10.1146/annurev-arplant-050213-040251
Sabatini S, Beis D, Wolkenfelt H, Murfett J, Guilfoyle T, Malamy J, Benfey P, Leyser O, Bechtold N, Weisbeek P, Scheres B (1999) An auxin-dependent distal organizer of pattern and polarity in the Arabidopsis root. Cell 99:463-472
Sabatini S, Heidstra R, Wildwater M, Scheres B (2003) SCARECROW is involved in positioning the stem cell niche in the Arabidopsis root meristem Genes & development 17:354-358 doi:10.1101/gad.252503
Sairanen I, Novák O, Pěnčík A, Ikeda Y, Jones B, Sandberg G, Ljung K (2012) Soluble Carbohydrates Regulate Auxin Biosynthesis via PIF Proteins in Arabidopsis. The Plant cell 24:4907-4916 doi:10.1105/tpc.112.104794
Santner A., Calderon-Villalobos L & Estelle M (2009) Plant hormones are versatile chemical regulators of plant growth. Nature chemical biology*, 5*, 301. doi: 10.1038/nchembio.165
Satoh-Nagasawa N, Nagasawa N, Malcomber S, Sakai H, Jackson D (2006) A trehalose metabolic enzyme controls inflorescence architecture in maize. Nature 441:227 doi:10.1038/nature04725
Sauer M, Robert S, Kleine-Vehn J (2013) Auxin: simply complicated Journal of Experimental Botany 64:2565-2577 doi:10.1093/jxb/ert139
Scheres B (2007) Stem-cell niches: nursery rhymes across kingdoms. Nature Reviews Molecular Cell Biology 8:345 doi:10.1038/nrm2164
Scheres B, Benfey P, Dolan L (2002) Root Development. The Arabidopsis Book American Society of Plant Biologists 1:e0101 doi:10.1199/tab.0101
Scheres B, Di Laurenzio L, Willemsen V, Hauser MT, Janmaat K, Weisbeek P, Benfey PN (1995) Mutations affecting the radial organisation of the Arabidopsis root display specific defects throughout the embryonic axis. Development 121:53
Sena G, Wang X, Liu H-Y, Hofhuis H, Birnbaum KD (2009) Organ regeneration does not require a functional stem cell niche in plants. Nature 457:1150-1153

doi:10.1038/nature07597
Shani E, Salehin M, Zhang Y, Sanchez SE, Doherty C, Wang R, Mangado CC, Song L, Tal I, Pisanty O, Ecker JR, Kay SA, Pruneda-Paz J, Estelle M (2017) Plant Stress Tolerance Requires Auxin-Sensitive Aux/IAA Transcriptional Repressors. Current Biology 27:437- 444 doi:https://doi.org/10.1016/j.cub.2016.12.016
Shanker AK, Cervantes C, Loza-Tavera H, Avudainayagam S (2005) Chromium toxicity in plants. Environment international 31:739-753 doi:10.1016/j.envint.2005.02.003
Shi X, Dalal NS (1990) On the hydroxyl radical formation in the reaction between hydrogen peroxide and biologically generated chromium(V) species. Archives of Biochemistry and Biophysics 277:342-350 doi:https://doi.org/10.1016/0003-9861(90)90589-Q
Shupack SI (1991) The chemistry of chromium and some resulting analytical problems. Environmental Health Perspectives 92:7-11
Smith FA (2007) Plant roots. Growth, activity and interaction with soils. Annals of Botany 100:151-152 doi:10.1093/aob/mcm099
Smith S, De Smet I (2012) Root system architecture: insights from Arabidopsis and cereal crops. Philosophical Transactions of the Royal Society B: Biological Sciences 367:1441- 1452 doi:10.1098/rstb.2011.0234
Steffens B (2014) The role of ethylene and ROS in salinity, heavy metal, and flooding responses in rice Frontiers in plant science 5:685 doi:10.3389/fpls.2014.00685
Steffens B, Rasmussen A (2016) The Physiology of Adventitious Roots. Plant physiology 170:603-617 doi:10.1104/pp.15.01360
Stepanova AN, Robertson-Hoyt J, Yun J, Benavente LM, Xie D-Y, Doležal K, Schlereth A, Jürgens G, Alonso JM (2008) TAA1-Mediated Auxin Biosynthesis Is Essential for Hormone Crosstalk and Plant Development. Cell 133:177-191 doi:https://doi.org/10.1016/j.cell.2008.01.047
Strasburger (2004) Tratado de botánica. 35ª Ed. Omega. España
Szemenyei H, Hannon M, Long JA (2008) TOPLESS Mediates Auxin-Dependent Transcriptional Repression During Arabidopsis Embryogenesis Science 319:1384-1386 doi:10.1126/science.1151461
Taiz L y Zeiger E (2006) Plant physiology 6ta Ed. Sinauer Associates Inc. Usa.
Teale WD, Paponov IA, Palme K (2006) Auxin in action: signalling, transport and the control of plant growth and development.Nature Reviews Molecular Cell Biology 7:847 doi:10.1038/nrm2020
Timilsena YP, Adhikari R, Casey P, Muster T, Gill H, Adhikari B (2015) Enhanced efficiency fertilisers: a review of formulation and nutrient release patterns. Journal of the Science of Food and Agriculture 95:1131-1142 doi:10.1002/jsfa.6812
van den Berg C, Willemsen V, Hendriks G, Weisbeek P, Scheres B (1997) Short-range control of cell differentiation in the Arabidopsis root meristem. Nature 390:287-289
Vanstraelen M, Benková E (2012) Hormonal Interactions in the Regulation of Plant Development. Annual Review of Cell and Developmental Biology 28:463-487 doi:10.1146/annurev-cellbio-101011-155741
Vieten A, Sauer M, Brewer PB, Friml J (2007) Molecular and cellular aspects of auxin- transport-mediated development. Trends in Plant Science 12:160-168 doi:https://doi.org/10.1016/j.tplants.2007.03.006
Villalobos LIAC, Lee S, De Oliveira C, Ivetac A, Brandt W, Armitage L, Sheard LB, Tan X, Parry G, Mao H, Zheng N, Napier R, Kepinski S, Estelle M (2012) A

combinatorial TIR1/AFB-Aux/IAA co-receptor system for differential sensing of auxin. Nature chemical biology 8:477-485 doi:10.1038/nchembio.926
Wang L, Ruan Y-L (2013) Regulation of cell division and expansion by sugar and auxin signaling. Frontiers in plant science 4:163 doi:10.3389/fpls.2013.00163
Wu X, Dabi T, Weigel D (2005) Requirement of Homeobox Gene STIMPY/WOX9 for Arabidopsis Meristem Growth and Maintenance. Current Biology 15:436-440 doi:10.1016/j.cub.2004.12.079
Yuan H-M, Huang X (2016) Inhibition of root meristem growth by cadmium involves nitric oxide-mediated repression of auxin accumulation and signalling in Arabidopsis. Plant, Cell & Environment 39:120-135 doi:10.1111/pce.12597

A. APÉNDICES

A.1. ARTÍCULO DE COLABORACIÓN:

Chromate induces adventitious root formation via auxin signalling and *SOLITARY-ROOT/IAA14* gene function in *Arabidopsis thaliana*.

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Chromate induces adventitious root formation via auxin signalling and SOLITARY-ROOT/IAA14 gene function in *Arabidopsis thaliana*

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Abstract Morphological root plasticity optimizes nutrient and water uptake by plants and is a promising target to improve tolerance to metal toxicity. Exposure to sublethal chromate [Cr(VI)] concentrations inhibits root growth, decreases photosynthesis and compromises plant development and productivity. Despite the increasing environmental problem that Cr(VI) represents, to date, the Cr tolerance mechanisms of plants are not well understood, and it remains to be investigated whether root architecture remodelling is important for plant adaptation to Cr(VI) stress. In this report, we analysed the growth response of *Arabidopsis thaliana* seedlings to concentrations of Cr(VI) that strongly repress primary and lateral root growth. Interestingly, adventitious roots started developing, branched and allowed seedlings to grow under highly growth-repressing Cr(VI) concentrations. Cr(VI) negatively regulates auxin transport and response gene expression in the primary root tip, as evidenced by decreased expression of auxin-related reporters *DR5::GFP*, *DR5::uidA* and *PIN1::PIN1::GFP*, and then, another auxin maximum is established at the site of adventitious root initiation that drives adventitious root organogenesis. Both primary root growth inhibition and adventitious root formation induced by high Cr(VI) levels are blocked by a gain-of-function mutation in the SOLITARY-ROOT/IAA14 gene of *Arabidopsis*. These data provide evidence that suggests a critical role for auxin transport and signalling via IAA14/SLR1 in the developmental program linking Cr(VI) to root architecture remodelling.

Keywords Adventitious roots · Chromate · Auxin · *Arabidopsis thaliana* · Heavy metals

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Introduction

Sustained plant growth is required to ensure food and feed supply and to respond to the need for biomass for renewable energy production. However, plant

productivity is greatly affected by environmental stresses, such as nutrient deficiency and/or metal toxicity (López-Arredondo et al. 2014; Giehl and von Wirén 2014). Root system architecture is emerging as a factor that can be influenced to increase tolerance to abiotic stresses because root characteristics determine water and nutrient acquisition, sensing of the local environment and direction of growth towards essential resources (López-Bucio et al. 2003; Giehl and von Wirén 2014). Although considerable variation exists, the root system usually consists of a taproot formed from the embryo, and then, lateral and/or adventitious roots emerge post-embryonically (Bellini et al. 2014).

Accumulating genetic evidence has indicated that biotic and abiotic factors modify root architecture by affecting endogenous hormone levels, homeostasis and signalling (Malamy 2005). Among plant hormones, auxin (indole-3-acetic acid, IAA) is a major factor regulating root organogenesis (Lavenus et al. 2013). IAA is distributed from the sites of synthesis in young leaves, as well as primary and lateral root tips to most plant tissues, where it is required to orchestrate cell division, cell elongation and differentiation processes (Davies 2010). Transport of this hormone occurs through the phloem by mass flow or via membrane transporter proteins from the PIN-formed family, which shows asymmetric distribution on root cells (Krecek et al. 2009). Auxin is perceived by TIR1 and related AFB1, AFB2 and AFB3 protein receptors, which, when associated with the SCF complex, marks proteins for degradation by the proteasome (Benjamins and Scheres 2008). Auxin-responsive genes are commonly activated by specific transcription factors termed auxin-response factors (ARFs) through binding to auxin response elements (AREs) present in the promoters. By contrast, the AUX/IAA repressors, which are encoded by 29 different genes in *Arabidopsis thaliana*, negatively regulate auxin responses via interaction with ARFs (Hagen and Guilfoyle 2002). Auxin acts as a glue to attach the AUX/IAA proteins with SCF^{TIR1}, resulting in ubiquitination and degradation of the AUX/IAA repressors by the proteasome (Quint and Gray 2006).

Morphological root plasticity optimizes plant adaptation towards better exploration of soils (Giehl and von Wirén 2014). Root growth in response to nutrients, such as phosphorus (P) or nitrate (N) availability, has been well studied (López-Bucio et al. 2002; Pérez-Torres et al. 2008; Gifford et al. 2008; Lima et al. 2010). An example of the intimate link between root development and nutrient transport systems is the function of the phosphate transporter 2 from *Arabidopsis thaliana* (*AtPT2*) in both phosphate uptake and in a phosphate-sensing mechanism that directs root growth towards local sources of phosphate (Sánchez-Calderón et al. 2005).

Nutrient transporter proteins in roots take up essential elements and also toxic non-essential elements via the transport systems for nutrients (Palmer and Guerinot 2009; Verbruggen et al. 2009). A stress-induced response, consisting of reduced primary root elongation and increased formation of lateral roots, has been observed in plants exposed to several biotic stress conditions, including low phosphate deficiency or exposure to aluminium (Al), rare earth elements lanthanum and gadolinium, or chromium (Cr), and it was postulated that this response redirects plant growth to diminish stress exposure limitations (López-Bucio et al. 2002; Potters et al. 2009; Ruiz-Herrera et al. 2012; Ruiz-Herrera and López-Bucio 2013; Martínez-Trujillo et al. 2014). Moreover, mineral nutrients, such as phosphate, sulphate and nitrate, have been reported to attenuate Cr(VI) toxicity, while Cr activates the expression of low-P inducible transporter genes *AtPT1* and *AtPT2* in *A. thaliana* seedlings. Primary root growth was inhibited in *AtPT2::uidA*-expressing seedlings upon exposure to micromolar Cr(VI) concentrations. However, increasing the P and sulphate supply to seedlings showing Cr(VI) toxicity symptoms restored root growth. These effects correlated with the Cr(VI)-induced *AtPT2::uidA* expression being completely reversed by the addition of phosphate and the decreased Cr content in leaves by phosphate supplementation (Ortiz-Castro et al. 2007; López-Bucio et al. 2014). Along with primary root growth inhibition, low Cr(VI) concentrations increased the formation of root hairs and lateral root primordia in *Arabidopsis* seedlings (Martínez-Trujillo et al. 2014). It was also found that the increasing concentration of Cr(VI) in the plant correlated with a decrease in the Fe content, whereas the supply of Fe to Cr(VI)-treated seedlings allowed the primary root to resume growth and alleviated toxicity symptoms, indicating that the Fe nutrition is a major target of Cr stress in plants by acting as a regulator of root architecture (Martínez-Trujillo et al. 2014). Interestingly, Cr(VI) specifically activated the expression in pericycle cells of a bHLH transcription

named POPEYE (PYE) and a putative E3 ligase protein, with metal ion binding and DNA binding domains termed BRUTUS (BTS), which are regulators of Fe deficiency responses (Long et al. 2010). The above mentioned information indicates that Cr toxicity impacts plant nutrition, likely affecting P, S and Fe sensing and uptake and/or distribution, but the influence on root system architecture has received little attention. Nevertheless, the precise position, morphology and extent of roots can influence metal uptake or mediate plant tolerance to toxic Cr(VI) levels due to stress-relieving capacities.

Adventitious roots may develop from different aerial organs, such as the hypocotyl, stem, or leaves, and from different tissues, such as the pericycle, mesophyll, parenchyma, cambium, non-differentiated secondary phloem, protoxylem, and epidermis (Gutiérrez et al. 2009). Although the formation of adventitious roots has common genetic elements with the formation of lateral roots, there are important differences at the level of genes and proteins involved in these processes (Bellini et al. 2014; Verstraeten et al. 2014). The importance of adventitious roots in the tolerance to metals has been analysed by Yang et al. (2000), who evaluated the performance of 229 lines of rice to Pb stress. The authors reported that tolerant lines could develop more adventitious roots in response to Pb treatment, whereas the sensitive varieties failed to activate this developmental response, showing the importance of adventitious roots in the mechanism of Pb tolerance.

Despite the significant problem that Cr(VI) toxicity poses for agriculture (Holland and Avery 2011), Cr tolerance mechanisms of plants are, to date, not well understood, and it remains to be investigated whether primary, lateral and adventitious roots differ in their sensitivity to Cr. In this report, we analysed the growth response of *A. thaliana* seedlings to concentrations of Cr(VI) that strongly repress primary and lateral root growth. Surprisingly, adventitious roots started developing, branched and allowed seedlings to grow in highly growth-repressing Cr(VI) concentrations. We present evidence that suggests a critical role for auxin transport, cell division and quiescent centre (QC) integrity in primary root growth inhibition by Cr(VI). Root architecture analyses of WT and *Arabidopsis* mutant lines *iaa28-1*, *iaa14/slr1*, *arf7arf19*, *gh3* and *arg7*, which have defects in genes involved in the auxin response, further showed that the repressor IAA14/SLR1 mediates the primary root growth and adventitious root responses to high Cr levels. Our data show the important role of auxin transport and signalling in the developmental program linking Cr(VI) to adventitious root formation and reveals a negative role of the IAA14/SLR1 *Arabidopsis* gene in root architecture remodelling by chromate.

Materials and methods

Plant material and growth conditions

Arabidopsis thaliana wild-type (Col-0); the transgenic lines *QC46::uidA* (Sabatini et al. 1999), *DR5::uidA* (Ulmasov et al. 1997), *DR5::GFP* (Ottenschläger et al. 2003), *PIN1::PIN1::GFP* (Benková et al. 2003), *PIN2::PIN2::GFP* (Blilou et al. 2005); and mutant lines *iaa28* (Rogg et al. 2001), *iaa14/slr1* (Fukaki et al. 2002), *arf7arf19* (Okushima et al. 2007), *tir1afb2afb3* (Dharmasiri et al. 2005), *gh3* (CS878718), and *arg7* (SALK_064784) were used for all of the experiments. The seeds were surface sterilized with 95 % (vol/vol) ethanol for 5 min and 20 % (vol/vol) bleach for 7 min. After five washes with sterile distilled water, the seeds were germinated and grown on agar plates containing 0.2× MS medium (Murashige and Skoog 1962). MS medium (Murashige and Skoog basal salt mixture; catalogue number M5524) was purchased from Sigma-Aldrich (St. Louis). The suggested formulation of salts is 4.3 g l^{-1}; we used 0.9 g l^{-1}, which we referred to as 0.2× MS. Phytagar (micropropagation grade) was purchased from Phytotechnology (Shawnee Mission, KS, USA). Plants were placed in a plant growth chamber (Percival Scientific AR-95L) with a photoperiod of 16 h of light and 8 h of darkness, a light intensity of 100 µmol m^2 s^{-1}, and a temperature of 22 °C. Media were supplemented with different concentrations of K_2CrO_4, which was referred to as chromium (VI); the stock solution of chromium (VI) was prepared with distilled water.

Analysis of plant growth and statistical analysis

The growth of primary roots was registered using a rule. The lateral root number was determined by counting the lateral roots present in the primary root from the tip to root/stem transition. The adventitious root number was determined by counting the

adventitious roots that emerged from the base of the hypocotyl. Univariate and multivariate analyses with Tukey's post hoc test were used for testing differences in growth and root developmental responses in the WT and *Arabidopsis* mutants. Different letters are used to indicate means that differ significantly ($P < 0.05$).

Microscopy

The *A. thaliana* root system was analysed using a stereoscopic microscope (Leica MZ6; Leica Microsystems, Wetzlar, Germany). The total number of lateral roots was counted at 30× magnification. The primary root meristems were analysed in semipermanent preparations of cleared roots using a microscope (Axiostar Zeiss Plus; Carl Zeiss, Göttingen, Germany) at 100× or 400× magnification. The images were captured using a Sony Cybershot DSC-S75 digital camera (Sony Electonics Inc., Oradell, NJ, USA), adapted to the microscope and processed with the Zeiss Axio Vision 4AC software (Carl Zeiss).

Histochemical analysis

Transgenic plants expressing the *uidA* reporter gene (Jefferson et al. 1987) were stained in 0.1 % 5-bromo-4-chlorium-3-indolyl, β-D-glucuronide in phosphate buffer (NaH_2PO_4 and Na_2HPO_4; 0.1 M each, pH 7) with 2 mM potassium ferrocyanide and 2 mM potassium ferricyanide for 12 h at 37 °C. Plants were cleared and fixed as previously described by Malamy and Benfey (1997). The processed roots were included in glass slips and sealed with commercial nail varnish. For each marker line and treatment, at least 10 transgenic plants were analysed.

PI staining and GFP detection

For confocal microscopy, solvent- or Cr(VI)-treated transgenic *Arabidopsis* seedlings expressing *DR5::GFP*, *PIN1::PIN1::GFP* and *PIN2::PIN2::GFP* were mounted on microscope slides into a solution of PI. For fluorescent staining with PI, seedlings with intact root systems were transferred to a solution of PI at 10 mg ml^{-1} for 3 min. Seedlings were rinsed in water and mounted in 50 % glycerol on microscope slides. The same sample was recorded separately at wavelengths specific to both PI fluorescence, with an 568 nm excitation line and an emission window of 585–610 nm, and GFP emission, with a 500- to 523-nm emission filter (488-nm excitation line), using a confocal microscope (Olympus FV1000), after which the two images were merged to produce the final image.

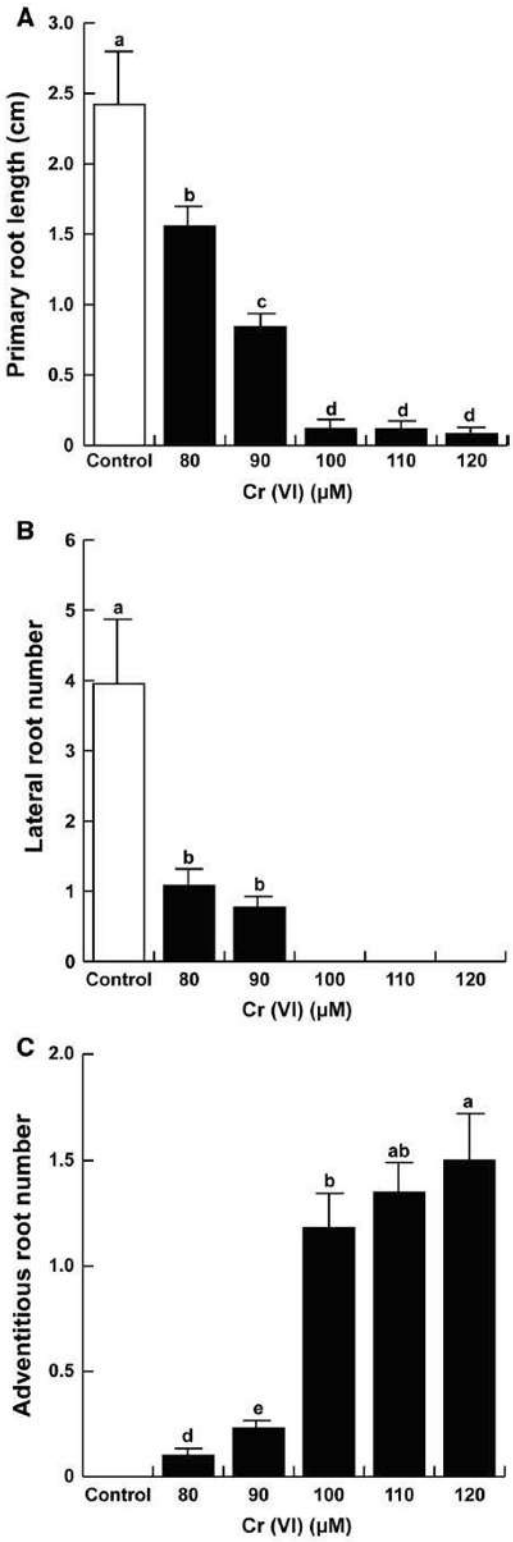
Results

Cr(VI) promotes adventitious root development at concentrations that inhibit primary and lateral root growth

We analysed different root traits of 12-day-old *A. thaliana* seedlings for Cr(VI) tolerance and sensitivity, which were initially assessed considering the inhibition of primary root growth that occurs from 80 to 120 μM Cr(VI) concentrations (Fig. 1a). Lateral root development was also affected in a dose-dependent manner and was correlated with stoppage of primary root growth (Fig. 1b). Interestingly, in the plates supplied with 100 μM or higher Cr(VI) treatments, the root system of the seedlings mostly comprised adventitious roots (Fig. 1c, Supplementary Fig. S1). The inhibition of primary root growth by Cr(VI) was related to decreased mitotic activity in the meristem, as shown by the reduced expression of the *CycB1::uidA* reporter construct in *Arabidopsis* transgenic seedlings (Fig. 2a), reduced number of cells expressing this marker (Fig. 2b), and loss of quiescent centre identity in primary root tips (Supplementary Fig. S2). Interestingly, as *CycB1::uidA* expression disappeared in the primary root meristems, adventitious roots, which express the cell cycle marker in normal levels, originated at the root/shoot junction and sustained normal growth at concentrations of 120 μM Cr(VI) (Fig. 2a). These data indicate that primary and lateral root development are highly sensitive to Cr(VI) toxicity and that adventitious roots differed in their sensitivity to Cr, allowing the root system to proliferate and support plant growth.

Cr(VI) modulates adventitious root development through auxin signalling

To understand the role played by Cr(VI) in root system architecture configuration and its possible relationship with auxin signalling, which regulates this organogenesis process, we analysed the expression of the



◀**Fig. 1** Effects of Cr(VI) on the *Arabidopsis* root system architecture. *Arabidopsis thaliana* seedlings were germinated and grown on MS 0.2× medium supplemented with different concentrations of Cr(VI) and were analysed 12 days after germination. **a** Primary root length. **b** Lateral root number. **c** Adventitious root number. Data show the mean ± SD (n = 30). *Different letters* indicate means that are significantly different ($P < 0.05$)

auxin responsive markers *DR5::uidA* and *DR5::GFP* in primary root tips and during adventitious root development in transgenic *Arabidopsis* seedlings expressing these markers and exposed to growth-inhibiting Cr(VI) treatments. *DR5::uidA* expression was strongly reduced in the shoot and root tips at 100 μM or higher Cr(VI) concentrations, which coincided with decreased shoot growth (Fig. 3a–l). By contrast, following primary root growth arrest, the development of a single adventitious root that normally expresses the *DR5::uidA* reporter gene occurred at the stem base (Fig. 3j–l). Further analysis of *DR5::GFP* expression in primary roots and at the site of adventitious root formation demonstrated that, at 100 μM Cr(VI), there is a decrease in auxin-inducible expression in the primary root tips and then another auxin maximum is established at the site of adventitious root initiation (Fig. 3m–r). These data suggest that Cr(VI) regulates auxin signalling during adventitious root development.

Adventitious root initiation is highly tolerant to Cr(VI) toxicity

To test the level of Cr(VI) tolerance of adventitious roots and determine whether auxin-inducible gene expression could be repressed by higher Cr(VI) doses, *DR5::GFP*-expressing seedlings were germinated and grown under 120–200 μM Cr(VI) supplementation. The growth of primary roots stopped immediately after germination; however, within another 5–6 days, new adventitious roots began developing again (Fig. 4a–e). The formation of newly formed adventitious roots coincided with auxin expression domains clearly established at the stem base and later on with strong auxin signatures in inner stem and root tissues (Fig. 4a–e). These data indicate that adventitious roots differ from primary roots in their sensitivity to Cr(VI) and that they represent new sinks for shoot-derived auxin.

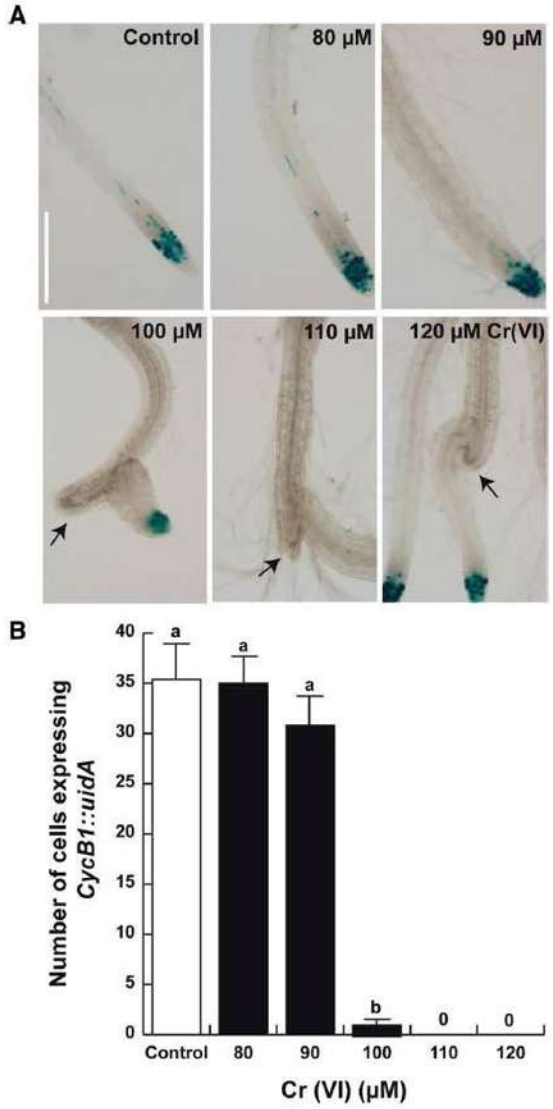


Fig. 2 Effects of Cr(VI) on cell division in the primary root. *A. thaliana* seedlings expressing the marker of mitotic activity *CycB1::uidA* were grown on MS 0.2× media supplemented with the indicated concentration of Cr(VI). **a** Histochemical *CycB1::uidA* expression. **b** Number of cells expressing *CycB1:uidA*. Histochemical staining was performed as described in the "Materials and Methods" section. Photographs are representative images of individuals from at least 15 seedlings analysed. Data represent the mean ± SD (n = 15). *Different letters* are used to indicate significant differences ($P < 0.05$). The experiment was repeated twice times with similar results. *Scale bar* 500 µm. The *arrow* shows the primary root tip

Cr(VI) affects the expression and distribution of auxin transporters PIN1 and PIN2

Auxin positively influences the PIN family of auxin transporters in a tissue-specific manner through an AUX/IAA-dependent signalling pathway (Vieten et al. 2005). PIN1 and PIN2 play important roles in lateral root formation and auxin-mediated gravitropism, respectively (Benková et al. 2003), but its role during adventitious root formation remains unknown. To test whether Cr(VI) could regulate primary root growth and/or adventitious root formation through differential expression of PIN1 or PIN2, we analysed the spatial pattern of PIN1 and PIN2 localization. In primary roots of seedlings expressing *PIN1::PIN1::GFP* (Vieten et al. 2005), grown in medium lacking Cr(VI), GFP fluorescence was detected in the stele and endodermal cells (Fig. 5a). In primary roots of seedlings supplied with 100 µM or higher Cr(VI) concentrations, the GFP fluorescence was strongly decreased at the stem base but was normally observed in mature adventitious roots (Fig. 5e–g). By contrast, *PIN2::PIN2::GFP* expression was detected in the cortex and epidermal cells of primary roots and emerging adventitious roots (Fig. 6a). An analysis of PIN2 localization during Cr(VI)-induced adventitious root initiation showed that, even considering the strongly primary root growth-repressing effects (Fig. 6b–d), adventitious roots exposed to 100–160 µM Cr(VI) still displayed the typical localization of PIN2 in most external cell layers (Fig. 6e–g). These findings suggest that Cr(VI) affects the expression and distribution of the PIN1 and PIN2 auxin transporters in primary roots but not in adventitious roots.

Effect of Cr(VI) on primary and adventitious root development of auxin-related *Arabidopsis* mutants

To further define the particular role of auxin in the *Arabidopsis* root response to Cr(VI) toxicity, we analysed the response of WT and *Arabidopsis* mutant lines affected in genes involved in the auxin response (*iaa28-1*, *iaa14/slr1*, *arf7arf19*, *gh3* and *arg7*) to 100 µM Cr(VI) treatment. Most of these genes have

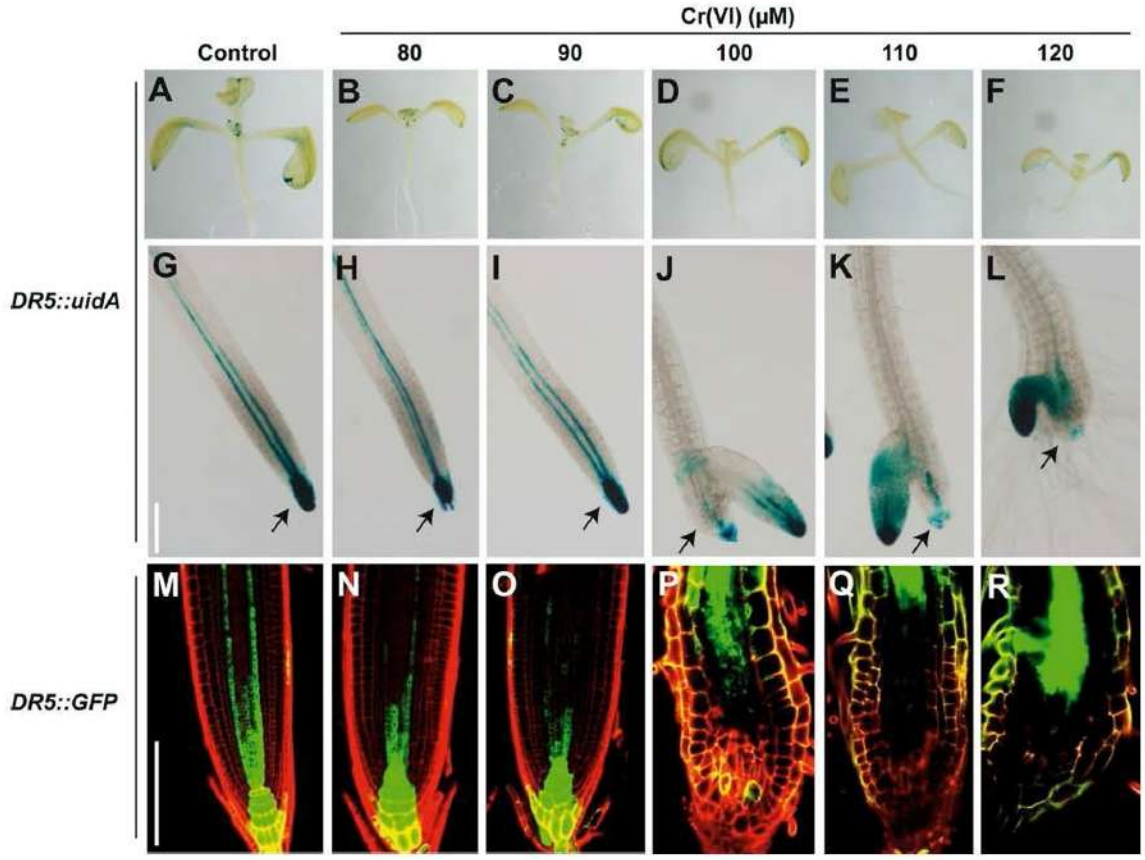


Fig. 3 Effects of Cr(VI) on plant growth and auxin-regulated gene expression. *A. thaliana* seedlings expressing the auxin markers *DR5::uidA* and *DR5::GFP* were germinated and grown on MS 0.2× agar-solidified medium supplemented with the indicated concentrations of Cr(VI). **a–l** Plants were stained for β-glucuronidase activity and cleared to show gene expression. **m–r** Transgenic *Arabidopsis* seedlings expressing the *DR5:GFP* marker were stained with propidium iodide to determine cell structure and viability. Photographs show representative individuals from at least 15 stained plants. The experiment was replicated twice with similar results. *Scale bar* 100 μm. The *arrow* shows the primary root tip

been reported to play a role in lateral root formation, but less is known about their role in adventitious root formation (Bellini et al. 2014). It was found that *arf7arf19, iaa28-1*, *gh3* and *arg7* mutants showed WT responses to Cr(VI), including the primary root growth inhibition and induction of adventitious root formation, while *iaa28-1* and *arf7arf19* seedlings showed primary root growth inhibition but reduced adventitious root formation (Fig. 7a, b). Interestingly, the *iaa14/slr1* mutant was clearly resistant to primary root growth inhibition even at growth-repressing concentrations of 100–200 μM Cr(VI) (Fig. 7c). These data indicate that IAA14/SLR1 mediates the primary root growth responses to high Cr levels.

Discussion

The root system has recently gained attention in strategies for improving plant growth and yield. Roots are directly involved in water and nutrient uptake efficiency and metal tolerance, and all of these responses are of adaptive value because water and nutrients will become increasingly limiting in the future (Lynch 1995; Den Herder et al. 2010). Plants can cope with nutrient limitation or localized metal pollution by altering their root system architecture to efficiently explore areas of available nutrients or to avoid metal-rich zones (López-Bucio et al. 2003; Potters et al. 2009; Giehl and von Wirén 2014). Plant

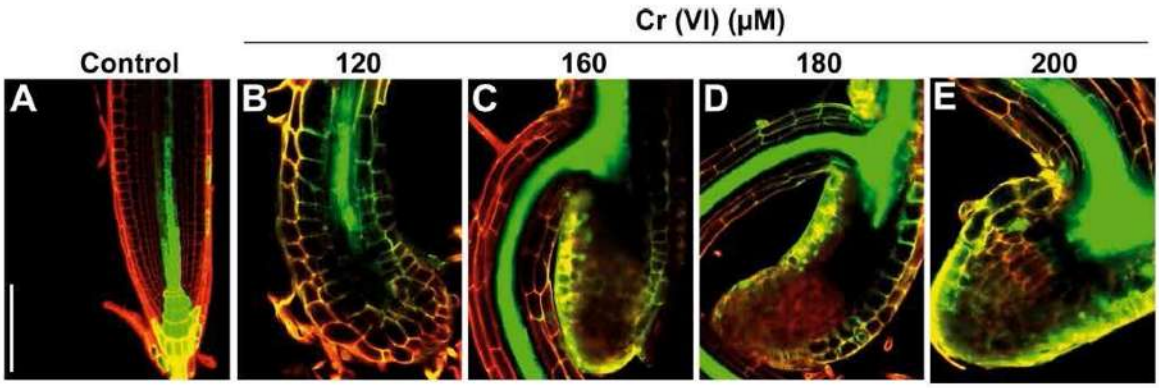


Fig. 4 Effect of Cr(VI) on auxin-regulated gene expression in adventitious roots. Transgenic *Arabidopsis* seedlings expressing the *DR5::GFP* marker were germinated and grown on MS 0.2× agar-solidified medium **a** or supplemented with 120, 160, 180 or 200 μM Cr(VI) **b–e**. Twelve-day-old transgenic *Arabidopsis* seedlings were stained with propidium iodide to determine cell structure and to analyse the expression of the *DR5::GFP* marker. Note the redistribution of auxin expression from the primary to the adventitious root, adopting apical dominance. Photographs are representative individuals of at least 15 stained plants. The experiment was replicated twice with similar results. *Scale bar* 100 μm

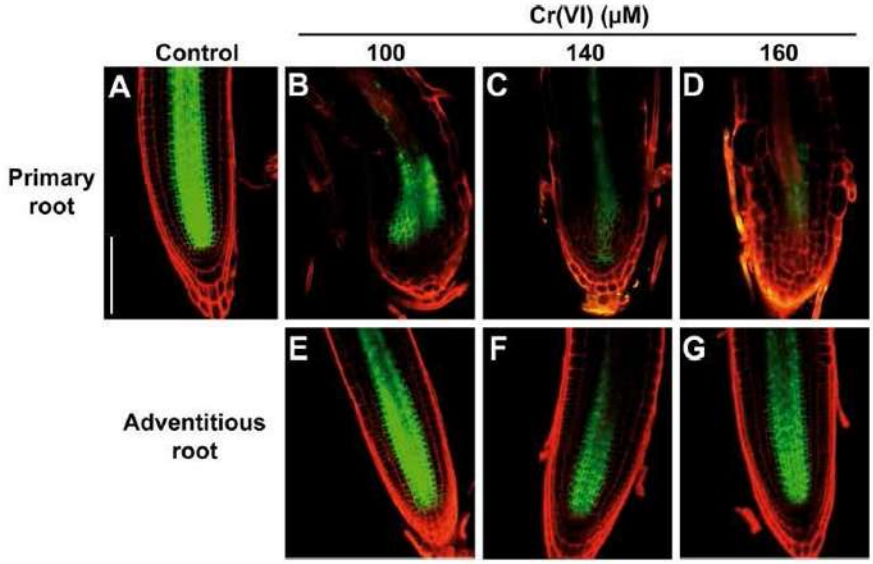


Fig. 5 Effect of Cr(VI) on PIN1 auxin-transporter expression. *A. thaliana* seedlings expressing the *PIN1::PIN1::GFP* marker were germinated and grown on MS 0.2× agar medium supplemented with the indicated concentrations of Cr(VI). Eight days after germination, the seedlings were stained with propidium iodide and analysed by confocal microscopy. **a** Primary root, **b–d** stem base, and **e–g** adventitious roots. Photographs are representative individuals of at least 15 stained plants. *Scale bar* 100 μm

responses to the toxic effects of excess Cr(VI) have been studied mainly at physiological, biochemical and molecular levels (Barceló et al. 1985; Dubey et al. 2010; Hayat et al. 2012; López-Bucio et al. 2014; Martínez-Trujillo et al. 2014), but little is understood regarding the mechanisms underlying root architectural remodelling to Cr(VI). Research towards a better understanding and improvement of plant growth in the presence of Cr(VI) contamination could contribute to future use of polluted areas for safe and efficient biomass production and to increase nutrient uptake efficiency and bioremediation strategies.

Root system architecture has been intensively studied in *Arabidopsis*. Its rather small root system is easy to dissect, and the availability of genome information and annotation, as well as development of molecular tools, has yielded fundamental insight into root development in this species (Malamy and Benfey 1997; López-Bucio et al. 2003). Here, we have shown that both primary and lateral root growth are

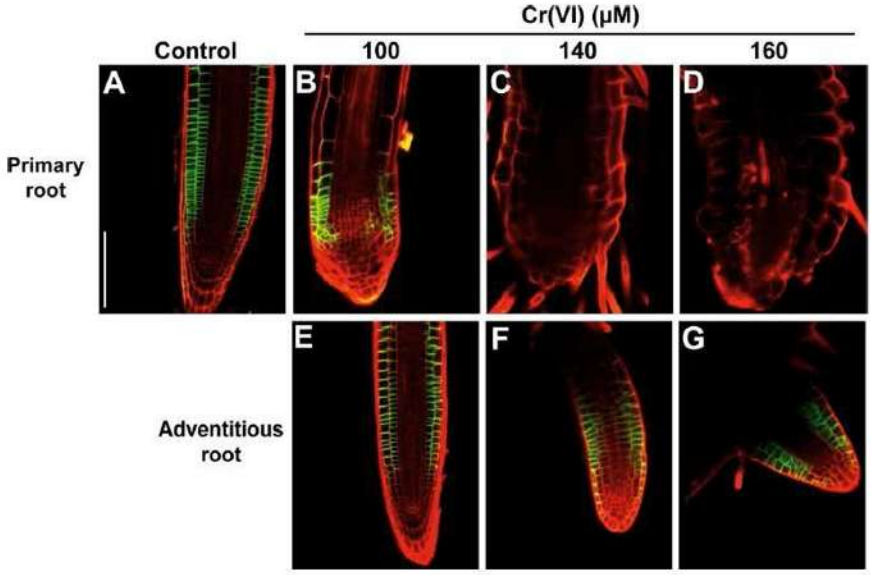
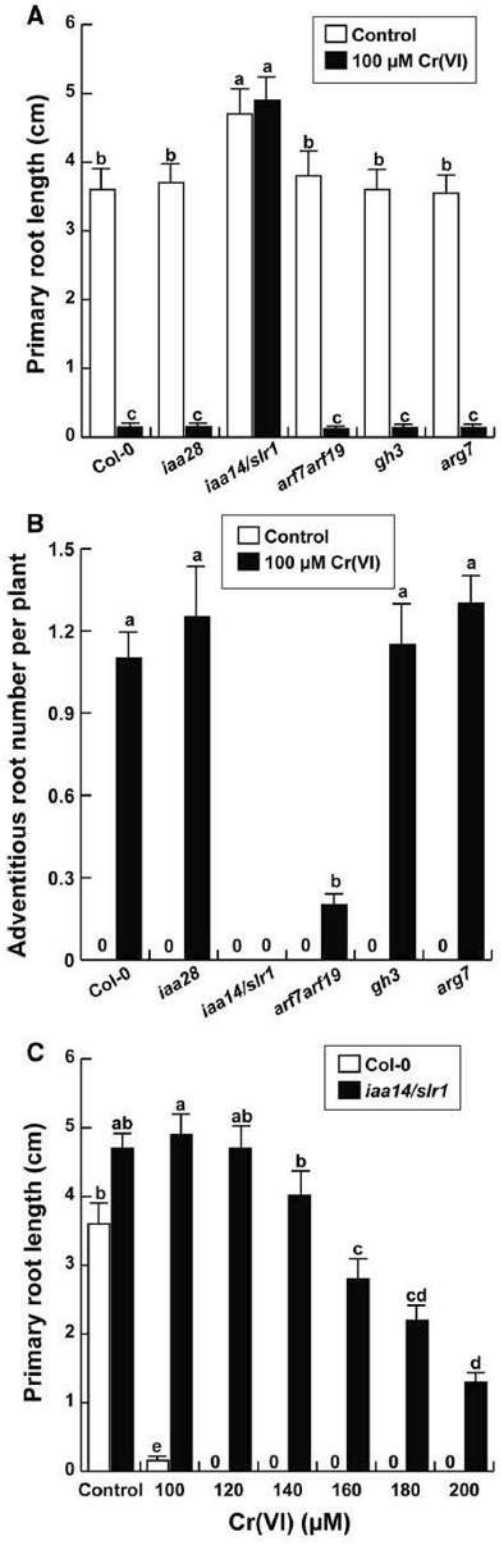


Fig. 6 Effect of Cr(VI) on PIN2 auxin transporter expression. *A. thaliana* seedlings expressing *PIN2::PIN2::GFP* marker were germinated and grown on MS 0.2× agar medium supplemented with different concentrations of Cr(VI). Eight days after germination, the seedlings were stained with propidium iodide and analysed by confocal microscopy. **a** Primary root apical meristem. **b–d** Stem base. **e–g** Adventitious roots. Note that *PIN2::PIN2::GFP* expression in adventitious roots persists, in marked difference to the primary root meristem, which is lost in high concentrations of Cr(VI). Photographs are representative individuals of at least 15 stained plants. *Scale bar* 100 μm

affected by Cr(VI) toxicity (Fig. 1a, b). This finding was correlated with decreased cell division, as evidenced by the loss of expression of *CycB1::uidA* in primary root meristems and quiescent centre (QC) cell identity (Fig. 2, S2). The primary root is the first root structure to emerge after germination and is derived from embryonically formed meristem tissue. Primary and lateral roots contain proliferative cells at the tip, where the root apical meristem is located, and form the basic stem cell pool for other cell types in the root. Cell division is regulated by the QC, an area of rarely dividing cells located distal to the root apical meristem, which signals surrounding cells to organize and maintain the population of initial stem cells (Dolan et al. 1993). These data indicate that the QC might be a primary target of Cr(VI) toxicity or that it might sense the changing metal levels for the root to activate alternate developmental pathways to survive under high Cr levels.

Root branching is essential to increase the surface area of the root system, enabling the plant to reach more distant reserves of water and nutrients and improve soil anchorage. In contrast to primary roots, lateral roots are formed post-embryonically from the pericycle, a tissue layer located between the central vascular cylinder and endodermis (Malamy and Benfey 1997). Lateral root initiation is the result of auxin-dependent cell cycle progression of pericycle cells to form 'founder cells' (Dubrovsky et al. 2008). In contrast to the formation of shoot lateral organs, the later stages of lateral root emergence require the de novo development of an apical meristem within the emerging lateral root. Concomitant with primary root growth inhibition, 80–100 μM Cr(VI) decreased lateral root formation (Fig. 1b) by 80–100 %, suggesting that auxin signalling in pericycle cells is affected by Cr(VI), thus inhibiting lateral root primordia initiation. Interestingly, 80–100 μM Cr(VI) or higher stimulated adventitious root formation in a dose-dependent manner (Fig. 1c).

Our data showed that upon primary and lateral root formation arrest, a novel developmental program is activated to tolerate increasing Cr(VI) levels, which implies de novo adventitious root organogenesis. Although adventitious root formation is important for horticultural practices, currently, the mechanisms by which adventitious roots are formed and their adaptive significance are still not well understood.



◀**Fig. 7** Effect of Cr(VI) on primary root growth and adventitious root formation of *Arabidopsis* wild-type (Col-0) and auxin-related mutants. *A. thaliana* wild-type (Col-0) and *iaa28, iaa14/slr1, arf7arf9, gh3* and *arg7* mutant seedlings were germinated and grown for 10 d on MS 0.2× agar medium supplemented with the indicated concentration of Cr(VI). **a** Primary root length. **b** Adventitious root number per plant. **c** Primary root length of wild-type and *iaa14/slr1* seedlings grown under different concentrations of Cr(VI). Values shown represent the mean primary root length of 30 seedlings ±SD. *Different letters* indicate means that differ statistically ($P < 0.05$). The experiment was repeated three times with similar results

Decreased primary root growth and increased adventitious root formation in *Arabidopsis* seedlings treated with high Cr(VI) concentrations, respectively, were correlated with alterations in *DR5:uidA*-driven reporter gene activity detected in the primary root tip and emerged adventitious roots (Fig. 3). The data from *DR5::GFP* analysis further showed that auxin accumulates at the sites of adventitious root initiation at the stem base (Fig. 3m–r). Subsequently, an auxin gradient is established with its maximum at the tip of the newly formed adventitious root even at concentrations up to 200 μM Cr(VI) (Fig. 4), coinciding with strong expression of the auxin transporter PIN1 (Fig. 5). It is tempting to speculate that Cr(VI) leads to enhanced auxin transport and/or distribution from producing cells in the shoot, causing auxin to accumulate at the stem base and the formation of an adventitious root primordium. Later in development, efficient transport of auxin by PIN1 may explain why under high Cr(VI), *Arabidopsis* seedlings show accelerated growth of these adventitious roots, which restores the damaged root system. Our results indicate that Cr(VI) activates a compensatory mechanism, negatively regulating primary root growth via decreasing auxin biosynthesis/transport in primary root tips, compromising QC cell identity. Subsequently, adventitious root initiation and development probably occur by Cr(VI) modulating an initial step during the establishment of a novel auxin response maximum at the stem base and promoting adventitious root growth via PIN1. Because adventitious roots formed in response to Cr(VI) treatment normally express auxin transporter PIN2 in external cell layers, an explanation is that these roots are developing normally and could tolerate sublethal Cr(VI) concentrations (Fig. 6). It is worth noting that auxin itself positively feeds back on

PIN gene expression in a tissue-specific manner through the AUX/IAA-dependent signalling pathway. Vieten et al. (2005) suggested a positive effect of IAA on PIN1 expression. Our data are in agreement with this previous report by showing that both auxin transport and signalling are important factors involved in root system remodelling, particularly in adventitious root formation in response to Cr(VI).

In this study, mutants were used in a reverse genetics approach to identify molecular components underlying quantitative changes in the root architecture after exposure to concentrations of chromate that repress primary root growth to understand the underlying consequences in adventitious root development. It was found that *arf7arf19, gh3* and *arg7* mutants showed WT responses to Cr(VI), including primary root growth inhibition and the induction of adventitious root formation, while *iaa28-1, arf7arf19* seedlings showed primary root growth inhibition but reduced adventitious root formation (Fig. 7a, b). Interestingly, the *iaa14/slr1* gain-of-function mutant was highly resistant to primary root growth inhibition even at growth-repressing concentrations of 100-200 μM Cr(VI) (Fig. 7c). These data indicate that IAA14/SLR1 is a repressor of the primary root growth and adventitious root formation processes that occur at high Cr levels.

Accumulating information has suggested that auxin signalling plays an important role in adventitious root formation from *Arabidopsis* seedlings and stem cuttings. Defects in adventitious rooting observed in the *argonaute1* (*ago1*) mutants, which can barely form adventitious roots, correlated with the deregulation of auxin homeostasis in hypocotyls (Sorin et al. 2005). In particular, a reduction in endogenous levels of free IAA and IAA conjugates was shown. This was correlated with down-regulation of the expression of several auxin-inducible *Gretchen Hagen 3* (GH3) genes in the hypocotyl of the *ago1-3* mutant. It was also found that the Auxin Response Factor17 (ARF17) gene, a potential repressor of auxin-inducible genes, was overexpressed in *ago1-3* hypocotyls. The characterization of an ARF17-overexpressing line showed that it produced fewer adventitious roots than the WT and retained a lower expression of GH3 genes. Thus, ARF17 negatively regulates adventitious root formation in *ago1* mutants by repressing GH3 genes, therefore, perturbing auxin homeostasis. In contrast to ARF17, the auxin response factors ARF6 and ARF8, targets of the microRNA miR167, are positive regulators of adventitious rooting (Gutiérrez et al. 2009, 2012). ARF6 and ARF8 have overlapping expression profiles during adventitious rooting, and they regulate each other's expression at the transcriptional and posttranscriptional levels by modulating the homeostasis of miR160 and miR167. Interestingly, these transcription factors regulate the expression of three GH3 genes, GH3.3, GH3.5, and GH3.6, which encode acyl-acid-amido synthetases that are required for fine-tuning adventitious root initiation in the *A. thaliana* hypocotyl, where they act by modulating jasmonic acid homeostasis (Gutierrez et al. 2012). Our data are consistent with the proposed role of auxin in adventitious root formation and extend previously published information by showing that specific factors, such as IAA14/SLR1, control auxin-induced adventitious root formation in response to Cr(VI). Although the molecular determinants by which IAA14/SLR1 controls primary root growth inhibition and the adventitious root number mediating plant tolerance to Cr(VI) remain to be identified, our data reveal a novel facet of Cr(VI) signalling to stimulate adventitious root growth, likely mediating surveillance to sublethal metal concentrations.

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References

Barceló J, Poschenrieder C, Gunse B (1985) Effect of chromium VI on mineral element composition of bush beans. J Plant Nutr 8:211–217

Bellini C, Pacurar DI, Perrone I (2014) Adventitious roots and lateral roots: similarities and differences. Annu Rev Plant Biol 65:639–666

Benjamins R, Scheres B (2008) Auxin: the looping star in plant development. Annu Rev Plant Biol 59:443–465

Benková E, Michniewicz M, Sauer M, Teichmann T, Seifertová D, Jürgens G, Friml J (2003) Local, efflux-dependent auxin gradients as a common module for plant organ formation. Cell 115:591–602

Blilou I, Xu J, Wildwater M, Willemsen V, Paponov I, Friml J, Heidstra R, Aida M, Palme K, Scheres B (2005) The PIN

auxin efflux facilitator network controls growth and patterning in *Arabidopsis* roots. Nature 433:39–44
Davies J (2010) Plant hormones: biosynthesis, signal transduction, action, 3rd edn. Kluwer Academic Publishers, Dordrecht
Den Herder G, Van Isterdael G, Beeckman T, De Smet I (2010) The roots of a new green revolution. Trends Plant Sci 15:600–607
Dharmasiri N, Dharmasiri S, Estelle M (2005) The F-box protein TIR1 is an auxin receptor. Nature 435:441–445
Dolan L, Janmaat K, Willemsen V, Linstead P, Poethig S, Roberts K, Scheres B (1993) Cellular organisation of the *Arabidopsis thaliana* root. Development 119:71–84
Dubey S, Misra P, Sanjay S, Chatterjee S, Bag SK, Mantri S, Assif MH, Rai A, Kumar S, Tripathi P, Tripathi R, Trivedi PK, Chakrabarty D, Tuli R (2010) Transcriptomic and metabolomics shifts in rice roots in response to Cr(VI) stress. BMC Genom 11:648–667
Dubrovsky JG, Sauer M, Napsucialy-Mendivil S, Ivanchenko MG, Friml J, Shishkova S, Celenza J, Benková E (2008) Auxin acts as a local morphogenetic trigger to specify lateral root founder cells. Proc Natl Acad Sci USA 105:8790–8794
Fukaki H, Tameda S, Masuda H, Tasaka M (2002) Lateral root formation in blocked by a gain-of-function mutation line in the SOLITARY-ROOT/IAA14 gene of *Arabidopsis*. Plant J 29:153–168
Giehl RFH, von Wirén N (2014) Root nutrient foraging. Plant Physiol 166:509–517
Gifford ML, Dean A, Gutiérrez RA, Coruzzi GM, Birnbaum KD (2008) Cell-specific nitrogen responses mediate developmental plasticity. Proc Natl Acad Sci USA 105:803–808
Gutiérrez L, Bussell JD, Pacurar DI, Schwambach J, Pacurar M, Bellini C (2009) Phenotypic plasticity of adventitious rooting in Arabidopsis is controlled by a complex regulation of AUXIN RESPONSE FACTOR transcripts and MicroRNA abundance. Plant Cell 21:3119–3132
Gutiérrez L, Mongelard G, Floková K, Pacurar DI, Novák O, Staswick P, Kowalczyk M, Pacurar M, Demailly H, Geiss G, Bellini C (2012) Auxin controls *Arabidopsis* adventitious root initiation by regulating jasmonic acid homeostasis. Plant Cell 24:2515–2527
Hagen G, Guilfoyle T (2002) Auxin-responsive gene expression: genes, promoters and regulatory factors. Plant Mol Biol 49:373–385
Hayat S, Khalique G, Irfan M, Wani AS, Tripathi BN, Ahmad A (2012) Physiological changes induced by chromium stress in plants: an overview. Protoplasma 249:599–611
Holland S, Avery SV (2011) Chromate toxicity and the role of sulfur. Metallomics 3:1119–1123
Jefferson RA, Kavanagh TA, Bevan MW (1987) GUS fusion: β-glucuronidase as a sensitive and versatile fusion marker in higher plants. EMBO J 6:3901–3907
Krecek P, Skupa P, Libus J, Naramoto S, Tejos R, Friml J, Zazimalová E (2009) The PIN-FORMED (PIN) protein family of auxin transporters. Genome Biol 10(249–249):11
Lavenus J, Goh T, Roberts I, Guyomarch S, Lucas M, De Smet I, Fukaki H, Beeckman T, Bennet M, Laplace L (2013) Lateral root development in *Arabidopsis*: fifty shades of auxin. Trends Plant Sci 18:450–458
Lima JE, Kojima S, Takahashi H, von Wirén N (2010) Ammonium triggers lateral root branching in *Arabidopsis* in an AMMONIUM TRANSPORTER1;3-dependent manner. Plant Cell 22:3621–3633
Long TA, Tsukagohi H, Busch W, Lahner B, Salt DE, Benfey PN (2010) The bHLH transcription factor POPEYE regulates response to iron deficiency in Arabidopsis roots. Plant Cell 22:2219–2236
López-Bucio J, Hernández-Abreu E, Sánchez-Calderón L, Nieto-Jacobo MF, Simpson J, Herrera-Estrella L (2002) Phosphate availability alters architecture and causes changes in hormone sensitivity in the *Arabidopsis* root system. Plant Physiol 129:244–256
López-Bucio J, Cruz-Ramírez A, Herrera-Estrella L (2003) The role of nutrient availability in regulating root architecture. Curr Opin Plant Biol 6:280–287
López-Arredondo DL, Leyva-González MA, González-Morales SI, López-Bucio J, Herrera-Estrella L (2014) Phosphate nutrition: improving low-phosphate tolerance in crops. Annu Rev Plant Biol 65:95–123
López-Bucio J, Hernández-Madrigal F, Cervantes C, Ortiz-Castro R, Carreón-Abud Y, Martínez-Trujillo M (2014) Phosphate relieves chromium toxicity in *Arabidopsis thaliana* plants by interfering with chromate uptake. Biometals 27:363–370
Lynch J (1995) Root architecture and plant productivity. Plant Physiol 109:7–13
Malamy JE (2005) Intrinsic and environmental response pathways that regulate root system architecture. Plant Cell Environ 28:67–77
Malamy JE, Benfey PN (1997) Organization and cell differentiation in lateral roots of *Arabidopsis thaliana*. Development 124:33–44
Martínez-Trujillo M, Méndez-Bravo A, Ortiz-Castro R, Hernández-Madrigal F, Ibarra-Laclette E, Ruiz-Herrera LF, Long TA, Cervantes C, Herrera-Estrella L, López-Bucio J (2014) Chromate alters root system architecture and activates expression of genes involved in iron homeostasis and signaling in *Arabidopsis thaliana*. Plant Mol Biol 86:35–50
Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol Plant 15:473–497
Okushima Y, Fukaki H, Onoda M, Theologis A, Tasaka M (2007) ARF7 and ARF19 regulate lateral root formation via direct activation of LBD/ASL genes in *Arabidopsis*. Plant Cell 19:118–130
Ortiz-Castro R, Martínez-Trujillo M, López Bucio J, Cervantes C (2007) Effects of dichromate on growth and root system architecture of *Arabidopsis thaliana* seedlings. Plant Sci 172:684–691
Ottenschläger I, Wolff P, Wolverton C, Bhalerao RP, Sandberg G, Ishikawa H, Evans M, Palme K (2003) Gravity-regulated differential auxin transport from columella to lateral root cap cells. Proc Natl Acad Sci USA 100:2987–2991
Palmer C, Guerinot ML (2009) Facing the challenges of Cu, Fe and Zn homeostasis in plants. Nature Chem Biol 5:333–340
Pérez-Torres CA, López-Bucio J, Cruz-Ramírez A, Ibarra-Laclette E, Dharmasiri S, Estelle M, Herrera-Estrella L (2008) Phosphate availability alters lateral root

development in *Arabidopsis* by modulating auxin sensitivity via a mechanism involving the TIR1 auxin receptor. Plant Cell 20:3258–3272

Potters G, Pasternak TP, Guisez Y, Jansen M (2009) Different stresses, similar morphogenic responses: integrating a plethora of pathways. Plant Cell Environ 32:158–169

Quint M, Gray WM (2006) Auxin signaling. Curr Opin Plant Biol 9:448–453

Rogg LE, Lasswell J, Bartel B (2001) A gain-of-function mutation in IAA28 suppresses lateral root development. Plant Cell 13:465–480

Ruíz-Herrera LF, López-Bucio J (2013) Aluminum induces low phosphate adaptive responses and modulates primary and lateral root growth by differentially affecting auxin signaling in *Arabidopsis* seedlings. Plant Soil 371:593–609

Ruíz-Herrera LF, Sánchez-Calderón L, Herrera-Estrella L, López-Bucio J (2012) Rare earth elements lanthanum and gadolinium induce phosphate-deficiency responses in *Arabidopsis thaliana* seedlings. Plant Soil 353:231–247

Sabatini S, Beis D, Wolkenfelt H, Murfett J, Guilfoyle T, Malamy J, Benfey P, Leyser O, Bechtold N, Weisbeek P, Scheres B (1999) An auxin-dependent distal organizer of pattern and polarity in the *Arabidopsis* root. Cell 99:463–472

Sánchez-Calderón L, López-Bucio B, Chacón-López A, Cruz-Ramírez A, Nieto-Jacobo MF, Dubrovsky J, Herrera-Estrella L (2005) Phosphate starvation induces a determinate developmental program in the roots of *Arabidopsis thaliana*. Plant Cell Physiol 46:174–184

Sorin C, Bussell JD, Camus I, Ljung K, Kowalczyk M, Geiss G, McKhann H, Garcion C, Vaucheret H, Sandberg G, Bellini C (2005) Auxin and light control of adventitious rooting in *Arabidopsis* require ARGONAUTE1. Plant Cell 17: 1343–1359

Ulmasov T, Murfett J, Hagen G, Guilfoyle TJ (1997) Aux/IAA proteins repress expression of reporter genes containing natural and highly active synthetic auxin response elements. Plant Cell 9:1963–1971

Verbruggen N, Hermans C, Schat C (2009) Molecular mechanisms of metal hyperaccumulation in plants. New Phytol 181:759–776

Verstraeten I, Schotte S, Geelen D (2014) Hypocotyl adventitious root organogenesis differs from lateral root development. Front Plan Sci. doi:10.3389/fpls.2014.00495

Vieten A, Vanneste S, Wisniewska J, Benková E, Benjamins R, Beeckman T, Luschnig C, Friml J (2005) Functional redundancy of PIN proteins is accompanied by auxin-dependent cross-regulation of PIN expression. Development 132:4521–4531

Yang Y, Jung JY, Song W, Suh HS, Lee Y (2000) Identification of rice varieties with high tolerance or sensitivity to lead and characterization of the mechanism of tolerance. Plant Physiol 124:1019–1026

A.2. ARTÍCULO DE DIVULGACIÓN:

Arabidopsis thaliana: una planta modelo
Publicado en la revista impresa algarabía niños número 19, febrero-marzo 2017.

Arabid thaliana

Una planta modelo

por Fátima Hernández M.

Arabidopsis thaliana o simplemente *Arabidopsis* es una pequeña planta, pariente de la col y el brócoli, que no es famosa por su belleza, sino porque ha atraído la mirada de muchos científicos que se interesan en entender mejor cómo funcionan las plantas.

Arabidopsis es la planta más conocida en el mundo de la ciencia, ya que es la favorita como modelo de estudio biológico, esto quiere decir que gracias a ella entendemos cómo funcionan la mayoría de las plantas con flor.

Esta planta es tan famosa que existen miles de publicaciones acerca de ella, incluso tiene varias páginas web. Como es toda una celebridad, su nombre aparece en miles de revistas científicas, y año con año, especialistas de todo el mundo se reúnen para dar a conocer sus últimas noticias.

opsis

Te preguntarás cuáles son esas características que hacen de *Arabidopsis* un modelo ideal para estudiar a las plantas; pues bien, empecemos:

- La pequeña que cabe en todos lados. *Arabidopsis* mide alrededor de 30 centímetros de altura cuando es adulta —el tamaño de una regla de tu juego de geometría—, por lo que permite a los científicos tener y analizar muchas plantas para que sus estudios sean muy similares a lo que pasa en la naturaleza.
- Crece rápido y produce muchas semillas o descendientes. Es ideal para estudiar cómo crece y se desarrolla una planta, ya que tiene un ciclo de vida corto: seis semanas. El ciclo de vida de una planta es el tiempo que transcurre desde que la planta surge de la semilla hasta que se convierte en una planta adulta capaz de generar descendientes y finalmente muere. Además, como *Arabidopsis* produce una gran cantidad de semillas, hay suficientes plantas para todos los experimentos.
- Conocimiento del número total de sus genes. Los genes son como las piezas de un rompecabezas para formar una planta, o sea que contienen toda la información

semillas de *Arabidopsis thaliana*

necesaria para que los organismos, en este caso las plantas, funcionen y sean tal y como las vemos. Los genes, junto con el ambiente, determinan el color, forma y función de las plantas. *Arabidopsis* fue la primera planta de la que se conoció la cantidad total de genes: ¡cerca de 25 mil! Este descubrimiento hizo mucho más fácil e interesante estudiarla y así saber qué función tiene cada gen en la planta, ya que es raro que se conozca ese tipo de información.

¿Por qué estudiar las plantas?

Las plantas son importantes para el ser humano, simplemente porque sin ellas no podríamos vivir. Gracias a ellas obtenemos alimentos y materiales para elaborar viviendas, ropa, medicamentos, entre otras muchas otras cosas. Las plantas nos obsequian paisajes hermosos y producen parte del oxígeno que respiramos. Son organismos extraordinarios que aunque no pueden moverse como nosotros, tienen poderes sorprendentes, como formar una planta completa a partir de una pequeña parte de la misma. Y si de competencias se trata, las plantas tienen varios récords mundiales: son los organismos más longevos —es decir que llegan a vivir muchos, muchísimos años— e increíblemente grandes.

Un *modelo de estudio biológico* es un organismo que por sus características es más fácil de analizar y gracias a él entendemos cómo funcionan todos los organismos de su tipo.

Observando y conociendo

Usando *Arabidopsis*, los científicos pueden estudiar cómo es que las plantas se las arreglan para sobrevivir en condiciones extremas. Fijándose muy bien en detalles de su aspecto, como el cambio en el color de la hoja y la forma en la que crece la raíz, pueden entendender mejor cómo es el comportamiento de las plantas y así generar nuevos conocimientos acerca de ellas.

Laralalá

En mi casa son todos deportistas
mi abuelita juega al basquet,
mi papá practica natación,
mi hermanita juega al futbol,
mi mamá levanta pesas,
pero yo no, pero yo no, porque estoy enamorado.
¡Ay Lilí! ¿No ves que estoy loco por ti?
si cuando te veo me hago pipí, mi Lilí...

«¡Ay Lilí!», letra y música de Luis María Pescetti.