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naturali e Ambiente**

Corso di Laurea magistrale in Scienze e Tecnologie Agrarie

Strigolactone regulation of maize root development seems to involve interactions with auxin and ethylene

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ANNO ACCADEMICO 2025/2026

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ABSTRACT

Strigolactones (SLs) are plant hormones derived from β -carotene that regulate plant growth, symbiotic interactions with arbuscular mycorrhizal fungi, and the germination of seeds of parasitic plants belonging to the Orobanchaceae family. Interest has increased due to their involvement in responses to abiotic stresses and in root system development and plasticity. However, the mechanisms through which SLs regulate different root types, particularly crown roots, remain poorly understood and represent the main focus of this study.

To investigate this role, two maize (*Zea mays* L.) genotypes were compared: the B73 inbred line, used as the wild type (WT), and the *zmccd8* knockout mutant, characterized by impaired SL biosynthesis. Plants were grown for 28 days and analysed at four sampling time points at phenotypic, physiological, and transcriptional levels. Shoot and root biomass were determined, chlorophyll, anthocyanin and flavonoid indices were monitored using the DUALEX SCIENTIFIC+™ instrument, and the expression of genes involved in auxin metabolism, transport and response, in lateral root formation and emergence and in ethylene biosynthesis and responses was analysed by RT-qPCR.

Overall, the results indicate that impaired SL biosynthesis is associated with altered maize root system development, particularly affecting crown roots, which showed reduced number and biomass. These changes, together with the observed physiological and transcriptional differences, suggest the involvement of multiple hormonal and developmental pathways.

RIASSUNTO

Gli strigolattoni (SLs) sono una classe di fitormoni derivati dal β -carotene che regolano la crescita delle piante, le interazioni con i funghi micorrizici arbuscolari e la germinazione dei semi di piante parassite della famiglia delle Orobanchaceae. Negli ultimi anni, l'interesse nei confronti di questi ormoni è cresciuto anche in relazione al loro coinvolgimento nelle risposte agli stress abiotici e nella regolazione della plasticità e dello sviluppo dell'apparato radicale. Tuttavia, i meccanismi attraverso cui gli SLs regolano lo sviluppo delle diverse tipologie di radici, specialmente le crown roots, rimangono ancora poco conosciuti e rappresentano il principale oggetto del presente lavoro.

A tal fine, sono stati confrontati due genotipi di mais (*Zea mays* L.): la linea pura B73, utilizzata come wild type (WT) e il mutante knockout *zmccd8*, caratterizzato da una compromissione della biosintesi degli strigolattoni. Le piante sono state coltivate per 28 giorni e analizzate in quattro diversi momenti di campionamento dal punto di vista fenotipico, fisiologico e trascrizionale. Sono state misurate la biomassa della parte aerea e dell'apparato radicale e sono stati monitorati gli indici di clorofilla, antociani e flavonoidi tramite il DUALEX SCIENTIFIC+™. Inoltre, mediante RT-qPCR, è stata analizzata l'espressione di geni coinvolti nel metabolismo, nel trasporto e nella risposta all'auxina, nella formazione e nell'emergenza delle radici laterali e nella biosintesi dell'etilene e nelle risposte a questo ormone.

Nel complesso, i risultati ottenuti evidenziano un'associazione tra la compromissione della biosintesi degli SLs e le alterazioni dello sviluppo dell'apparato radicale del mais, suggerendo un possibile coinvolgimento degli strigolattoni nella regolazione dello sviluppo delle crown roots attraverso l'interazione con diverse vie ormonali.

1. INTRODUCTION

1.1 MAIZE

1.1.1. Taxonomy

Maize (*Zea mays* L., 1753) is an annual monocotyledonous plant of the Poaceae family and belongs to the Andropogoneae tribe. This tribe includes, in addition to the genus *Zea* L., approximately 90 genera and more than 1,000 species, among which are sorghum (*Sorghum bicolor* L.) and sugarcane (*Saccharum officinarum* L.) (CFIA, 2021; Figure 1.1). Within the genus *Zea* L., five species are recognized: *Z. perennis*, *Z. diploperennis*, *Z. luxurians*, *Z. nicaraguensis* and *Z. mays*.

The species *Zea mays* L. is further subdivided into four subspecies: *Z. mays* ssp. *mays* (maize), *Z. mays* subsp. *parviglumis* (teosinte), *Z. mays* subsp. *mexicana*, and *Z. mays* subsp. *huehuetenangensis* (CFIA, 2021).

Taxon	Scientific name and common name
Kingdom	Plantae (plants)
Subkingdom	Tracheobionta (vascular plants)
Superdivision	Spermatophyta (seed plants)
Division	Magnoliophyta (flowering plants)
Class	Liliopsida (monocotyledons)
Subclass	Commelinidae
Order	Cyperales
Family	Poaceae / Gramineae (grass family)
Tribe	Andropogoneae
Genus	<i>Zea</i> L.
Species	<i>Zea mays</i> L.

Figure 1.1: Taxonomy of *Zea mays* L., source: Canadian Food Inspection Agency - CFIA (2021).

1.1.2. Origin, domestication and life cycle

The area of origin of maize is Central America. It is in this region – more precisely in the Balsas River valley, located in the southwestern part of Mexico – that the domestication of this species began, carried out by indigenous populations approximately 9,000 years ago (Matsuoka et al., 2002; Piperno et al., 2009). The term “domestication” refers to the selective pressure exerted by humans on specific traits and phenotypes that meet their needs, often at the expense of features associated with competition, colonization ability, and overall biological fitness. Maize is one of the crops most profoundly affected by domestication, having undergone a radical restructuring of the female inflorescence that ultimately made it highly dependent on human cultivation and management (Doebley et al., 2006). The ancestor of modern maize has been molecularly identified as *Zea mays* subsp. *parviglumis* (Piperno et al., 2009), commonly known as teosinte, a general term broadly used to indicate plants belonging to any of the species within the genus *Zea* (Doebley & Iltis, 1980). For a long time, the major differences between maize and teosinte were studied exclusively at the phenotypic level, whereas only in recent decades has it become possible to characterize them from a genetic and molecular perspective. The most evident morphological differences between these two subspecies are observed in the ear and include the following:

- In maize, both spikelets, which are borne in pairs at each internode of the female inflorescence, develop completely, whereas in teosinte only one reaches full maturity (Kellogg, 2000; Wu et al., 2009; Stitzer & Ross-Ibarra, 2018). As each fertile spikelet produces a single kernel, maize ears contain approximately twice as many kernels as teosinte ears.
- Maize is polystichous, producing at least four rows of kernels along the ear axis, whereas teosinte is distichous and develops only two rows (Stitzer & Ross-Ibarra, 2018). This

difference reflects the distinct organization of the inflorescence meristem (Bartlett & Thompson, 2014).

- In teosinte, abscission layers form between internodes, causing the ear to break into individual fruitcases that facilitate seed dispersal (Figure 1.2). In maize, the rachis remains intact, keeping the kernels attached to the cob and making maize dependent on humans for seed dispersal (Iltis, 2000; Doebley, 2004).

- In maize, the glumes (the bracts subtending the flower) are reduced and papery, leaving the kernels exposed, whereas teosinte glumes completely enclose and protect each kernel. Their reduction during domestication allowed maize kernels to increase in mass by approximately tenfold (Figure 1.2; Stitzer & Ross-Ibarra, 2018; Flint-Garcia et al., 2009; Preston et al., 2012).

- Compared with teosinte, maize exhibits a strong apical dominance and limited vegetative branching, producing fewer but considerably larger ears as a result of domestication (Figure 1.3; Stitzer & Ross-Ibarra, 2018; Doebley, 2004; Studer et al., 2011; Wills et al., 2018).

The life cycle of maize can be divided into three main developmental phases: the juvenile and adult vegetative phases and the reproductive phase (Vollbrecht & Schmidt, 2009). Following seed germination, the plant enters the juvenile vegetative stage, which is characterized by rapid growth and the formation of leaves, internodes and axillary buds. During this period, the shoot apical meristem (SAM), made up of undifferentiated and self-renewing cells, continuously produces new vegetative organs while supporting stem elongation.

As development progresses, maize enters the adult vegetative phase, during which previously initiated phytomers mature and the plant acquires the competence to transition to reproduction. A key event of this transition is the change in meristem identity: the SAM

ceases the production of vegetative structures and differentiates into an inflorescence meristem, redirecting its resources toward the development of reproductive organs (Bommert & Jackson, 2015).

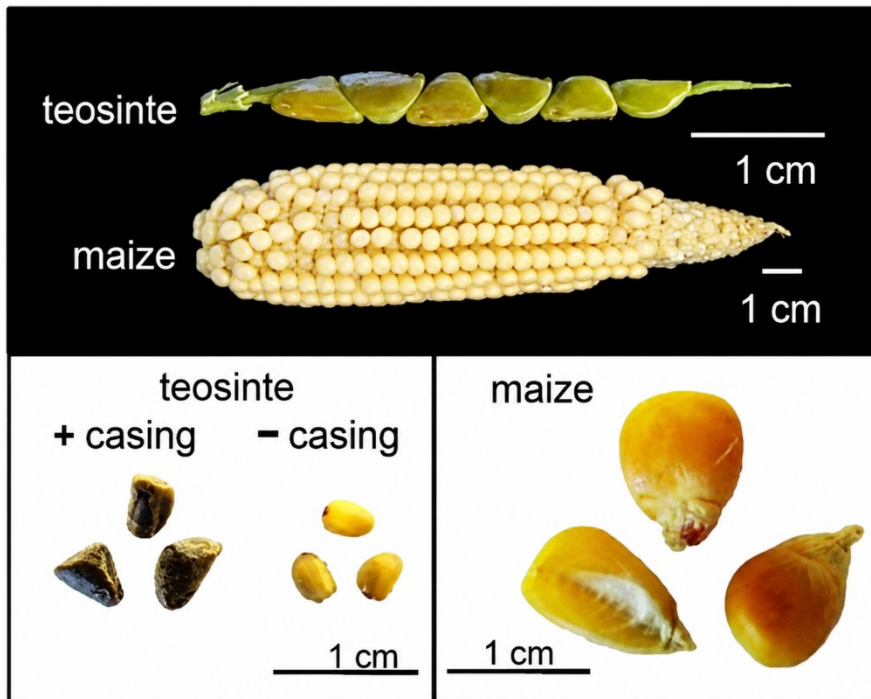


Figure 1.2: Morphological differences between maize and teosinte ears and kernels, with scale reference (Guan et al., 2023).

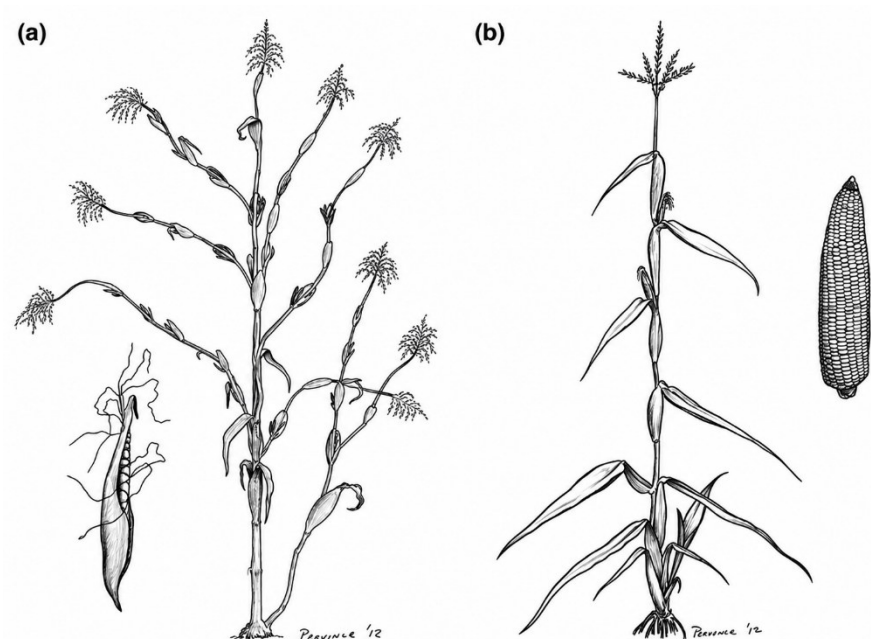


Figure 1.3: Differences between plant architecture and branching between maize (b) and teosinte (a) (Stitzer & Ross-Ibarra, 2018).

1.1.3. Agronomic relevance and crop productivity of maize

The importance of maize can already be inferred from the name given to this plant species: *Zea* is a word of Greek origin meaning “to live”, while *mays* derives from the Native American word *mahiz*, meaning “bread of life” (Marocco & Lorenzoni, 2007). Together with rice and wheat, maize represents one of the most important cereal crops worldwide, both for human and animal nutrition, as well as for its numerous industrial applications, such as the production of starch, bioethanol and bioplastics (FAO, 2022; Shiferaw et al., 2011).

Over the last decades, global maize production has shown a constant increase, rising over a 20-year period from approximately 728 million tonnes to about 1218 million tonnes (Figure 1.4). A similar trend can also be observed in cultivated areas, which increased by more than 60 million hectares between 2004 and 2024 (Figure 1.4; FAOSTAT, 2026).

One of the main factors underlying the agronomic success of maize has been the exploitation of heterosis (or hybrid vigor), a phenomenon whereby hybrids obtained from crosses between genetically divergent lines exhibit superior performance compared with their parent lines. This phenomenon was first described at the beginning of the twentieth century by George Harrison Shull and Edward Murray East, who demonstrated that self-pollination led to the depression of desirable traits, whereas crossing inbred lines restores vigor and productivity (Shull, 1908; East, 1908). In maize, the practical application of hybrid vigor led to the development of commercial hybrids, whose large-scale adoption began in the United States during the 1930s and resulted in a substantial increase in yields. Today, the production of highly heterotic hybrids represents the cornerstone of modern maize cultivation and breeding programs are focused on the selection of highly performing and genetically divergent parental lines, as well as on their combinations, in order to maximize heterosis (Duvick, 2001). Maize hybrids provide several agronomic advantages, including

higher productivity, greater phenotypic uniformity and improved adaptability to environmental stresses (Tollenaar & Lee, 2002). Their use has significantly increased crop yields and continues to represent a key strategy for addressing future challenges, including climate change and the growing demand for food (Duvick, 2005). The development of commercial hybrids relies on the inbred lines, obtained through repeated generations of self-pollination until a nearly homozygous and genetically stable genotype is achieved. Although inbred lines generally exhibit reduced vigor and productivity, they represent an essential genetic resource because they provide uniform parental material for hybrid seed production and allow breeders to accurately evaluate combining ability and agronomic performance (Duvick, 2005; Hirsch et al., 2016). Among these, B73 is one of the most extensively studied and widely used maize inbred lines. Developed in 1972 through recurrent selection from the Iowa Stiff Stalk Synthetic (BSSS) population, B73 is an elite maize inbred line characterized by high combining ability and has been extensively used as a parental line in commercial hybrid breeding (Schnable et al., 2009). Its importance increased when it became the first maize line to be completely sequenced, providing the reference genome for maize and establishing itself as the standard genotype for genetic, genomic and transcriptomic studies (Schnable et al., 2009). Thanks to the extensive genetic and molecular information available, B73 is currently considered a reference inbred line for experimental and comparative studies (Hirsch et al., 2016), including the one presented in this thesis.

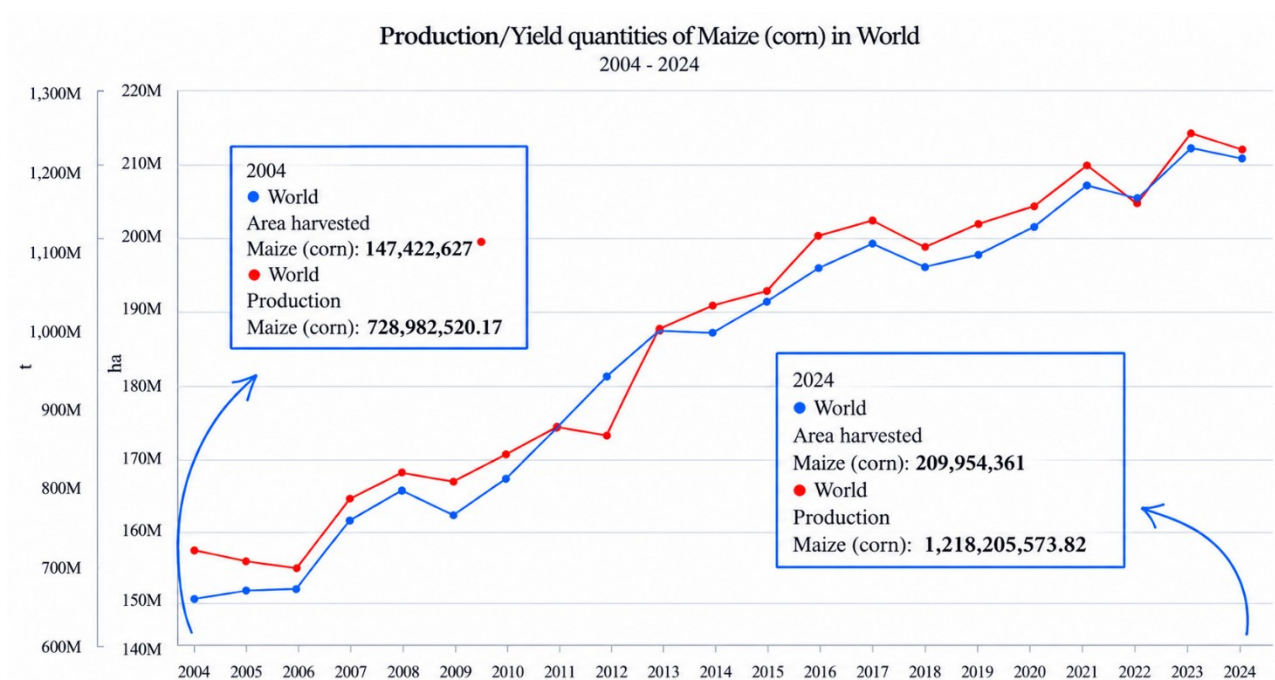


Figure 1.4: Trends in global maize production (red line) and harvested area (blue line) from 2004 to 2024, highlighting the two years. Source: FAOSTAT, 2026.

1.1.4. Root morphology

Roots play an essential role in plant development and fitness, as they mediate water and nutrient uptake from the soil, provide mechanical support and interact with microbial communities within the rhizosphere (Beeckman & Eshel, 2024; George et al., 2024). As the primary site of soil-plant interactions, roots play a key role in plant responses to climate change and influence important agronomic traits such as nutrient use efficiency, lodging resistance and plant responses to abiotic stresses (George et al., 2024; Lynch, 2013).

The maize root system exhibits a complex architecture and consists of both embryonic and post-embryonic roots. Embryonic roots include the primary root and seminal roots, whereas post-embryonic roots comprise lateral roots and adventitious roots originating from the stem, including crown roots and brace roots (Figure 1.5; Hawkes et al., 2007; Zhu et al., 2011; Villordon et al., 2014).

In maize, five main root types can therefore be distinguished:

- Primary root forms at the basal pole of the embryo (Figure 1.5) and generally emerges during the first 2-3 days of the germination process (Hochholdinger et al., 2004). It is the first root to establish contact with the soil, exploring it and providing the initial anchorage of the seedling. During germination, primary root also acts as the main axial pathway for nutrient and water transport from the soil to the emerging shoot, ensuring seedling establishment before the development of additional root classes (Hochholdinger & Tuberosa, 2009).
- Seminal roots develop at the scutellar node (Figure 1.5) and emerge approximately one week after germination. Up to 12 seminal roots may form, although their number varies depending on the genetic background (Sass, 1977; Erdelská & Vidovencová, 1993). Their rapid elongation increases the volume of soil explored during the first weeks after germination, improving the acquisition of water and mobile nutrients, such as nitrate. Seminal roots are particularly important under limited water availability conditions, where they sustain early seedling growth until the post-embryonic root system becomes fully established (Hochholdinger & Tuberosa, 2009; Hostetler et al., 2022).
- Lateral roots originate from cells of the pericycle and endodermis in both embryonic and post-embryonic roots (Figure 1.5; Fahn, 1990). Their function is to enhance soil exploration and increase the efficiency of resource uptake. Because of their high surface-to-volume ratio and extensive branching pattern, lateral roots represent the main interface between the plant and the surrounding soil environment. They constitute the primary absorptive surface of the maize root system and play a central role in water and nutrient acquisition. Lateral roots also display a remarkable developmental plasticity, adjusting their length, density and spatial distribution in response to water gradients and nutrient availability (Lynch, 2013; Yu et al., 2019).

- Crown roots develop from underground stem nodes within the crown region (Figure 1.5); the first whorl generally appears 10-14 days after germination and, as the plant grows, they progressively replace the embryonic root system (Hochholdinger et al., 2004). Crown roots, by the exploration of deeper soil layers, become the principal organs responsible for water and nutrient uptake in adult plants. Their large diameter and extensive branching also provide mechanical support and improve plant stability, allowing maize to sustain vegetative growth and reproductive development (Hochholdinger & Tuberosa, 2009; Hostetler et al., 2022).

- Brace roots arise adventitiously from nodes located above the soil surface (Figure 1.6; Reneau et al., 2020). Traditionally considered only as support structures, brace roots are now recognized as functionally active organs involved in both anchorage and resource acquisition: when they penetrate the soil, they develop lateral branches and actively absorb water and nutrients. The lower brace root whorls contribute most to mechanical stability, increasing resistance to stem lodging caused by wind or heavy rainfall, whereas the upper aerial whorls, depending on environmental conditions, may remain aboveground or eventually enter the soil (Figure 1.6; Hostetler et al., 2021; Sparks, 2023).

An adult maize root system typically develops approximately 70 stem-borne roots, generally organized into six whorls of crown roots and two to three whorls of aerial brace roots. During the first weeks following germination, the root system mainly consists of the primary and seminal roots, which are therefore crucial for early vigor, whereas post-embryonic adventitious roots determine the architecture of the adult root system (Martin & Harris, 1976).

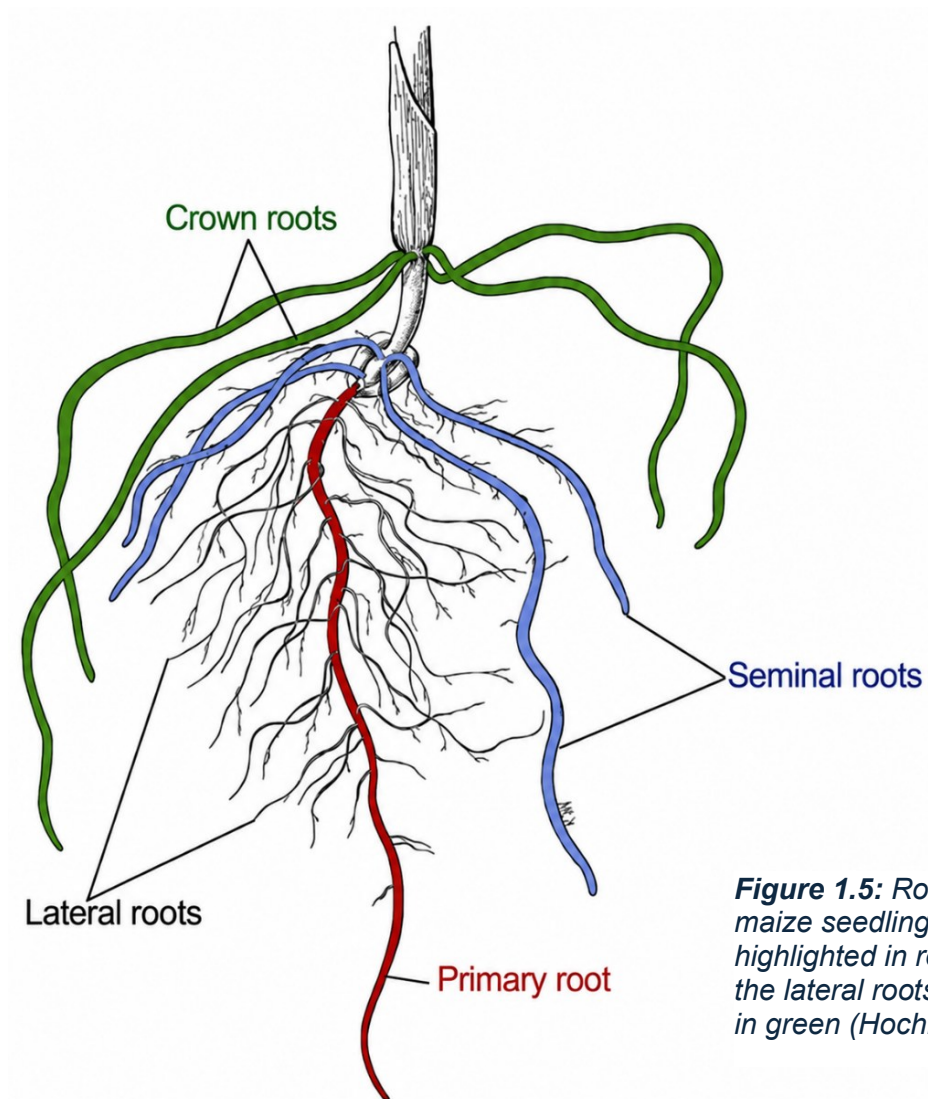


Figure 1.5: Root system of a 14-day-old maize seedling, with the primary root highlighted in red, the seminal roots in blue, the lateral roots in black and the crown roots in green (Hochholdinger & Tuberosa, 2009).

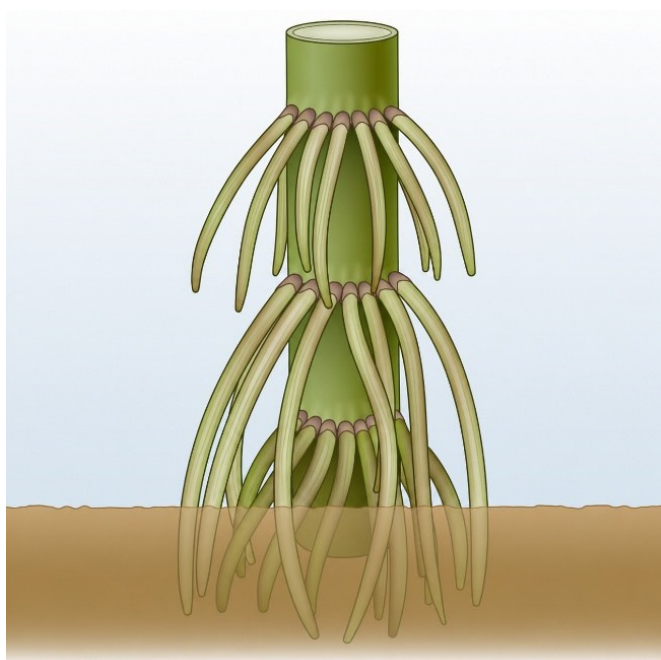


Figure 1.6: Illustration of maize brace root whorls developing from aerial stem nodes (Sparks, 2023).

1.2 ABIOTIC STRESSES

Abiotic stresses refer to environmental conditions of a non-biological nature that alter the normal physiological functioning of plants (Mittler, 2006). They represent one of the major limiting factors for crop productivity, as they can negatively affect plant growth, development and yield. The most significant abiotic stresses include drought, extreme temperatures, soil salinity and waterlogging; unfortunately, these conditions are becoming increasingly severe due to climate change and growing environmental variability (Zhao et al., 2017; Lesk et al., 2016). At the physiological level, these stresses interfere with fundamental processes such as photosynthesis, nutrient uptake and transpiration, leading to metabolic imbalances and the production of reactive oxygen species (ROS), which are responsible for oxidative damage to the cellular structures (Mittler, 2002). Plants respond to these stressful conditions by activating a range of highly complex adaptive mechanisms, including morphophysiological modifications, hormonal regulation and the expression of specific genes involved in stress tolerance (Figure 1.7; Atkinson & Urwin, 2012).

1.2.1. Stress and climate change

Plants, being sessile organisms, are strongly influenced by the surrounding environment. Adverse environmental changes are rapidly perceived and recognized and must be counteracted through appropriate physiological and molecular responses (Abbas et al., 2021). Abiotic factors are fundamental components of the environment, determining plant distribution and productivity by limiting croplands worldwide and negatively affecting crop performance (Zhang et al., 2022). Abiotic stresses are estimated to be the major cause of yield loss in cultivated plants worldwide, reducing the productivity of major food and commercial crops by more than 50% and causing substantial economic losses (Bray et al., 2000). Furthermore, several abiotic stress conditions have been shown to weaken plant

defence mechanisms, thereby increasing susceptibility to pathogen infections (Suzuki et al., 2014).

Abiotic stresses, often acting simultaneously with biotic stresses, are becoming increasingly severe as a consequence of the extreme events associated with rising global temperatures and, more generally, climate change. The increase in average temperatures, alterations in precipitation patterns and the growing frequency of extreme events, such as heat waves and prolonged drought periods are deeply modifying the environmental conditions under which agricultural crops develop, resulting in significant yield losses and threatening global food security (Mittler, 2006; Zandalinas et al., 2021). Globally, it has been estimated that, over recent decades, approximately 1.82 billion tons of cereals, including maize, wheat and rice, have been lost due to climate-related factors (Guoyong & Hall, 2019). Moreover, global wheat production is projected to decrease by approximately 6% for every 1°C increase in temperature (Asseng et al., 2015).

Maize is among the crops most vulnerable to climate change-related losses. Due to its high susceptibility to drought and because it's cultivated during increasingly warm growing seasons, rising temperatures and more frequent heat waves may lead to yield reductions exceeding 40% (Tigchelaar et al., 2018). In Italy, maize production experienced a decline in 2024 due to adverse weather conditions, with yield losses reaching up to 35% (Figure 1.8). Although maize production showed a slight recovery in 2025, accompanied by a 9% increase in cultivated areas, it still represented the fourth consecutive year in which national production remained below 6 million tons (CREA, 2025).

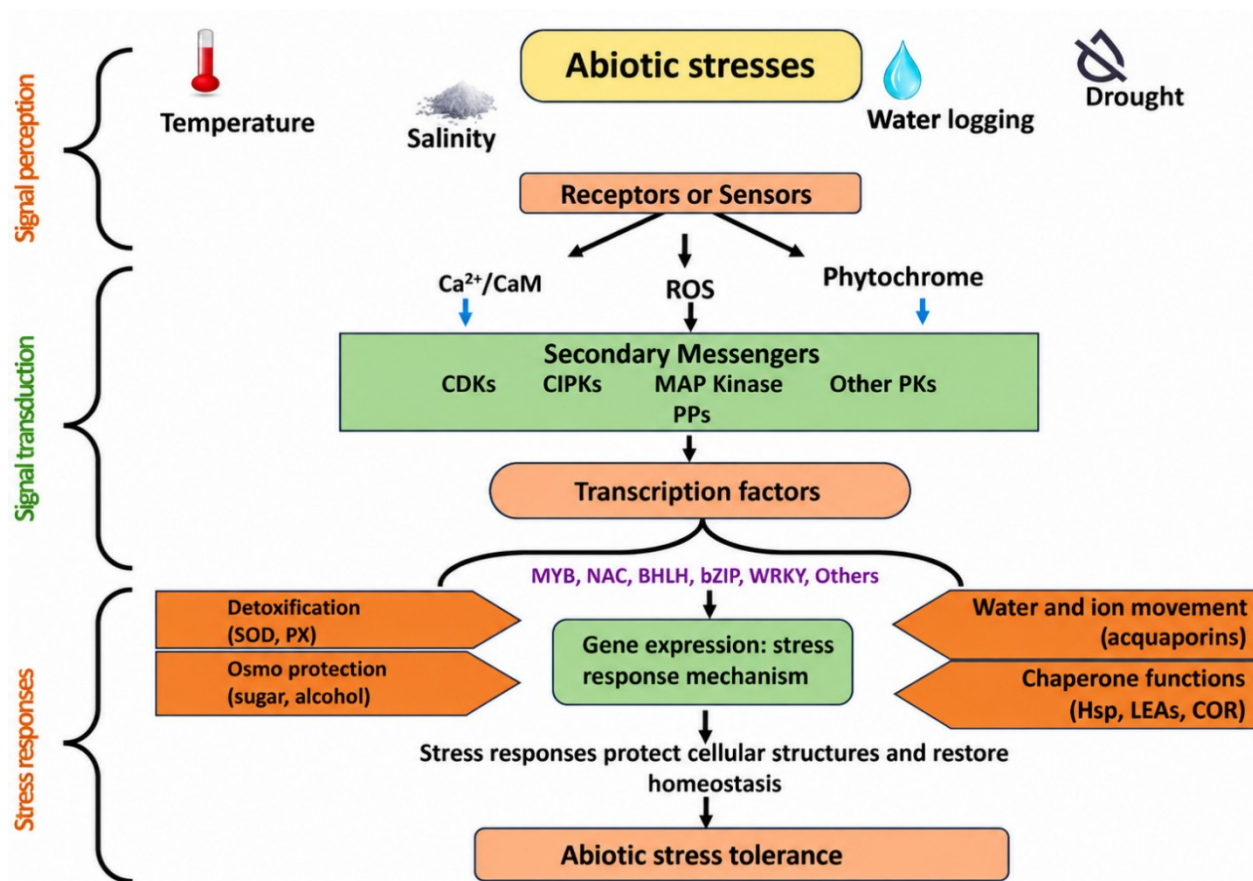


Figure 1.7: General signalling pathways and adaptive responses activated by plants under major abiotic stresses, leading to the induction of stress-responsive genes and the establishment of abiotic stress tolerance (Ramzan et al., 2026).



Figure 1.8: Recent trends in maize production, yield and cultivated area in Italy (2021-2025). (a) Maize production (blue bars) and average yield (red line) exhibited marked interannual fluctuations, with a pronounced decline in 2022, a recovery in 2023 and a subsequent reduction in 2024. (b) Cultivated area showed a slight contraction between 2022 and 2024 followed by a moderate recovery in 2025. Source: CREA, 2025.

1.2.2. Main abiotic stresses

The environmental stresses that most significantly affect plant metabolism are the following:

- Drought stress is among the most widespread and damaging forms of abiotic stress, as it directly limits the availability of water required for normal physiological processes. Drought induces stomatal closure, reducing CO₂ uptake and transpiration, consequently decreasing photosynthetic activity and plant growth (Farooq et al., 2012).

Typical symptoms of drought stress include loss of cellular turgor, resulting in reduced leaf expansion, leaf rolling and senescence, increased abscission and decreased transpiration flow (Ramzan et al., 2026). At the cellular level, water deficiency interferes with cell elongation and division and impairs water transport through the xylem and phloem, thereby limiting leaf expansion and biomass accumulation (Fahad et al., 2017). Water deficit also induces production of reactive oxygen species (ROS), which cause severe damage to proteins, cellular membranes and nucleic acids (Mittler, 2002). To counteract these negative effects, plants respond to drought through morphological adaptation, such as deeper root system development, and through osmotic adjustment, namely the accumulation of soluble solutes (osmoprotectants) within the cytoplasm. The mechanism of osmotic adjustment helps maintain cellular water balance and protects essential metabolic functions under stress conditions (Figure 1.7; Shanker et al., 2014). In maize, drought stress is particularly critical during the flowering stage, as it compromises kernel set and may result in severe yield losses (Boyer & Westgate, 2004).

- Thermal stress negatively affects plant physiology by altering the stability of cellular membranes and the activity of enzymes involved in photosynthesis.

Low temperatures may cause extracellular ice formation, intracellular dehydration, increased electrolyte leakage and alterations in stomatal and transpiratory activity (Ramzan

et al., 2026). These disturbances reduce photosynthetic capacity while simultaneously promoting oxidative damage due to excessive ROS accumulation (Moore et al., 2021).

High temperatures, on the other hand, reduce the activity of important enzymes, such as glucose-1-phosphate adenylyltransferase and β -fructofuranosidase, which are involved in sucrose phosphorylation, thereby compromising sucrose and starch synthesis and accumulation. Heat also causes the inactivation of photosystem II, impairing photosynthetic activity, slowing plant growth and inducing premature leaf senescence (Wahid et al., 2007). Moreover, high temperatures compromise pollen vitality and consequently reproductive success. Maize is particularly vulnerable to rising global temperatures, as thermal stress during the reproductive stage reduces the number of kernels per ear and consequently decreases the final yield (Zhao et al., 2017).

- Salinity stress refers to the negative effects caused by excessive accumulation of soluble salts in the soil or plant tissues. Salinity toxicity not only impairs cell division but also causes ionic imbalances, loss of membrane integrity, accumulation of reactive oxygen species (ROS) and biochemical alterations (Munns & Tester, 2008). In maize, salinity limits water and nutrient uptake and leads to reduced photosynthetic efficiency and vegetative growth, which are directly associated with decreased productivity, while also increasing oxidative stress due to ROS accumulation (Farooq et al., 2015).

- Waterlogging stress occurs when the soil becomes saturated with water, typically following intense rainfall events. Water stagnation drastically reduces oxygen availability in the soil, causing hypoxic or anoxic conditions at the root level. Under these conditions, aerobic respiration is impaired and energy production decreases, negatively affecting nutrient uptake and plant growth (Setter & Waters, 2003).

- Heavy metal stress occurs when toxic metals, such as lead (Pb), cadmium (Cd) and mercury (Hg) accumulate within the apoplast and symplast of plants.

These metals cause two closely related problems: they directly interfere with the activity of enzymes and proteins and induce oxidative stress through excessive ROS production (Hasanuzzaman et al., 2020).

- Nutrient deficiency stress takes place when one or more essential mineral elements are unavailable in sufficient quantities to sustain normal plant growth and development. Since nutrients are required for the synthesis of structural components, enzymes, nucleic acids and photosynthetic pigments, their deficiency disrupts many metabolic and physiological processes (Marschner, 2012). Nutrient limitation generally results in reduced photosynthetic activity, impaired carbon assimilation and consequently decreased biomass accumulation. Insufficient nutrient availability, in addition, may alter chlorophyll biosynthesis, protein synthesis and energy metabolism, limiting plant growth and productivity (Hawkesford et al., 2023). Plants subjected to nutrient deficiency exhibit typical morphological symptoms, such as chlorosis, necrosis, reduced leaf expansion, stunted growth and alterations in root system architecture (Lynch, 2019).

At the cellular level, nutrient deficiency induces metabolic reprogramming and activates adaptive responses that optimize nutrient uptake, transport and remobilization. These responses include changes in root morphology, increased expression of nutrient transporters and the accumulation of metabolites involved in stress tolerance (Kant et al., 2011). Prolonged nutrient limitation is also associated with alterations in cellular redox homeostasis, leading to the accumulation of ROS and the consequent oxidative damage of proteins, lipids and nucleic acids (Foyer & Noctor, 2005). In maize, nutrient deficiency reduces vegetative growth, delays reproductive development and negatively affects kernel number and grain filling, resulting in significant yield losses. Among the essential macronutrients, deficiencies of nitrogen (see Section 1.2.3), phosphorus and potassium have the greatest impact on crop productivity because of their fundamental roles in

photosynthesis, energetic metabolism, enzyme activation and osmotic regulation (Hawkesford et al., 2023).

1.2.3. Nitrogen deficiency

Nitrogen (N) is one of the most important macronutrients for plants, as it is a fundamental component of key biological molecules such as nucleic acids, amino acids and proteins.

Although nitrogen is the most abundant gas in the atmosphere, its stable form (N_2) can't be directly assimilated by plants, since roots are able to absorb this element only in the form of nitrate ion (NO_3^-), ammonium ion (NH_4^+) or organic compounds such as free amino acids and proteins (Abbas et al., 2021; Nacry et al., 2013). The conversion of atmospheric N into forms directly available for plant uptake occurs, biologically, through the activity of nitrogen-fixing microorganisms (either free-living or symbiotic) and nitrifying bacteria; the first convert nitrogen into ammonium, whereas the latter transform it into nitrate (Giehl et al., 2017).

In natural ecosystems, therefore, the nitrogen absorbed by a plant originates from the decomposition of residues or entire organisms that had previously assimilated it, thus maintaining a balanced nutrient cycle. In agroecosystems, however, the N removed through harvest is not naturally replenished (Lüdemann et al., 2022). Consequently, soil nitrogen availability is often limiting, making N deficiency one of the major factors negatively affecting crop growth and productivity worldwide (Mishra et al., 2024) and necessitating systemic replenishment of this macronutrient.

Despite the continuously increasing demand for nitrogen fertilizers (Schroeder et al., 2013), a significant proportion of applied N is not absorbed by plants, contributing to environmental issues such as soil acidification and water eutrophication. This has made the improvement of Nitrogen Use Efficiency (NUE) in plants an increasingly important area of research (Shafiq

et al., 2024). Current NUE values range between 30% and 50%, meaning that more than half of the nitrogen supplied to crops is not assimilated. In addition to being essential for the synthesis of enzymatic proteins involved in cellular metabolism, N is a constituent of chlorophyll, the pigment required for photosynthesis. Furthermore, as a structural component of nucleic acids (DNA and RNA), it also plays a crucial role in the regulation of gene expression and cell division (Mishra et al., 2024). Nitrogen deficiency causes physiological alterations that impair plant growth and development. One of the most evident symptoms is leaf chlorosis, resulting from reduced chlorophyll content, which initially appears in older leaves due to the mobility of N within the plant. From a metabolic perspective, nitrogen deficiency leads to reduced protein synthesis and alterations in carbon metabolism, resulting in the accumulation of carbohydrates that can't be effectively used for plant growth (Amoah et al., 2025).

In cereals, nitrogen deficiency is associated with reductions in tillering and in the number of spikes per unit area and kernel size, mainly due to the limited availability of carbon resources (Abbas et al., 2021). In maize, besides a decrease in chlorophyll, N deficiency also induces increased anthocyanin synthesis, which is phenotypically visible as a reddish-purple coloration of the leaves (Abbas et al., 2021).

1.3 ROOT RESPONSES TO STRESS

Among the various plant organs, the root system represents the first point of contact with the soil and plays a crucial role in the perception and response to environmental stresses, particularly abiotic stresses such as drought, nutrient deficiency, soil salinity and hypoxia (Smith & De Smet, 2012; Koevoets et al., 2016). Despite their importance, the study of roots has historically been limited by the technical difficulties associated with observing them under natural conditions. However, in recent years, interest in root biology has increased considerably, also in relation to the challenges posed by climate change to global agriculture and the need to develop crops with greater resilience (Den Herder et al., 2010).

Roots are equipped with highly sophisticated systems for the perception of external signals, enabling them to detect variations in water availability, nutrient concentration, soil pH and the presence of toxic compounds. These external stimuli are rapidly translated into physiological and molecular responses through the action of signalling molecules involved in root-to-shoot communication, such as abscisic acid (ABA), signalling peptides and reactive oxygen species (ROS) (Dodd, 2005). In addition to sensing their physical and chemical environment, roots establish complex interactions with the rhizosphere microbiome. Through the release of root exudates, plants shape the microbial communities surrounding their roots, promoting the establishment of beneficial microorganisms, including Plant-Growth-Promoting-Rhizobacteria (PGPR) and mycorrhizal fungi. These interactions enhance nutrient acquisition, improve tolerance to abiotic stresses and contribute to root growth and development (Bais et al., 2006; Smith & Read, 2008; Berendsen et al., 2012; Fitzpatrick et al., 2020).

In responses to stress conditions, roots exhibit not only architectural plasticity (Section 1.3.1), but also significant anatomical adaptations. For instance, under hypoxic conditions, they develop aerial tissues (aerenchyma) to facilitate gas transport (Colmer, 2003).

Additionally, drought or salinity stress can trigger the deposition of suberin and lignin in endodermal cells to reinforce the apoplastic barrier and limit uncontrolled water and ion fluxes. At the same time, the regulation of the Casparian strip is adjusted to maintain precise control over solute transport (Barberon, 2017).

1.3.1. Root plasticity and hormones involved

Root plasticity is another fundamental aspect of plant responses to stress and refers to the ability of the root system to modify several parameters of its architecture, including root length, growth angle, density and the distribution of lateral roots. Root system plasticity enables plants to adapt to highly heterogeneous soil conditions, such as deeper root growth under water deficit conditions, increased development of superficial roots and root hairs under phosphorus (P) deficiency and the avoidance of toxic zones during root growth (Smith & De Smet, 2012; Rogers & Benfey, 2015).

These adaptations are mainly regulated by phytohormones:

- Absciscic acid (ABA) is the primary regulator of plant responses to abiotic stresses, particularly under drought and salinity. Roots represent a major site of ABA biosynthesis; following the perception of reduced soil water potential, root cells rapidly increase its biosynthesis, and the hormone is transported through the xylem to the aerial parts of the plant. Once it reaches the leaves, ABA induces stomatal closure, thereby reducing leaf transpiration and water loss (Tardieu et al., 2014). At the root level, ABA regulates the inhibition of primary root growth under severe stress conditions, modulates lateral root formation and, together with other hormones, especially auxin, contributes to the regulation of root architecture (Rowe et al., 2016).

- Auxin is the main regulator of root development and plasticity, as it controls fundamental processes such as cell division within the root meristem, lateral root formation and the gravitropic response of roots. When plants are exposed to stress conditions, auxin distribution within the root is profoundly altered. For example, under drought or salinity stress, auxin polar transport, mainly mediated by transporters belonging to the *PIN* family, is reorganized, leading to changes in root branching patterns (Koevoets et al., 2016). Auxin and ABA interact closely, forming a balance that enables the plant to coordinate resource conservation with soil exploration; under mild stress conditions, adaptive root growth is promoted, whereas increasing stress intensity leads to growth inhibition (Rowe et al., 2016).
- Ethylene is a gaseous hormone that plays a crucial role in stress responses, particularly under conditions of root hypoxia, as it induces the formation of aerial tissues (aerenchyma), which facilitate oxygen transport from aerial tissues to the roots (Colmer, 2003). Stress conditions increase ethylene production, and this hormone also functions as an inhibitor of cell elongation. Through interactions with auxin and abscisic acid, ethylene contributes to the regulation of root system growth (Huang et al., 2022).
- Cytokinins (CKs) are hormones associated with cell division and with balancing root and shoot development. They act antagonistically to auxin and contribute to the modulation of meristematic activity and lateral root formation. Under stress conditions, cytokinin levels in roots decrease, thereby favouring root growth rather than shoot development in order to enhance soil exploration for limiting resources (Werner et al., 2010).

1.3.2. Molecular responses to abiotic stress and gene regulation

Following the perception of environmental stress, root cells rapidly activate response programs involving changes in gene expression, intracellular signalling and metabolism (Karlova et al., 2021). One of the earliest events is transcriptional regulation, which leads to the induction or repression of thousands of genes. These include genes encoding stress-responsive proteins, such as heat shock proteins (HSP) and late embryogenesis abundant (LEA) proteins, as well as various enzymes involved in cellular detoxification and secondary metabolism. These changes are highly dynamic and strongly dependent on the type of stress and on the specific root tissue involved (Kang et al., 2022). Transcriptional reprogramming is mediated by specific families of transcription factors, including DREB/CBF, NAC, MYB and bZIP, which coordinate the activation of groups of stress-related genes, forming complex Gene Regulatory Networks (GRNs) capable of interacting with environmental and hormonal signals (Hoang et al., 2020).

In addition to transcriptional regulation, the role of post-transcriptional regulation mediated by non-coding RNAs is becoming increasingly recognized. During this process, microRNAs (miRNA) and long non-coding RNAs (lncRNA) modulate the stability and translation of specific target mRNAs, thereby contributing to the fine and rapid regulation of stress responses (Jha et al., 2020). Reactive oxygen species (ROS) also play a key role in transcriptional regulation, since, at low concentrations, they act as signalling molecules capable of activating signal transduction pathways and redox-sensitive transcription factors (Karlova et al., 2021).

Another important aspect is the regulation of membrane transporters, which are essential for maintaining ionic and water homeostasis. Aquaporins, which control water flux across cellular membranes, are subject to both transcriptional and post-transcriptional regulation in

response to water stress. Likewise, ion transporters are modulated in order to limit ionic toxicity and optimize nutrient uptake under restrictive environmental conditions (Bárzana & Carvajal, 2020).

1.4 CROWN ROOTS

Crown roots are adventitious roots characteristic of the Poaceae family. Their name derives from the region from which they originate, namely the crown, corresponding to the basal stem nodes located near the soil surface (Sebastian et al., 2016). As previously mentioned (Section 1.1.3), together with brace roots, crown roots constitute the majority of the functional root system in adult plants. In addition to playing a major role in plant anchorage, they are essential for water and nutrient uptake (Reneau et al., 2020). Indeed, it has been demonstrated by Muthreich et al. (2013) that the *zmrtcs* mutant, which fails to develop both crown and seminal roots but produces a normal primary root with lateral roots, exhibits overexpression of stress-related genes, highlighting the inability of primary and lateral roots alone to adequately sustain plant development. Additional studies have confirmed this evidence by showing that, in five-week-old maize seedlings, the primary source of water uptake is represented by the lateral roots of crown roots (Ahmed et al., 2018).

1.4.1. Crown root formation

Unlike the primary root, which originates during embryogenesis, crown roots are formed post-embryonically from differentiated cells, known as founder cells. These cells are located adjacent to the peripheral vascular cylinder of the stem vascular bundles and, through periclinal divisions, give rise to the crown root primordium. The inner cells of the primordium

undergo periclinal divisions to generate the newly formed tissues of the endodermis/epidermis and the central cylinder, whereas the outer cells divide anticlinally to form the root cap. Subsequently, the initial endodermal/epidermal cells undergo further periclinal divisions, leading to the formation of the two distinct layers of epidermis and endodermis. The endodermal cells then undergo additional asymmetric periclinal divisions, generating the cortical tissue layers. In parallel, the initial cells of the root cap divide periclinally to form the columella, while the cells of the central cylinder continue dividing both anticlinally and periclinally, increasing in size and gradually acquiring a dome-shaped structure. Once the fundamental root organization has been established, the cells of the different tissues elongate and undergo vacuolization. Finally, the crown root primordium emerges after establishing vascular connections with the stem vascular system (Itoh et al., 2005).

1.4.2. Regulation of crown root development

The genetic regulation of crown root development has been investigated much more in rice (*Oryza sativa* L.) than in maize (Singh et al., 2022; Hochholdinger & Tuberosa, 2009). Crown root development can be broadly divided into three sequential stages: initiation of the crown root primordium, outgrowth of the primordium and the emergence of the crown root through the surrounding stem tissues (Figure 1.9; Singh et al., 2022). Throughout these developmental phases, auxin acts as the major positive regulator by promoting cell dedifferentiation and specification as founder cells and primordium formation. Coordinated auxin biosynthesis and transport generate a local auxin maximum, essential for the activation of developmental programs leading to crown root initiation. In rice, several transcription factors of the *LBD* (LATERAL BOUNDARIES DOMAIN) family, including *CRL1* (CROWN ROOTLESS1), function downstream of auxin signalling and represent key regulators of crown root initiation. This confirms the importance of auxin-responsive

transcription factors during the early stages of crown root development (Inukai et al., 2005; Singh et al., 2022).

Auxin signalling is coordinated with cytokinin, which generally acts as an antagonist by limiting root initiation and regulating the number of crown roots that are produced (Figure 1.9). Auxin and cytokinin do not act independently but interact through a coordinated regulatory network in which auxin promotes root initiation whereas cytokinin contributes to regulating the spatial and temporal progression of primordium development. Several rice regulators, such as *WOX11*, *CRL5* and *ERF3*, are involved in this hormonal crosstalk by modulating cytokinin-responsive genes and coordinating the transition from primordium initiation to root outgrowth (Kitomi et al., 2011; Zhao et al., 2009, 2015; Singh et al., 2022). Following primordium formation, successful crown root emergence requires coordinated growth through the overlying cortical and epidermal tissues. Ethylene plays a major positive role during this phase by promoting ROS accumulation and programmed cell death in the external cell layers. This facilitates crown root emergence without damaging the root apex.

Gibberellins (GA) act synergistically with ethylene to promote this process, while abscisic acid (ABA) generally exerts an antagonistic effect by restricting crown root elongation and emergence (Figure 1.9). Polar auxin transport is also essential during these later developmental stages, as demonstrated by the reduced crown root emergence observed in rice mutants with altered *OsPIN1* activity (Steffens & Rasmussen, 2016; Xu et al., 2005; Singh et al., 2022).

Compared with rice, the molecular regulation of crown root development in maize is still only partially understood and relatively few genetic regulators have been functionally characterized. The best-known regulator is *RTCS* (ROOTLESS CONCERNING CROWN AND SEMINAL ROOTS): an LBD transcription factor, homologous to rice *CRL1*, which is required for the initiation of crown roots, brace roots and seminal roots. Mutations in *RTCS*

impair the formation of the post-embryonic root system, demonstrating its central role in maize root architecture (Taramino et al., 2007; Singh et al., 2022). Its paralog *RTCL* acts together with *RTCS* during adventitious root elongation, highlighting the importance of auxin-responsive LBD transcription factors in maize root development (Xu et al., 2015; Singh et al., 2022).

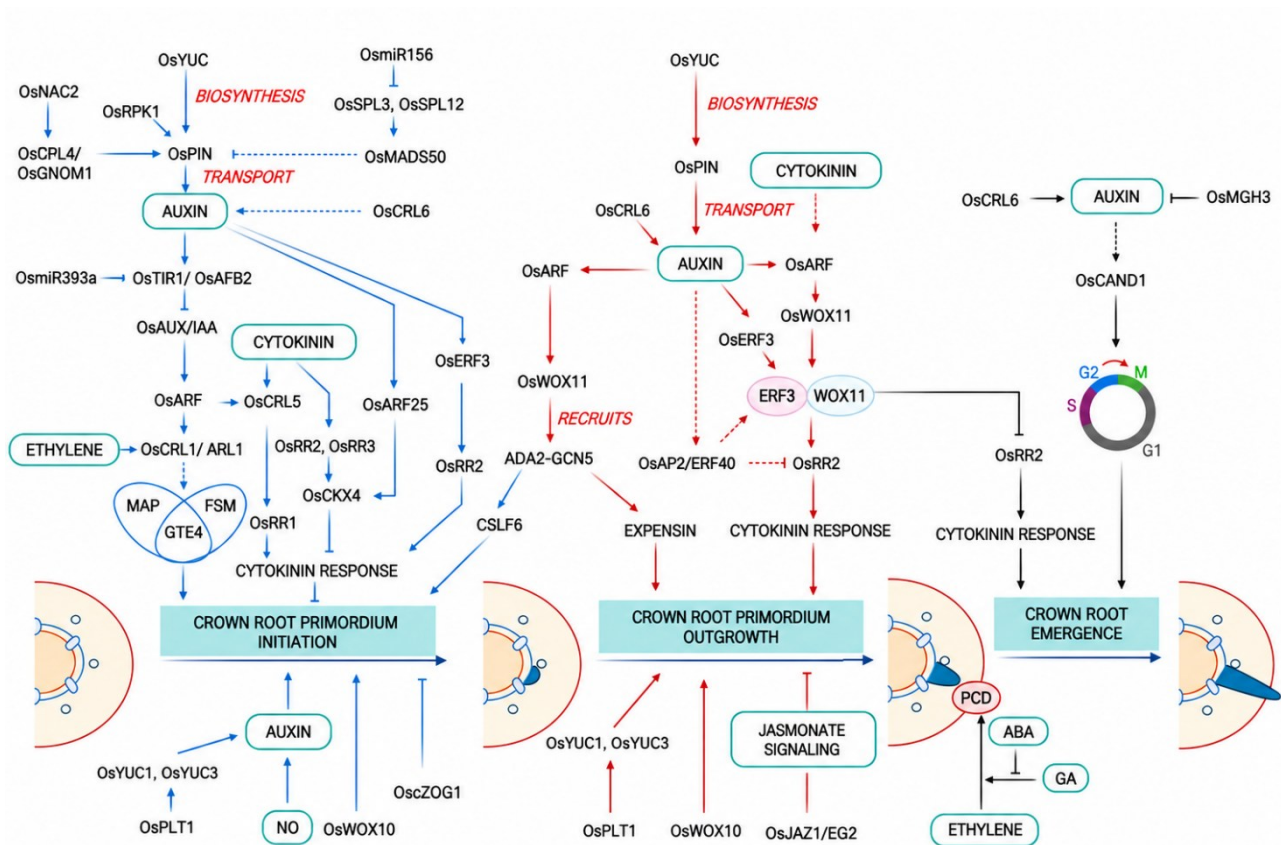


Figure 1.9: Schematic representation of the hormonal and genetic regulatory network controlling crown root development in rice, showing the main signalling pathways involved in primordium initiation, outgrowth and emergence. Arrows indicate positive regulation, lines with flat head represent negative regulation (Singh et al., 2022).

1.5 STRIGOLACTONES

1.5.1. Identification and chemical structure

Strigolactones (SLs) are terpenoid lactones derived from carotenoid metabolism (Al-Babili & Bouwmeester, 2015). Since 2008, they have been recognized as a phytohormone and key regulators of plant development and plant-environment interactions. Their discovery dates back to 1966, when compounds capable of inducing the germination of parasitic plant seeds, such as witchweeds (*Striga* spp.) and broomrapes (*Orobanche* and *Phelipanche* spp.), were isolated from the root exudates of cotton (*Gossypium hirsutum* L.) (Cook et al., 1966). The first compound identified was named strigol (a name that was subsequently extended to the entire class of strigolactones) because of its ability to stimulate the germination of witchweeds. Species belonging to the genus *Striga* are parasitic plants of the Orobanchaceae family and represent a major biotic threat to global food security. In Africa, they severely affect cereal production, causing annual yield losses estimated between 7 and 10 billion dollars (Parker, 2012). Strigol was subsequently identified in the root exudates of sorghum (*Sorghum bicolor* L.), maize (*Zea mays* L.), and millet (*Panicum miliaceum* L.) (Ćavar et al., 2015). In 1998, Yokota et al. isolated additional compounds from red clover (*Trifolium pratense* L.) capable of stimulating the germination of *Orobanche* species. These molecules were named orobanchol and orobanchyl acetate and are among the most widespread strigolactones in the plant kingdom (Ćavar et al., 2015).

Initially, these compounds were considered exclusively as rhizospheric signalling molecules involved in communication between plants and parasitic plants. Subsequent studies demonstrated that SLs released by plant roots stimulate hyphal branching in arbuscular mycorrhizal fungi (AMF), thereby attracting them and promoting the establishment of symbiotic associations (Akiyama et al., 2005). In this mutualistic interaction, fungi improve

plant water and mineral nutrient uptake, and plants give them reduced carbon compounds. This symbiosis occurs in more than 80% of terrestrial plant species (Kodama et al., 2022) and is particularly important under nutrient-limiting conditions, as AMF facilitate the solubilization and transfer of nitrogen and phosphorus to the host plant in the form of nitrates and phosphates, which can be readily assimilated (Zwanenburg et al., 2016). The inclusion of SLs among the recognized phytohormones in 2008 was supported by the characterization of mutants displaying semi-dwarf and highly branched phenotypes in three genetically distant model species: *Arabidopsis thaliana*, *Pisum sativum*, and *Oryza sativa*. These studies demonstrated that exogenous application of the synthetic strigolactone analogue GR24 was able to restore the wild-type phenotype in mutants defective in SL biosynthesis, but not in mutants impaired in SL perception, thereby providing direct evidence for the involvement of strigolactones in the regulation of apical dominance and shoot branching (Gomez-Roldan et al., 2008; Umehara et al., 2008). Since then, numerous studies have revealed the involvement of strigolactones in a wide range of physiological processes, including leaf senescence, stomatal closure, responses to biotic and abiotic stresses, and the regulation of root system architecture (Al-Babili & Bouwmeester, 2015).

From a chemical perspective, all strigolactones share a conserved core structure consisting of a butenolide ring (D-ring) connected through an enol-ether bridge to a second variable moiety. Based on the nature of this variable portion, the approximately 35 naturally occurring strigolactones identified to date are classified into two major groups: canonical and non-canonical strigolactones (Figure 1.10). Canonical SLs contain a tricyclic lactone structure composed of three rings (A, B, and C rings) linked to the D-ring, whereas non-canonical SLs lack one or more of these rings, which are replaced by alternative structural motifs (Figure 1.10; Al-Babili & Bouwmeester, 2015; Yoneyama, 2020). An example of a non-canonical strigolactone is zealactone, which was identified in maize. The A and B rings may differ in

the presence of additional functional groups, such as $-\text{CH}_3$, $-\text{OH}$, and $-\text{C}(\text{O})\text{CH}_3$, whereas the C and D rings are highly conserved and, together with the enol-ether bridge, are essential for the biological activity of SLs. Furthermore, the stereochemistry of the C-ring in canonical strigolactones allows their classification into two additional subgroups: strigol-type SLs, which possess a β -oriented C-ring (upward orientation) with an 8bS configuration, and orobanchol-type SLs, characterized by an α -oriented C-ring (downward orientation) with an 8bR configuration (Figure 1.10; Jia et al., 2018). Strigolactones can also be chemically synthesized, and the most widely used synthetic analogue is rac-GR24 (Figure 1.10). Rac-GR24 consists of an equimolar mixture of two enantiomers: (+)-GR24, which mimics both the biological activity and stereochemical configuration of natural 5-deoxystrigol (5DS), and (–)-GR24, which possesses a 2'S stereochemistry that is not found among naturally occurring strigolactones. In addition to SLs, karrikins (KARs) also contain a butenolide ring; however, their overall chemical structure differs substantially from that of strigolactones. Karrikins (Figure 1.10) are smoke-derived compounds produced during the combustion of plant material and are well known for their ability to stimulate seed germination, particularly in species adapted to post-fire environments. Despite their structural differences, both KARs and SLs are perceived through homologous α/β -hydrolase receptors and activate partially overlapping signalling pathways, suggesting a common evolutionary origin for these two classes of butenolide-containing molecules (Waters et al., 2017).

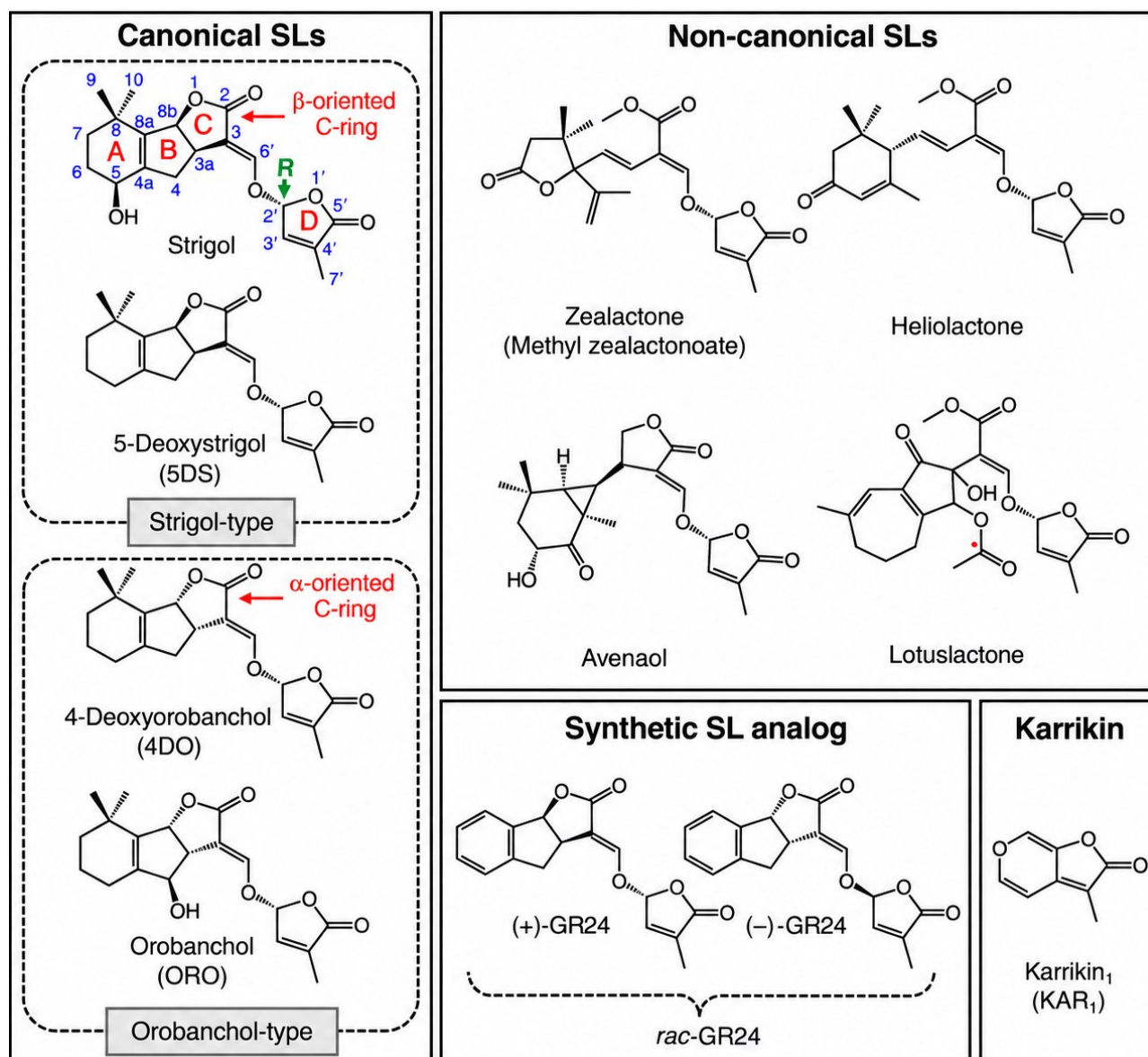


Figure 1.10: Chemical structures of strigolactones (SLs), including canonical strigol-type and orobanchol-type molecules, non-canonical strigolactones, the synthetic SL analogue GR24 and Karrikin (Mashiguchi et al., 2021).

1.5.2. Strigolactone biosynthesis and signalling

The elucidation of the strigolactone biosynthetic pathway began following the discovery that root exudates from maize carotenoid-deficient mutants, as well as from wild-type plants treated with fluridone, a carotenoid biosynthesis inhibitor, induced significantly lower germination rates of *Striga lutea* seeds. These observations suggested that SLs are directly derived from carotenoids (Matusova et al., 2005). Despite the remarkable structural diversity of SLs among plant species, the early steps of their biosynthesis are highly conserved throughout evolution. The precursor of strigolactones is the same as abscisic acid (ABA), namely all-trans- β -carotene (Liu et al., 2020). The first stage of strigolactone biosynthesis consists of the conversion of all-trans- β -carotene into carlactone (CL). This process takes place within plastids and involves the sequential activity of three enzymes: DWARF27 (D27), CAROTENOID CLEAVAGE DIOXYGENASE 7 (CCD7), and CAROTENOID CLEAVAGE DIOXYGENASE 8 (CCD8). D27 catalyzes the reversible isomerization of all-trans- β -carotene into 9-cis- β -carotene (Figure 1.11), whereas CCD7 and CCD8 subsequently cleave and convert 9-cis- β -carotene into carlactone via the intermediate 9-cis- β -apo-10'-carotenal (Al-Babili & Bouwmeester, 2015). Carlactone represents the central intermediate of strigolactone biosynthesis and can be considered the simplest non-canonical SL, as it contains the characteristic butenolide ring (D-ring) linked to an enol-ether bridge in the biologically active R configuration that is conserved among natural strigolactones (Alder et al., 2012). Following its synthesis, carlactone is exported from plastids to the cytosol, where a cytochrome P450 monooxygenase belonging to the More Axillary Growth 1 (MAX1) family catalyzes its oxidation to carlactonic acid (CLA) and subsequently generates species-specific non-canonical SLs (Figure 1.11). Depending on the plant species, MAX1 homologs may further catalyze additional oxidation and structural modification reactions, contributing

to the remarkable diversity of naturally occurring SLs (Wakabayashi et al., 2019; Yoneyama & Brewer, 2021).

In maize (*Zea mays* L.), the conversion of β -carotene into carlactone is mediated by the enzymes ZmD27, ZmCCD7, and ZmCCD8 (Li et al., 2023). In vitro studies have demonstrated that the cytochrome P450 monooxygenase ZmMAX1b oxidizes carlactone to produce carlactonic acid (CLA), while the methyltransferase ZmCLAMT1 catalyzes methylation of the C-19 carboxyl group of CLA, yielding methyl carlactonoate (MeCLA), a key intermediate in the biosynthesis of maize non-canonical SLs (Figure 1.12; Li et al., 2023). Subsequently, the cytochrome P450 enzyme ZmCYP706C37 catalyzes sequential oxidation reactions on MeCLA, leading to the formation of zealactone (Figure 1.12; Li et al., 2023). Interestingly, in vivo evidence suggests the existence of an alternative biosynthetic route. In this pathway, ZmCYP706C37 initially oxidizes carlactone at both the C-3 and C-19 positions, generating 3-oxo-19-hydroxy-carlactone (3-oxo-19-OH-CL). The same enzyme subsequently converts this intermediate into zealactol, which is then oxidized by ZmMAX1b to form zealactonoic acid (ZA). Finally, ZmCLAMT1 methylates ZA to produce zealactone (Figure 1.12; Li et al., 2023). Consistent with this model, mutations in ZmCLAMT1 result in the accumulation of zealactonoic acid (ZA) rather than carlactonoic acid (CLA), indicating that methylation occurs predominantly at the final stages of zealactone biosynthesis (Figure 1.12; Li et al., 2023). Zealactone can undergo further structural modifications to generate zeapyranolactone, another maize-specific non-canonical SL. However, the enzymatic mechanisms underlying this conversion remain unknown. Current evidence suggests that the formation of zeapyranolactone may occur spontaneously through non-enzymatic rearrangements, although this hypothesis has yet to be experimentally confirmed (Figure 1.12; Charnikhova et al., 2017, 2018; Xie et al., 2017).

SL signal transduction depends on the α/β -hydrolase receptor D14 (DWARF14), which undergoes a conformational change upon strigolactone binding. This conformational change enables the interaction of D14 with the F-box protein MAX2/D3 and with transcriptional repressors belonging to the SMXL/D53 family (Waters et al., 2017). The resulting complex promotes the ubiquitination and subsequent proteasomal degradation of SMXL repressors, thereby activating genes involved in stress responses (Figure 1.13). In rice (*Oryza sativa* L.), D53 represents one of the major repressors of the strigolactone signalling pathway, whereas in *Arabidopsis thaliana* this role is performed by the proteins SMXL6, SMXL7, and SMXL8 (Soundappan et al., 2015).

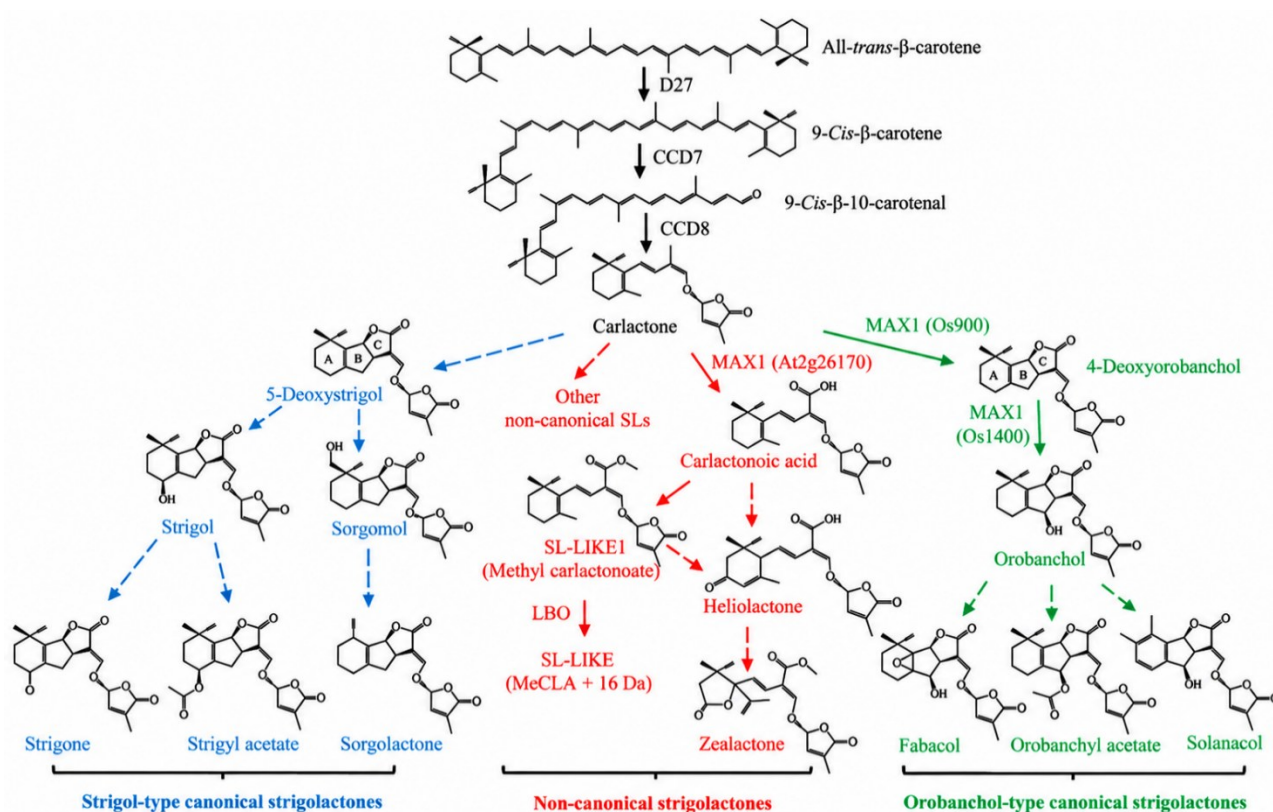


Figure 1.11: Biosynthetic pathway of canonical and non-canonical SLs from β -carotene. Carlactone acts as the central intermediate from which different enzymatic reactions produce canonical SLs (strigol-type in blue and orobanchol-type in green) and non-canonical strigolactones in red (Wang & Bouwmeester, 2018).

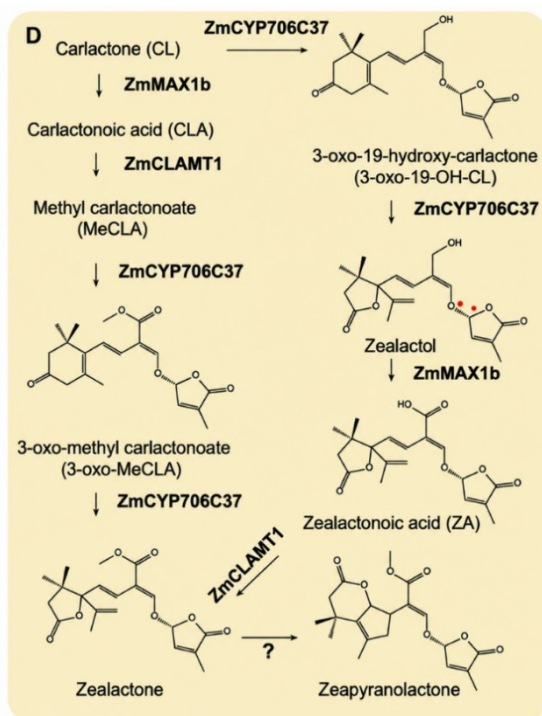


Figure 1.12: Biosynthetic pathway of maize SLs showing the conversion of carlactone (CL) into zealactone through the action of the enzymes ZmMAX1b, ZmCLAMT1 and ZmCYP706C37. The final step leading to zeapyranolactone is still unsolved (Ou et al., 2026).

1.5.3. Strigolactone transport

Since SLs were initially identified in root exudates as signalling molecules involved in interactions between plants, arbuscular mycorrhizal fungi (AMF), and parasitic weeds, it is likely that specific transporters facilitate the secretion of SLs from roots into the rhizosphere (Ou et al., 2026). Subsequently, grafting studies conducted in *Arabidopsis thaliana*, *Pisum sativum*, and *Petunia hybrida* demonstrated the mobility of SLs from the root system to the aerial parts of the plant, suggesting the existence of long-distance transport mechanisms (Beveridge et al., 1994, 1996; Napoli, 1996; Turnbull et al., 2002; Booker et al., 2004). Nevertheless, the number of characterized SL transporters remains limited, and the mechanisms regulating their allocation are still poorly understood (Ou et al., 2026). The first SL transporter to be characterized was *Petunia hybrida* Pleiotropic Drug Resistance 1 (*PhPDR1*), a member of the ABCG transporter family (Kretzschmar et al., 2012). This transporter is localized in the plasma membrane and is predominantly expressed in root tissues and nodal tissues of the shoot, where it mediates both the secretion of SLs into the

rhizosphere and their transport from roots to shoots (Kretzschmar et al., 2012). The physiological role of *PhPDR1* in SL transport is supported by genetic evidence. The *pdrl* petunia mutant exhibits a phenotype partially overlapping that of the *dad1* mutant (Napoli, 1996), which is defective in SL production, displaying reduced AMF colonization and increased shoot branching. Furthermore, studies performed in *Arabidopsis thaliana* showed that heterologous expression of *PhPDR1* attenuates the effects of exogenously applied SLs (GR24) on root system growth (Kretzschmar et al., 2012). The involvement of strigolactones and their transporter *PhPDR1* in the establishment of symbiotic interactions between plants and arbuscular mycorrhizal fungi is further supported by the observation that *PhPDR1* is predominantly expressed in hypodermal passage cells, which correspond to the entry sites of AMF hyphae (Kretzschmar et al., 2012). Moreover, *PhPDR1* displays a pronounced apical polarity in the plasma membrane of root cells, a feature that enables directional SL transport (Sasse et al., 2015). Homologs of *PhPDR1* have subsequently been characterized as SL transporters in tobacco (*Nicotiana tabacum* L.) and tomato (*Solanum lycopersicum* L.) (Xie et al., 2015; Ban et al., 2025). In tomato, the genes *SIABCG44* and *SIABCG45*, orthologs of *PhPDR1*, mediate both the secretion of SLs into the rhizosphere and their transport toward the shoot. Under phosphate-deficient conditions, the expression of these genes is upregulated by *SINSP1* and *SINSP2*, thereby enhancing strigolactone export (Ban et al., 2025).

In maize, Trevisan et al. (2015) identified a set of genes potentially involved in SL transport, namely *ZmPDR1*, *ZmPDR3*, and *ZmWBC33*. These genes were identified through transcriptomic analysis of the transition zone (TZ), a highly specialized root region involved in the perception of endogenous and exogenous stimuli and in the coordination of adaptive responses. Ravazzolo et al. (2019) demonstrated a strong induction of *ZmWBC33* transcription under nitrogen and phosphorus deficiency, whereas its expression was

downregulated in the presence of ammonium and nitrate. In addition to being detected in vascular tissues and in the apical meristem of lateral root primordia (LRPs), *ZmWBC33* transcripts were found co-localized with those of *ZmCCD8* in the epidermis and cortex of the stem (Ravazzolo et al., 2019). The co-localization of *ZmWBC33* and *ZmCCD8* within shoot vascular tissues suggests that SL biosynthesis occurs not only in roots but also in aerial organs, supporting the hypothesis that *ZmWBC33* is involved in the intercellular transport of strigolactones in maize (Ravazzolo et al., 2019).

Recently, Shi et al. (2025) identified two novel SL transporters in sorghum through the integration of transcriptomic analyses under phosphate starvation and treatments with GR24^{5DS}, a synthetic stereoisomer of strigolactones. These two ABCG transporters, designated *SbSLT1* and *SbSLT2* (Sorghum bicolor Strigolactone Transporter 1 and 2), are localized to the plasma membrane and are predominantly expressed in lateral epidermal cells (Shi et al., 2025). Although double knockout mutants for *SbSLT1* and *SbSLT2* display normal plant growth and development, they exhibit complete resistance to parasitism by *Striga* spp., highlighting the essential role of these transporters in mediating SL release into the rhizosphere (Shi et al., 2025).

1.5.4. Role of strigolactones in abiotic stress response

To date, SLs are recognized not only as regulators of plant development but also as important mediators of plant responses to abiotic stresses. They act through a complex network involving biosynthetic genes, signalling components, and hormonal interactions. Numerous studies conducted in recent years have demonstrated that abiotic stress conditions, such as phosphorus and nitrogen deficiency, drought, extreme temperatures, salinity, and oxidative stress, induce the expression of genes involved in strigolactone

biosynthesis, including *D27*, *CCD7*, *CCD8*, and *MAX1* (Marzec, 2016; Yoneyama & Brewer, 2021). Phosphorus deficiency in rice (*Oryza sativa* L.) significantly increases the expression of *D27*, *CCD7*, and *CCD8*, resulting in enhanced accumulation and root exudation of strigolactones into the rhizosphere (Umehara et al., 2010). This stress-induced response promotes colonization of the rhizosphere by arbuscular mycorrhizal fungi (AMF), thereby improving water and nutrient uptake from the soil (Umehara et al., 2010). In abiotic stress responses, SLs interact closely with other phytohormones, particularly abscisic acid (ABA), with which they share the biosynthetic precursor β -carotene. Under drought conditions, SLs and ABA exhibit strong cooperation in the regulation of stomatal closure and water homeostasis (Figure 1.13; Haider et al., 2018). Studies in *Arabidopsis thaliana* have shown that mutants defective in SL biosynthesis (*max3* and *max4*) or perception (*d14*) exhibit increased susceptibility to water deficit, accompanied by altered expression of several ABA-responsive genes, highlighting the close interaction between strigolactones and ABA signalling pathways during drought response (Ha et al., 2014; Li et al., 2020). Furthermore, SLs enhance the antioxidant capacity of plants by inducing the expression of genes involved in the detoxification of reactive oxygen species (ROS). These genes increase the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD), thereby contributing to the preservation of cellular membrane integrity and reducing oxidative damage (Dong et al., 2025). Under high salinity conditions, strigolactones modulate MAPK (Mitogen-Activated Protein Kinase) signalling pathways and other genes involved in osmotic protection, promoting proline accumulation and membrane stabilization (Figure 1.13; Barbier et al., 2023a). In response to nutrient limitation, SLs regulate auxin transport and distribution through the control of *PIN* transporter localization, thereby influencing lateral root and root hair development (Barbier et al., 2023a). *Arabidopsis thaliana max* mutants exhibit altered lateral root density and reduced root plasticity under

phosphate-deficient conditions, highlighting the essential role of SL–auxin signalling in root morphological adaptation (Mayzlish-Gati et al., 2012).

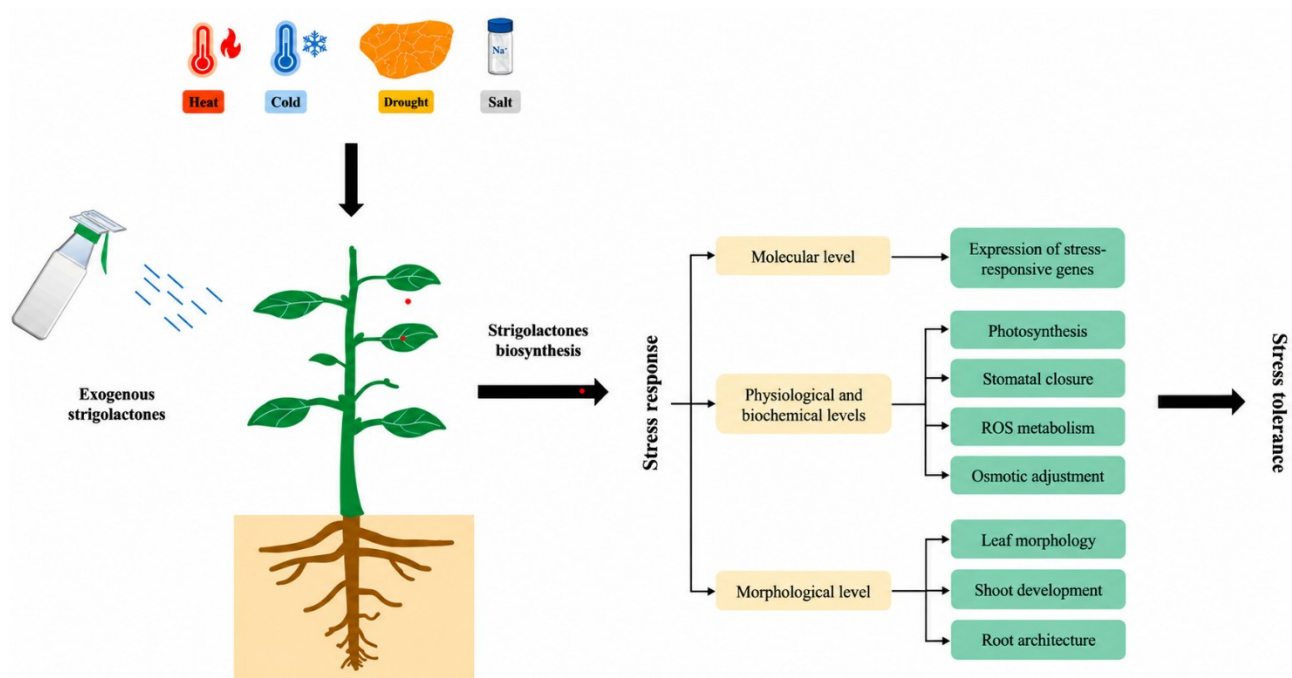


Figure 1.13: Overview of strigolactone-mediated responses to abiotic stresses. SLs modulate plant responses at the molecular, physiological and morphological levels, ultimately enhancing stress tolerance (Dong et al., 2025).

1.5.5. Strigolactones and root system architecture

Further insights into the role of strigolactones in root system regulation have highlighted how SLs, through a close interaction with auxin, cytokinins, and other hormonal pathways, contribute to the regulation of root system architecture (RSA) in response to abiotic stresses. These phytohormones modulate the development, spatial distribution, and plasticity of the primary root, seminal roots, lateral roots, root hairs, and, most importantly, adventitious roots, including crown roots (Kapulnik & Koltai, 2014; Sun et al., 2022). Strigolactones initially influence primary root growth by promoting the maintenance of meristematic activity and, consequently, root elongation. Studies performed using *Arabidopsis thaliana* mutants defective in strigolactone biosynthesis (*max3* and *max4*) or perception (*d14*) revealed that

mutant plants exhibited shorter primary roots and reduced meristematic activity compared with the wild type. Treatment with the synthetic strigolactone analogue GR24 restored the wild-type phenotype in biosynthetic mutants but not in *d14*, indicating that these hormones contribute to the maintenance of cell proliferation within the root apical meristem (Mehmood Jan et al., 2024). At the molecular level, this regulation is strongly associated with the interaction between strigolactones and auxin transport through the modulation of *PIN* transporters, which control IAA distribution within the root system (Barbier et al., 2023a).

Strigolactones also play a fundamental role in the regulation of lateral roots, although their effects strongly depend on plant species and nutritional status. In *Arabidopsis*, SLs generally repress lateral root initiation and density under optimal nutrient conditions by limiting local auxin accumulation within lateral root primordia. However, under phosphate-deficient conditions, strigolactones accumulate in root tissues and contribute to the remodelling of root architecture, promoting root plasticity and improving soil exploration capacity (Marzec & Melzer, 2018). In rice and maize, by contrast, SLs often exert an inhibitory effect on lateral root development, suggesting the existence of species-specific differences in auxin signalling regulation and hormonal sensitivity (Wang et al., 2024).

Particularly relevant is the role of SLs in the development of crown roots in rice and maize. The rice mutants *d10* and *d3* exhibit a significantly lower number of crown roots compared with wild-type plants (Figure 1.14; Sun et al., 2015). Furthermore, the exogenous application of GR24 increases crown root number in the biosynthetic mutant (*d10*) but not in the signalling mutant (*d3*) (Sun et al., 2015). These results indicate that proper strigolactone biosynthesis and perception are required for normal crown root development in rice. The *zmccd8* mutant (Section 1.5.6), which is defective in strigolactone biosynthesis, exhibits reductions in both crown root number and crown root length compared with wild-type plants, indicating that strigolactones promote both the initiation and elongation of this root type

(Guan et al., 2012). Collectively, these findings suggest that SLs contribute to the adaptive remodelling of crown root development and to the plasticity of root system architecture under nutrient deficiency conditions, thereby improving soil exploration and enhancing phosphorus acquisition. This response is considered of agronomic relevance because it may enhance nutrient acquisition and improve maize performance under nutrient-deficient conditions (Barbier et al., 2023b; Wang et al., 2024). Finally, strigolactones are also involved in the regulation of root hair development, which plays a crucial role in phosphorus acquisition by increasing the absorptive surface of the root system. Early studies showed that SLs promote root hair elongation under phosphorus-deficient conditions through signalling pathways in which ethylene, reactive oxygen species (ROS) and nitric oxide (NO) participate (Kapulnik et al., 2011; Villaécija-Aguilar et al., 2019).

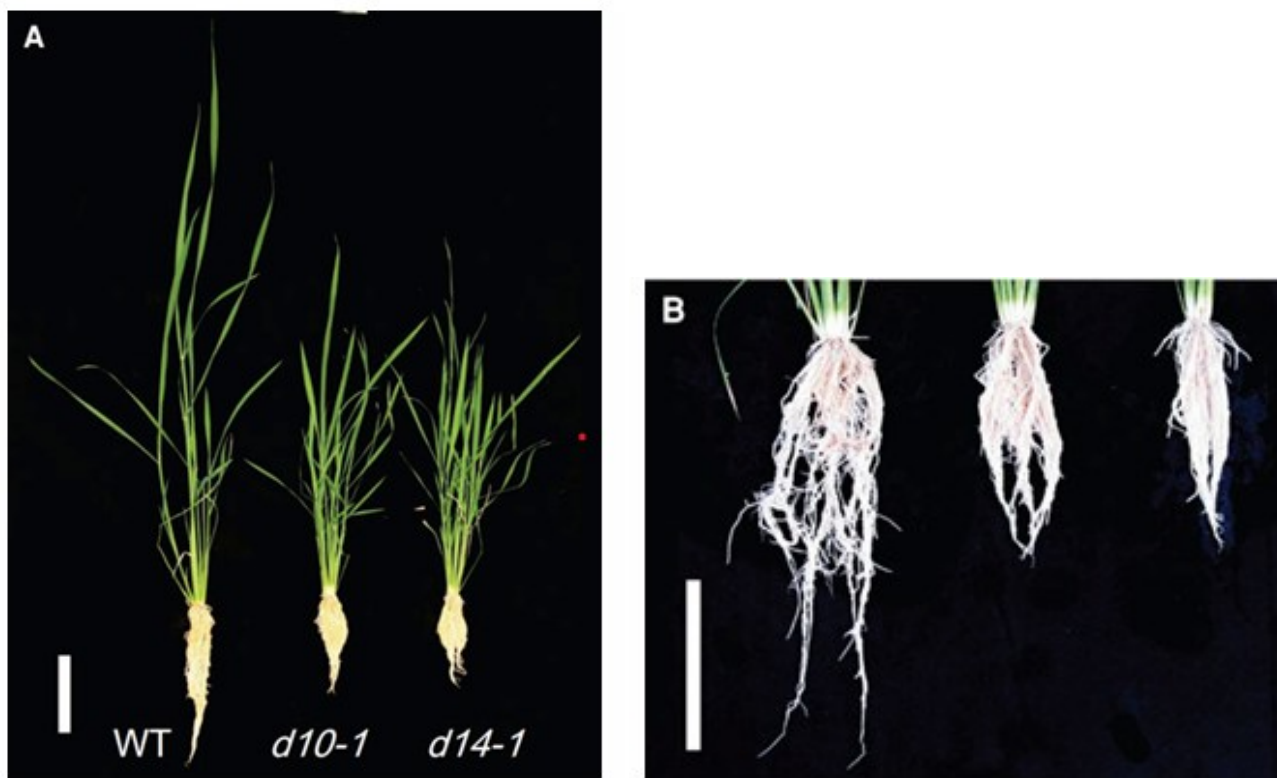


Figure 1.14: Phenotypic comparison between wild type (WT) rice plants and SL mutants *d-10* (biosynthesis mutant) and *d-14* (signalling mutant). **(A)** Shoot phenotype showing increased tillering and reduced plant height in the mutants compared with the WT. **(B)** Root phenotype showing less developed root systems in mutants (Arite et al., 2012).

1.5.6. *zmccd8* - The mutant that does not produce strigolactones

The identification of strigolactones (SLs) as carotenoid-derived metabolites was initially supported by studies conducted on maize mutants deficient in carotenoid biosynthesis. The root exudates of these mutants exhibited a reduced ability to induce the germination of *Striga lutea* seeds compared to wild-type plants (Matusova et al., 2005). Subsequently, the characterization of the SL biosynthetic pathway highlighted the central role of the enzyme CCD8 (CAROTENOID CLEAVAGE DIOXYGENASE 8), which is responsible for the conversion of 9-cis- β -apo-10'-carotenal into carlactone (CL), the main precursor of all natural strigolactones (Section 1.5.2; Alder et al., 2012; Al-Babili & Bouwmeester, 2015). In maize, the *ZmCCD8* gene is present as a single-copy gene on chromosome 3 and encodes a 572 amino-acid protein that shares a high degree of homology with *MAX4* from *Arabidopsis thaliana* and *D10* from *Oryza sativa*, suggesting a strong conservation of the strigolactone biosynthetic pathway between dicotyledonous and monocotyledonous species (Guan et al., 2012).

The biological importance of *ZmCCD8* was demonstrated by Guan et al. (2012) through the characterization and use of the insertional mutant *zmccd8*, in which the gene is inactivated by a transposon, thereby impairing strigolactone biosynthesis. As observed in the *max* mutants of *A. thaliana* and the *d10* mutant of rice, *zmccd8* plants exhibit marked alterations in shoot architecture, characterized by early axillary bud outgrowth and a substantial increase in lateral branching. In *zmccd8* mutants, axillary buds are visible and more developed than in wild-type plants (Figure 1.15) from the earliest stages of development. Treatment with GR24 suppresses this phenotype, confirming that the excessive branching is a direct consequence of strigolactone deficiency (Guan et al., 2012).

Adult mutant plants display approximately three times more axillary branches than wild-type plants, a characteristic accompanied by a reduction in stem diameter, plant height, and ear size. The internodes of mutant plants are also shorter than those of the wild type, due to an altered growth rate that was reported to be 45% lower throughout all stages of stem elongation (Figure 1.15; Guan et al., 2012). Furthermore, the *zmccd8* mutant exhibits profound alterations in root development (Figure 1.16). The primary root is significantly shorter in mutant plants than in the wild type and crown root development is severely impaired, with both crown root length and emergence being markedly reduced (Figure 1.16). Wild-type plants already display emerging nodal roots between the coleoptile and mesocotyl, whereas these structures are absent or poorly developed in *zmccd8* mutants (Figure 1.16).

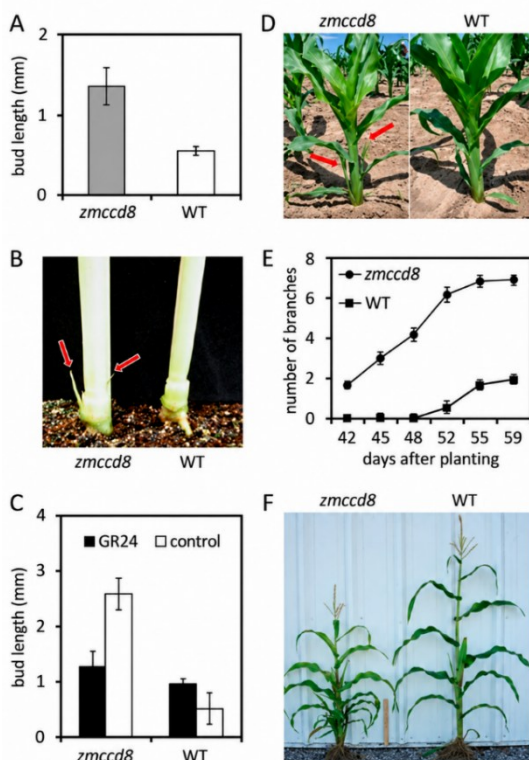


Figure 1.15: Phenotypic characterization of the maize *zmccd8* mutant. (A-C) Axillary bud growth under control conditions and after treatment with the synthetic SL analogue GR24. (D-F) Plant architecture and branch development in *zmccd8* and WT plants, showing the role of SLs in suppressing axillary bud outgrowth and shoot branching (Guan et al., 2012).

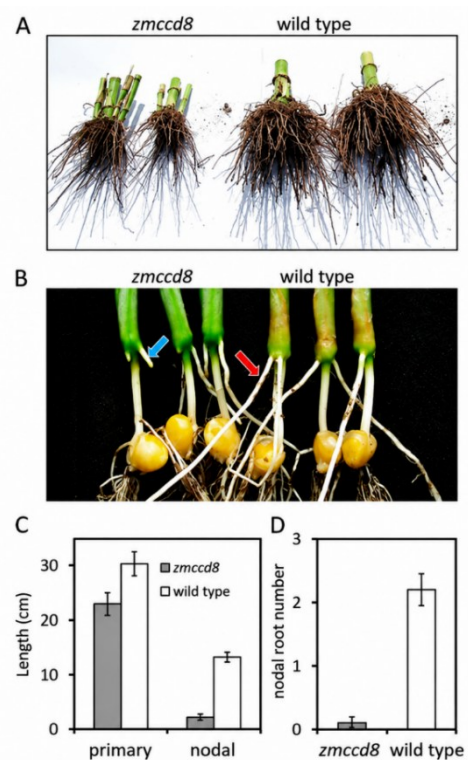


Figure 1.16: Comparison of root system architecture between maize *zmccd8* and WT plants. (A) Representative root systems showing reduced root development in *zmccd8*. (B) Seedling root phenotype showing the absence or strong reduction of crown roots in the mutant. (C-D) Primary root length and nodal root number (Guan et al., 2012).

2. AIM OF THE STUDY

The aim of the present study was to investigate the role of strigolactones (SLs) in regulating the development and plasticity of the maize (*Zea mays* L.) root system, focusing on the early stages of plant development. Although several studies have demonstrated the involvement of these phytohormones in plant responses to abiotic stresses and in the regulation of root system architecture, the mechanism through which strigolactones regulate the development of the different root types, particularly crown roots, is still not fully understood.

For this purpose, a comparative analysis was performed between two maize genotypes: the B73 inbred line, used as the wild type (WT) and the *zmccd8* knockout mutant, which is unable to synthesize strigolactones due to a mutation in the *ZmCCD8* gene, a key component of the biosynthetic pathway of SLs. Both genotypes were grown in vermiculite under controlled growth chamber conditions for 28 days.

Throughout the experiment, phenotypic, physiological and molecular analyses were performed at four different time points in different stages of vegetative development.

To assess the plant physiological state, leaf chlorophyll (Chl), anthocyanin (Anth) and flavonoid (Flav) were monitored using the DUALEX SCIENTIFIC+™ instrument, while biometric measurements for both the shoot and the root systems were carried out at each sampling time point. These analyses were followed by RNA extraction, cDNA synthesis and the expression analysis of genes involved in auxin metabolism, polar transport and response, in lateral root formation and emergence and in ethylene pathways by Reverse Transcription-quantitative PCR (RT-qPCR).

By integrating phenotypic, physiological and transcriptional analyses, this study elucidates how strigolactone deficiency alters plant development, advancing our understanding of these phytohormones in regulating maize root system architecture.

3. MATERIALS AND METHODS

3.1. Plant growth

This study was conducted using 60 maize (*Zea mays* L.) seedlings: 30 belonging to the B73 inbred line, used as the wild type (WT), and 30 belonging to the *zmccd8* knockout mutant line. Plants of both genotypes were grown under the same environmental conditions. Subsequently, six plants from each genotype were sampled at four different time points and phenotypic, physiological and molecular analyses were performed.

Seeds of both genotypes were soaked in deionized water for two hours to initiate imbibition and promote germination (Figure 3.1A). After being rinsed twice with deionized water, the seeds were placed on strips of moist paper, following their natural polarity (pedicel facing downward) and maintaining approximately 0.5 cm between adjacent seeds (Figure 3.1B). The paper strips were then rolled up and placed inside beakers containing 400 mL of deionized water (Figure 3.1C). The beakers were wrapped in aluminium foil to simulate dark conditions and kept in a growth chamber at 24°C for four days.

Following germination, the seedlings were selected to obtain a homogeneous plant population, and each selected seed was individually transplanted into a pot containing about 80 g of vermiculite at a depth of approximately 1.5 cm. Vermiculite is a porous substrate with a high water-holding capacity that ensures adequate root aeration while allowing the root system to remain intact during plant removal from the pots. Moreover, since vermiculite is free of nutrients, it allows precise control of nutrient supply, creating growth conditions that represent an intermediate system between in vitro culture and field cultivation.

From the day of transplanting onwards, each plant received 100 mL of nutrient solution on the days indicated in Table 3.2, and any excess solution was removed. The nutrient solution was prepared by dissolving the following components in deionized water:

Macroelements (μM)	
KNO_3	1000
MgSO_4	200
CaCl_2	200
KH_2PO_4	40

Microelements (μM)	
FeNaEDTA	10
H_3BO_3	4,6
MnCl_2	0,9
CuCl_2	0,036
ZnCl_2	0,086
NaMoO_4	0,011

Table 3.1: Nutrient concentrations used for the preparation of 1 L of nutrient solution.

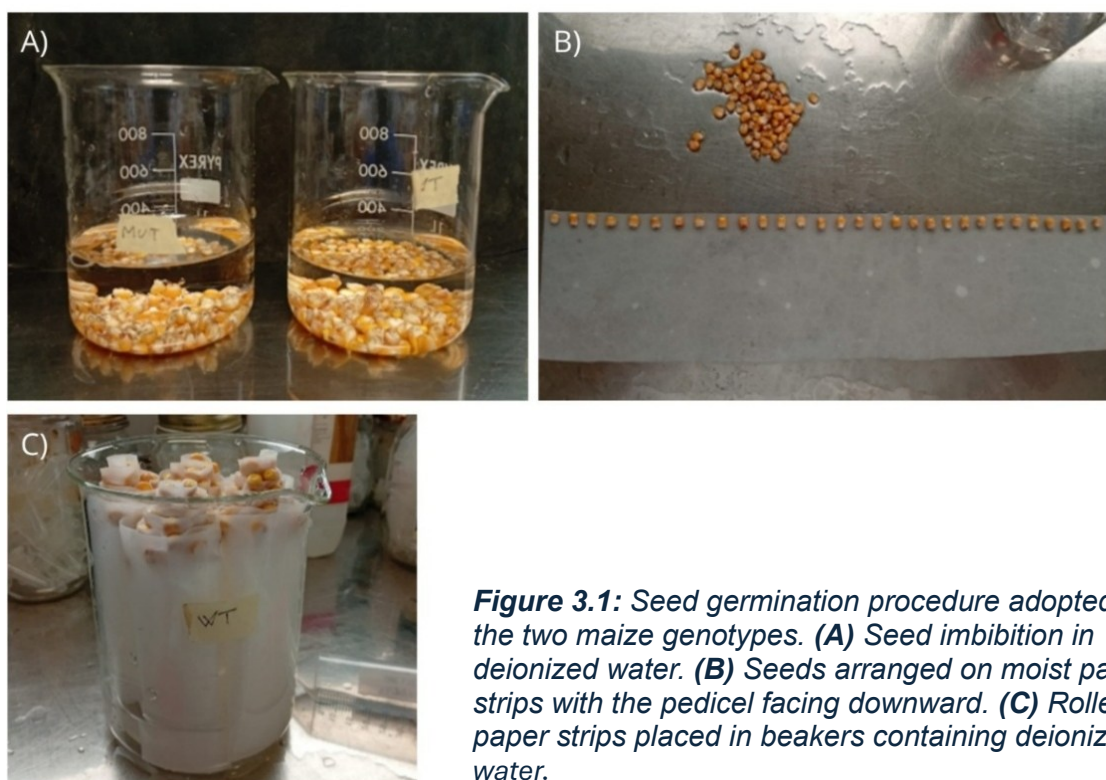


Figure 3.1: Seed germination procedure adopted for the two maize genotypes. **(A)** Seed imbibition in deionized water. **(B)** Seeds arranged on moist paper strips with the pedicel facing downward. **(C)** Rolled paper strips placed in beakers containing deionized water.

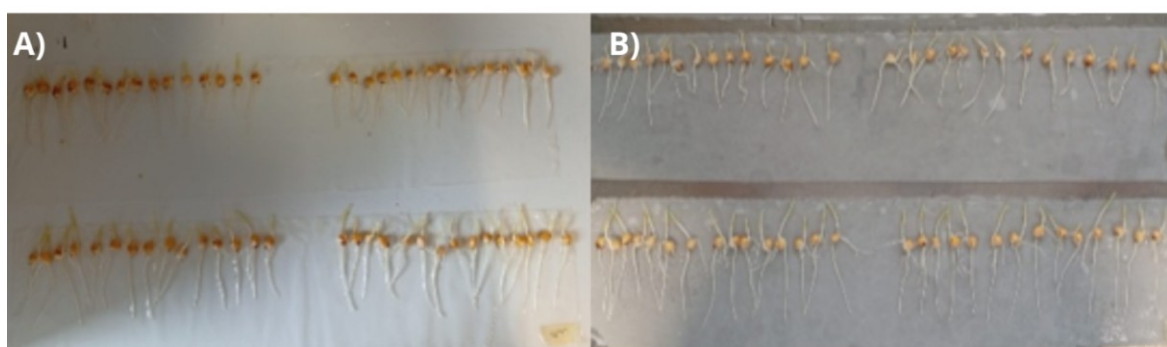


Figure 3.2: Selection of homogeneous seedlings for the two maize genotypes based on primary root length. **(A)** Wild type (B73) and **(B)** *zmccd8* knockout mutant.

3.2. Experimental layout

Plant cultivation in pots was carried out according to a scheduled timeline for a total period of 28 days, as shown in Table 3.2. DAT is the acronym for Days After Transplant and indicates the number of days elapsed since transplanting, which corresponds to 0 DAT.

DAT	ACTIVITY	DAT	ACTIVITY
0	Transplant in pot Nutrient solution	16	DUALEX Nutrient solution
2	Nutrient solution	18	DUALEX Nutrient solution Sampling (T3) Fresh weight measurement Morphological traits measurement
4	Nutrient solution		
7	DUALEX Nutrient solution		
8	Sampling (T1) Fresh weight measurement Morphological traits measurement	21	DUALEX Nutrient solution
9	DUALEX Nutrient solution	23	DUALEX Nutrient solution
11	DUALEX Nutrient solution Sampling (T2) Fresh weight measurement Morphological traits measurement	25	DUALEX Nutrient solution
14	DUALEX Nutrient solution	28	DUALEX Sampling (T4) Fresh weight measurement Morphological traits measurement

Table 3.2: Timeline of the experimental activities performed throughout the 28-day cultivation period. DAT = Days After Transplanting.

3.3. Optical Assessment of Leaf Pigments

Starting from the seventh day after transplanting, chlorophyll (Chl), anthocyanin (Anth), flavonoid (Flav) contents and the Nitrogen Balance Index (NBI) were measured using the DUALEX SCIENTIFIC+™ instrument (Force-A, Orsay, France; Figure 3.3). Measurements were performed on the days when the nutrient solution was supplied. At the same time points, seedling growth and the number of leaves were also assessed and documented by photographs. Dualex measurements were always carried out at the same time and on the same portion of the leaf. Initially, measurements were performed only on the first leaf of each seedling; as the plants developed and produced additional leaves, up to five leaves per plant were measured.



Figure 3.3: DUALEX SCIENTIFIC+™ instrument used for the non-destructive measurement of chlorophyll, anthocyanin, flavonoid indexes.

3.4. Weight and Length Analysis and Sampling

A total of four sampling events were carried out at 8 (T1), 11 (T2), 18 (T3) and 28 (T4) days after transplanting collecting six plants per genotype at each time point. The first two time points were selected because, according to the literature, crown root formation occurs during this developmental stage. The subsequent samplings were carried out 10 and 20 days after T1 to evaluate how the two genotypes differed over time at both phenotypic and molecular levels.

Plants were divided into five different portions using a blade: primary root, seminal roots, crown roots, nodal meristem and total shoot. Each tissue was weighed using a precision balance to determine fresh weight, immediately transferred to liquid nitrogen and stored at -80 °C to prevent tissue degradation

Digital photographs acquired at the four sampling time points were used to perform morphological measurements using ImageJ software (National Institutes of Health, Bethesda, MD, USA). This software allows quantitative measurements to be obtained from digital images after image calibration. Therefore, a ruler was included in each picture to provide a reference scale. The morphological traits measured at each time point were stem diameter, stem length and the average internode length, which was calculated as the ratio between the distance from the first to the last leaf and the total number of leaves per plant.

3.5. RNA extraction

Tissues collected during the sampling time points and stored at -80°C were used for RNA extraction. Since RNA is highly susceptible to degradation, it's essential to maintain the cold chain throughout the entire procedure. For this reason, liquid nitrogen was used during

sample preparation. In addition, all equipment used during tissue homogenization, including mortars, pestles and spatulas (Figure 3.4), was previously sterilized by heating at 200°C for 4 hours.

Two individual plants were pooled to obtain each biological replicate and for each sample 100 mg of tissue were weighed and transferred to a mortar. The tissue was initially crushed coarsely with a pestle and subsequently ground into a fine powder by gradually increasing the grinding force. Throughout the procedure, liquid nitrogen was replenished as it evaporated to ensure that the tissue remained completely frozen during homogenization. Once grinding was completed, tissue residues adhering to the pestle and the inner surface of the mortar were carefully collected using a spatula as the liquid nitrogen evaporated. The resulting powdered tissue was then transferred into a 2 mL Eppendorf tube and stored at -80°C until RNA extraction.

Total RNA was extracted using the SPECTRUM™ Plant Total RNA Kit (Sigma-Aldrich, St. Louis, MO, USA), following the manufacturer's protocol step by step (Figure 3.5).

RNA concentration and purity were assessed using a NanoDrop™ 1000 spectrophotometer (Thermo Scientific, NanoDrop Products, Wilmington, DE, USA), which quantifies RNA concentration (ng/μl) and evaluates sample purity based on the absorbance ratios at 260/280 and 260/230 nm. RNA integrity was subsequently assessed by agarose gel electrophoresis (Figure 3.6).

Once the purity and integrity of the extracted RNA had been assessed, reverse transcription was performed to synthesize complementary DNA (cDNA), which was subsequently used as the template for quantitative PCR (qPCR). The cDNA was synthesized according to the laboratory's internal protocol using Promega M-MLV Reverse Transcriptase. Briefly, 500 ng of total RNA were mixed with 1 μL of oligo(dT) (10 μM), as described by Manoli et al. (2012).



Figure 3.4: Equipment used for tissue grinding and sample preparation before RNA extraction, including mortars, pestles, spatulas and liquid nitrogen containers.

Figure 3.5: Filtration Column (blue retaining ring) and Binding Column (red retaining ring) of SPECTRUM™ Plant Total RNA Kit (Sigma-Aldrich, St. Louis, MO, USA). The Filtration Column removes residual cellular debris, whereas the Binding Column selectively binds total RNA for subsequent washing and elution.

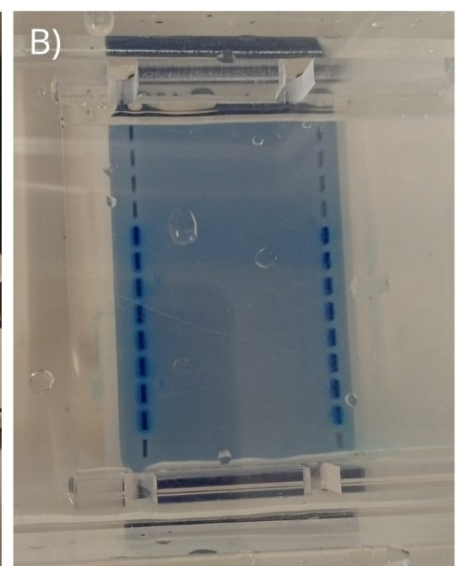


Figure 3.6: Experimental setup for agarose gel electrophoresis. **(A)** Electrophoresis system used for agarose gel analysis. **(B)** Agarose gel showing the wells loaded with RNA samples before electrophoresis.

3.6. qPCR

The cDNA obtained by reverse transcription was used for gene expression analysis by RT-qPCR System (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA). PowerUp™ SYBR™ Green Master Mix (Applied Biosystems, Monza, Italy) was used according to the manufacturer's instructions and the laboratory standard protocol. RT-qPCR is a molecular technique that enables the detection and quantification of target gene expression through the amplification of specific cDNA sequences. Gene expression analysis was performed on both genotypes (B73 and *zmccd8*) using cDNA obtained from primary root and crown root tissues collected at 11 and 28 DAT and from the nodal meristem at 8 and 11 DAT. For each genotype and sampling time, three biological replicates were analysed, and each biological replicate was run in three technical replicates for each gene. To investigate the molecular mechanisms underlying the observed phenotypes, three functional groups of genes were selected for expression analysis: genes involved in auxin signalling and transport, genes regulating lateral root emergence and genes associated with ethylene biosynthesis and signalling (Table 3.3).

Gene name	Gene ID	Primers		Reference	Group
PIN1a	Zm00001eb372180	For	GGTTTCGGCTTCAGATCAGGCGGCAAG	Forestan et al., 2012	1
		Rev	GCCACGACCGCCGGCATGGAGATGTG		
PIN2	Zm00001eb372180	For	AGGTGGCCAACAAGTTCGCGTCTGGG	Forestan et al., 2012	
		Rev	CCTTCTTGCGCGGGGCCACGTACG		
PIN1b	Zm00001eb254390	For	ACGAAGCTGCGGGAGCCGTTT	Forestan et al., 2012	
		Rev	CTCCGCTGGCTGCCGCACAGGGACA		
PIN1c	Zm00001eb194640	For	CCAGGGGGGACGTCGAGCTCGAGGCC	Forestan et al., 2012	
		Rev	CCTCGCGCTCGTCTTGGGGTAC		
PIN5c	Zm00001eb102370	For	GCGGCGGCAAGGGCAAGGACAGAG	Forestan et al., 2012	
		Rev	TGTGACCAGCGGCCATAACGACTGAGCC		
PIN8a	Zm00001eb154930	For	GCACCATAACATCTTGCCGAGACTCCT	Forestan et al., 2012	
		Rev	AGGAGTCTCGGCAAGATGTTATGGTGC		
PIN9	Zm00001eb150590	For	CACCGTCGCTCGTCTCCATGCTCC	Forestan et al., 2012	
		Rev	GGAGCATGGAGAGCGAGGCGACGGTG		
LAX1	Zm00001eb026490	For	CGAGTTTGCTCCACAGTAGTT	Zhang et al., 2014	
		Rev	TACAACAGGGCATTTGACGA		
LAX2	Zm00001eb010460	For	ATGAGAGACGATGAAATGAAC	Zhang et al., 2014	
		Rev	TAGAGGAGAGAACGAAGATG		
YUCCA6	Zm00001eb332870	For	AGGAGGAGGTGATCGTCGT	Ravazzolo et al., 2021	
		Rev	TTCTTGGTGAGGTGGAGGTG		
IAA19	Zm00001eb276990	For	CAGAGTGTGTGCTGGAAGAGG	Ludwig et al., 2013	
		Rev	ACCAAAGCCAAGTATGAAAGAAC		
IAA24	Zm00001eb301590	For	ATCCCAGTCCCACACAAAG	Ravazzolo et al., 2021	
		Rev	ATCGGAAGCGAAAGGAGAG		
ARF20	Zm00001eb232120	For	TATCGCCACGAAAGGGT	Ravazzolo et al., 2021	
		Rev	CAGCAACAGCTCTAGGTAAGT		
ARF25	Zm00001eb227100	For	TCTGGGGAACAACATTCTCC	Zhang et al., 2016	
		Rev	AACATTCTGCACCTGCCATC		
ARF34	Zm00001eb373970	For	GCACGAACCAACAAAACCAG	Xu et al., 2015	
		Rev	ATCCGTGATCCTTCATCTCG		
RTCS	Zm00001eb003920	For	AGAGAGATGACGGGGTTCG	Xu et al., 2015	2
		Rev	CCTCGTAGGAGATGGTGACG		
RTCL	Zm00001eb403030	For	GCAAGAACACGAAGTGATCG	Xu et al., 2015	
		Rev	TTCCGGCACTACGAGTAAGT		
ARGOS7	Zm00001eb244140	For	ACGATGACGACCCAAGCAC	Ravazzolo et al., 2021	
		Rev	AGCAGCACCGCCAACATT		
RSL4	Zm00001eb392530	For	CTCCTTGAACCTTTACTGCCG	Ravazzolo et al., 2021	
		Rev	TGCCCTCACAAGCCTCTCAT		
RAL1	Zm00001eb020110	For	TACTCGCCTGACAGCACTTG	Zhang et al., 2016	
		Rev	AACGGCTTCTTCGATGACCA		
RAP1	Zm00001eb393120	For	AACTGGCAACTTCCTTACG	Zhang et al., 2016	
		Rev	CGGTGTTCACGATTCAGAC		
LBD7	Zm00001eb051620	For	GTCTTCGTCGCCAAGTTCTT	Trevisan et al., 2015	
		Rev	AGTTGCCCGTCCACATGAG		

Gene name	Gene ID	Primers		Reference	Group
ACS7	Zm00001eb428490	For	CCTCACATGACTGCAAACAAG	Buzzicotti et al., 2026	3
		Rev	GTGCAACGTCTCTGTAC		
ACO2	Zm00001eb314610	For	CACTTCCACTCACC GCACTC	Buzzicotti et al., 2026	
		Rev	CGCCATGTTGCTCTGTCTGT		
SAMS4	Zm00001eb130870	For	CTTCTCTGGCTCTGCTGTT	Buzzicotti et al., 2026	
		Rev	TCATCATCTTCTCTCCGTTCA		
EREB54	Zm00001eb413930	For	ATGCAAATCAAGAAATCGACGGG	Buzzicotti et al., 2026	
		Rev	AATTTAGATGAAGCCGCTGTGA		
EREB174	Zm00001eb146650	For	GCCAGTAGCGGTCTTCTTCT	Buzzicotti et al., 2026	
		Rev	TCTCTCTTGTGATCTGCT		
EREB209	Zm00001eb171050	For	ACAGACAGATCAGACCTCACCT	Buzzicotti et al., 2026	
		Rev	ACAGACGGGACAGGACCAAG		
MPE	Zm00001eb257640	For	TGTACTCGGCAATGCTCTTG	Manoli et al., 2012	Housekeeping gene
		Rev	TTTGATGCTCCAGGCTTACC		

Table 3.3: Genes selected for RT-qPCR analysis, together with their Gene ID, forward and reverse primer sequences and the corresponding literature references. Based on their biological function, the genes were grouped into three categories: **Group 1**, genes involved in auxin biosynthesis, transport and signalling; **Group 2**, genes associated with lateral root emergence and development; and **Group 3**, genes involved in ethylene biosynthesis and signalling.

3.7. Statistical analyses

Statistical analyses were conducted using R software (version 2025.09.1+401) within the RStudio environment (<https://cran.rstudio.com>). Differences between the B73 wild type (WT) and *zmccd8* mutant were evaluated for phenotypic traits, gene expression levels, and DUALEX measurements using Student's t-test. Statistical significance was set at $P < 0.05$ and is indicated by asterisks as follows: * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$. Data are presented as mean $\pm$ standard error (SE).

4. RESULTS

4.1. PHENOTYPIC ANALYSES

4.1.1. Shoot biomass

Regarding shoot biomass, as shown in Figure 4.1, B73 (WT) plants exhibited higher shoot fresh weight than the *zmccd8* mutant throughout the entire experimental period except for T1 (8 DAT), when the difference between the two genotypes was not significant. However, as plant growth progressed, the difference became significant at T2 and highly significant at both T3 and T4. This trend is also reflected by the increasing divergence between the growth curves over time (Figure 4.1). At the final sampling time (T4), the difference between the mean shoot fresh weight of the six plants analysed for each genotype approached 1 g, with the wild-type plants showing the greater biomass (Figure 4.1 and 4.2).

4.1.2. Root biomass

Similarly, total root biomass was consistently higher in WT plants than in the *zmccd8* mutant throughout the experimental period, as shown in Figure 4.1. Highly significant differences between the two genotypes were observed at all sampling times except the first. At 28 days after transplanting (DAT), the total root biomass of the *zmccd8* mutant was approximately 55% of that of the WT plants (Figure 4.1).

When the root system was further divided into its individual components, the same overall trend was observed, with WT plants exhibiting greater biomass than the mutant across all root types (Figure 4.1). The biomass of the primary root began to diverge between the two genotypes from the third sampling time (18 DAT), whereas seminal root biomass showed significant differences as early as the second sampling time (11 DAT) (Figure 4.1). Among

the different root types, crown roots exhibited the greatest differences in biomass between the two genotypes. In addition to being fewer in number than those of the WT, crown roots of the *zmccd8* mutant showed an approximately 40% reduction in biomass compared with the WT at T4 (28 DAT) (Figure 4.1).

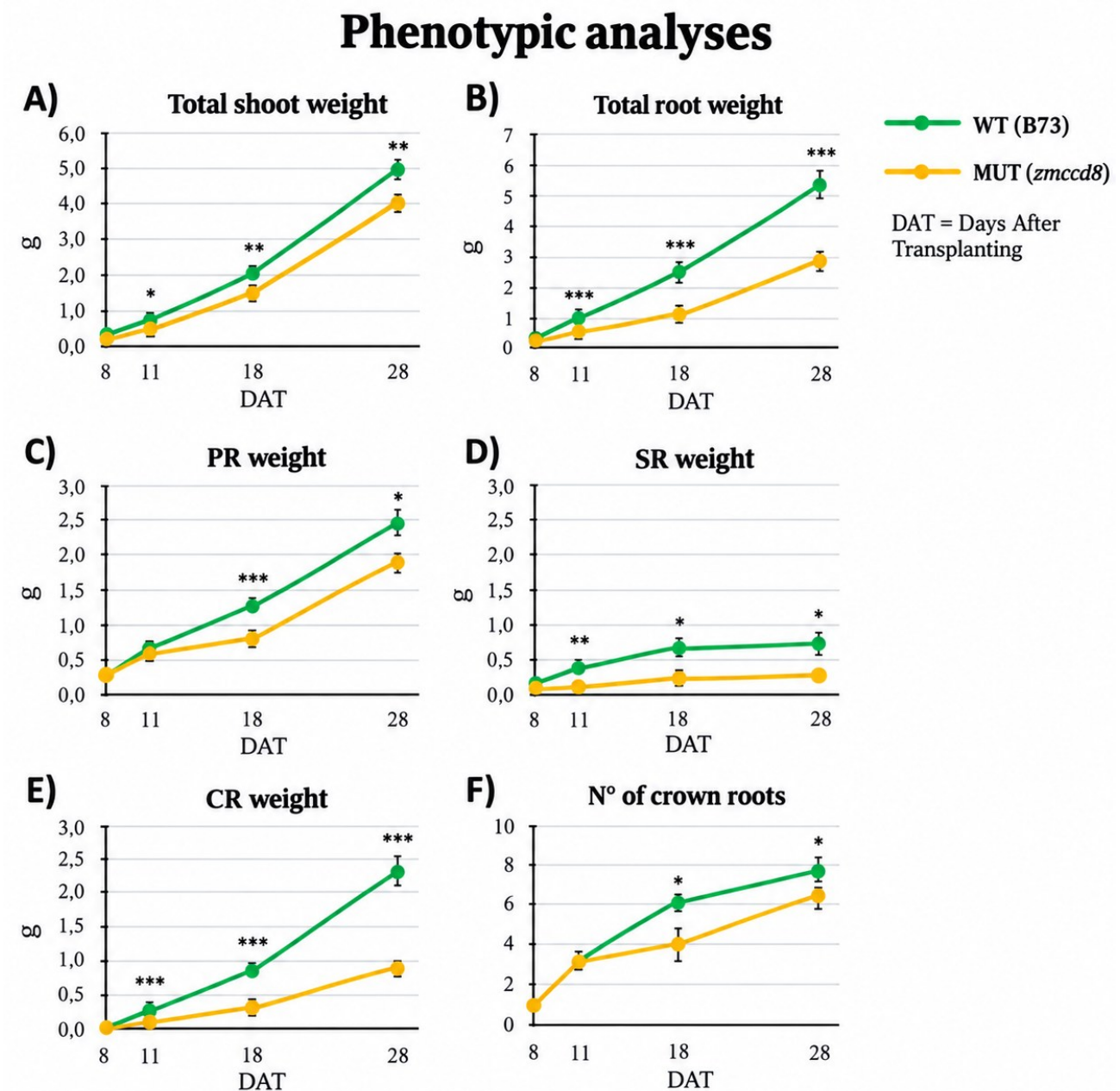


Figure 4.1: Shoot and root system traits of B73 wild type (WT) and *zmccd8* mutant (MUT) maize plants measured at 8, 11, 18, and 28 days after transplanting (DAT). (A) Total shoot fresh weight, (B) total root fresh weight, (C) primary root (PR) fresh weight, (D) seminal root (SR) fresh weight, (E) crown root (CR) fresh weight, and (F) number of crown roots. Data are presented as mean $\pm$ SE. Asterisks indicate statistically significant differences between genotypes at each sampling time (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).



Figure 4.2: Representative comparison of the aerial phenotype of *zmccd8* mutant and B73 wild type plants at the final sampling time (28 days after transplanting). The *zmccd8* mutant exhibited a reduced shoot development relative to the WT plants. Scale bar = 10 cm.

4.2. DUALEX ANALYSES

Physiological analyses were performed using the DUALEX SCIENTIFIC+™ instrument starting from the seventh day after transplanting. Measurements were carried out on the first, second, third, fourth, and fifth leaves, and chlorophyll (Chl), anthocyanin (Anth), and flavonoid (Flav) indices were assessed.

4.2.1. Chlorophyll

The analysis of the chlorophyll index in the first five leaves revealed distinct patterns between the two genotypes throughout plant development. In the older leaves, particularly the first and second leaves, the WT showed a progressive reduction in the chlorophyll index during the later stages of plant development compared with the *zmccd8* mutant (Figure 4.3). In the first leaf, after initially showing similar values between the two genotypes, the chlorophyll index of the WT progressively decreased, reaching a mean value of

approximately $18 \mu\text{g cm}^{-2}$ at the last measurement, whereas the mutant showed a progressive increase, reaching values close to $50 \mu\text{g cm}^{-2}$. A similar pattern was observed in the second leaf, where the WT showed a reduction in the chlorophyll index during the final measurements, while the values in the *zmccd8* mutant increased to approximately $35 \mu\text{g cm}^{-2}$ and subsequently remained relatively stable. In the third leaf, the values of the two genotypes progressively converged during plant development, with slightly higher values observed in the mutant at the later time points. In the fourth leaf, chlorophyll index values were more similar between the two genotypes and tended to converge at the final measurement. In the fifth leaf, the WT showed slightly higher chlorophyll index values than the *zmccd8* mutant. However, measurements for this leaf were only performed during the early stages of its development, as the fifth leaf was still at an early developmental stage during the final measurements preceding the last sampling.

Chlorophyll (DUALEX units)

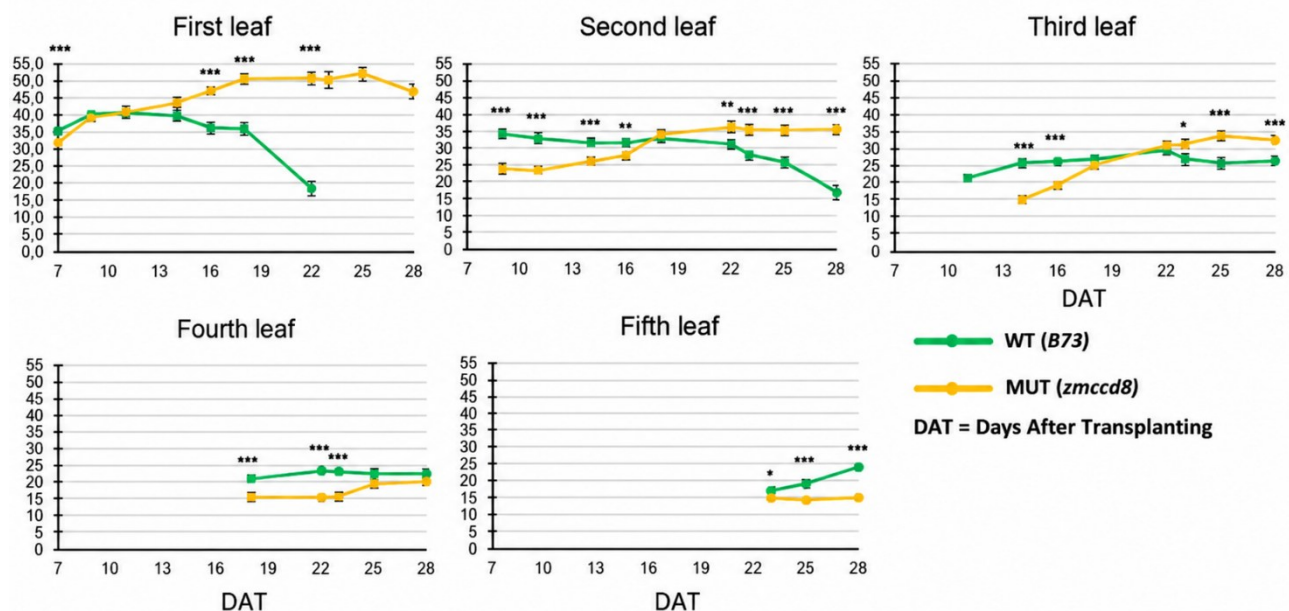


Figure 4.3: Changes in chlorophyll index in the first five leaves of WT (B73) and *zmccd8* (MUT) maize plants throughout plant development. Measurements were performed using the Dualex Scientific+™ at different days after transplanting (DAT). Data are presented as mean $\pm$ SE. Asterisks indicate statistically significant differences between genotypes at each sampling time (*P < 0.05; **P < 0.01; ***P < 0.001).

4.2.2. Anthocyanins

For anthocyanins, as shown in Figure 4.4, the analysis performed using the Dualex revealed a distinct pattern between the two genotypes. In the first leaf, the initial values were similar between WT and *zmccd8*, whereas during development the mutant showed a slight decrease in the index, which subsequently remained relatively stable at around 0.35 Dualex units. In contrast, the WT, after an initial phase with values remaining around 0.4, showed a marked increase during the final measurements, reaching a value of approximately 0.68 (Figure 4.4). A similar pattern was observed in the second leaf, where the mutant showed a progressive decrease in the index, whereas the WT, after an initial decrease, showed a progressive increase, reaching values close to 0.68 at the last measurement. Regarding the third, fourth, and fifth leaves, similar patterns were observed, with an overall decrease in the anthocyanin index throughout development (Figure 4.4). It is important to consider that, similarly to what was observed for the chlorophyll index, only a limited number of measurements were available for the fourth and, particularly, the fifth leaf, as these leaves were still at early developmental stages at the time of the final sampling.

Anthocyanin (DUALEX units)

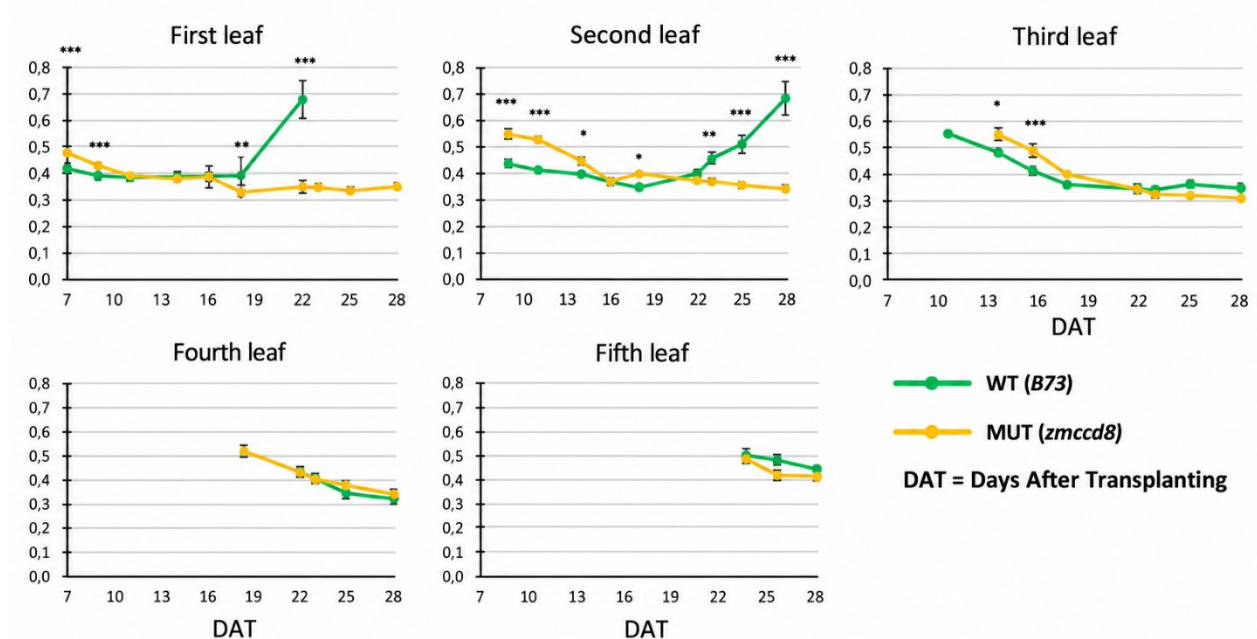


Figure 4.4: *Changes in anthocyanin index in the first five leaves of WT (B73) and zmccd8 (MUT) maize plants throughout plant development. Measurements were performed using the Dualex Scientific+™ at different days after transplanting (DAT). Data are presented as mean ± SE. Asterisks indicate statistically significant differences between genotypes at each sampling time (*P < 0.05; **P < 0.01; ***P < 0.001).*

4.2.3. Flavonoids

The analysis of the flavonoid index also revealed a distinct pattern between the two genotypes throughout plant development (Figure 4.5). In the first leaf, the *zmccd8* mutant showed slightly lower values than the WT and maintained relatively stable values, ranging between approximately 0.3 and 0.4 Dualex units, whereas the WT showed a progressive increase in the index, reaching a value of 1.0 (Figure 4.5). A similar pattern was observed in the second leaf, where the WT showed initial values close to 0.75, followed by a progressive increase, reaching approximately 1.15 at the last measurement; the mutant, in contrast, showed a more variable pattern, with values ranging between 0.6 and 0.8 and lower values than the WT during the final measurements. In the third leaf, both genotypes showed an overall increasing trend in the flavonoid index, with a more pronounced increase in the WT. In the fourth leaf, the values, which were initially lower in the mutant, progressively increased in both genotypes, with slightly higher final values in *zmccd8* (Figure 4.5). Regarding the fifth leaf, only a limited number of observations were available, during which both genotypes showed a progressive increase in the index, with higher values in the WT than in the mutant.

Flavonoids (DUALEX units)

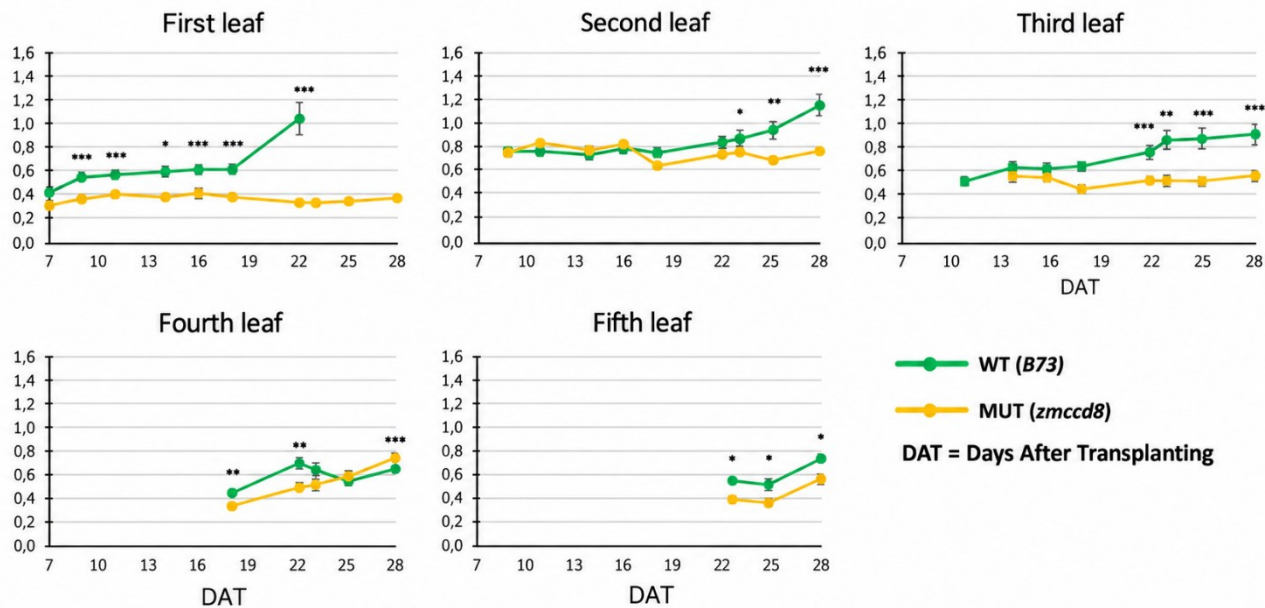


Figure 4.5: Changes in flavonoid index in the first five leaves of WT (B73) and *zmccd8* (MUT) maize plants throughout plant development. Measurements were performed using the Dualex Scientific+™ at different days after transplanting (DAT). Data are presented as mean $\pm$ SE. Asterisks indicate statistically significant differences between genotypes at each sampling time (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

4.3. GENE EXPRESSION ANALYSES

Gene expression analysis was performed by RT-qPCR to evaluate potential changes of expression of selected genes (Table 3.3). The analyses were carried out on tissues from the nodal meristem of the crown zone, the primary root, and the crown roots, considering three main functional groups of genes. The first group comprised genes involved in auxin metabolism, transport, and response; the second group comprised genes associated with lateral root formation and emergence, and the third group consisted of genes involved in ethylene biosynthesis and the regulation of ethylene-mediated responses (See table 3.3).

4.3.1. Genes involved in auxin metabolism, transport and response

4.3.1.1. Auxin-related genes in nodal meristem

The analysis of the expression of genes involved in auxin metabolism, transport, and response in the nodal meristem revealed differences between the WT (B73) and *zmccd8* mutant genotypes at the two time points considered, 8 and 11 DAT (Figure 4.6). Among the genes analysed, *PIN1A* and *PIN2* showed the most pronounced differences, with higher expression levels in the WT than in the mutant at both time points and a decrease in expression in the WT between 8 and 11 DAT. For *PIN5C* and *PIN9*, an increase in expression was observed in the mutant between 8 and 11 DAT, whereas expression levels in the WT remained relatively stable or showed a slight decrease; the differences between genotypes at 11 DAT were statistically significant. For *YUCCA6*, *IAA19*, *PIN1B*, *PIN1C*, *IAA24*, *ARF20*, *ARF25*, *ARF34*, *PIN8A*, *LAX1* and *LAX2*, no statistically significant differences in expression were observed between the two genotypes at the time points considered. Overall, the results indicate differential regulation of several genes associated with auxin metabolism, transport, and response in the *zmccd8* mutant, with particularly evident differences observed for some members of the *PIN* family. Considering that the *zmccd8* mutant is characterized by impaired strigolactone biosynthesis, the differences observed in the expression of genes associated with the auxin pathway may indicate altered regulation of this pathway under conditions of reduced SL biosynthesis.

4.3.1.2. Auxin-related genes in root tissues

Differences in the expression of genes involved in auxin transport in the crown roots and primary root, between WT and *zmccd8* were observed at the two time points considered, 11 and 28 DAT (Figure 4.7). Among the genes analysed, *PIN1A* showed a marked increase in expression in the WT between 11 and 28 DAT in both crown roots and the primary root, reaching higher expression levels than the mutant; the latter, in contrast, maintained relatively stable or slightly reduced expression levels. The differences between the two genotypes at 28 DAT were statistically significant in both tissues. A similar pattern was observed for *PIN2*, for which expression increased in the WT between 11 and 28 DAT, whereas it decreased in the mutant, with significant differences between the genotypes at 28 DAT in both crown roots and the primary root. *PIN9* showed the most marked changes: in the crown roots, expression increased in both genotypes, with higher levels in the WT at 28 DAT, whereas in the primary root, the WT showed a very high expression level at 11 DAT, followed by a marked decrease at 28 DAT, when expression levels were similar between the two genotypes. *PIN1C* showed an increase in expression in both genotypes in the crown roots, with higher levels in the WT at 28 DAT. In the primary root, however, the WT showed a marked increase, whereas the mutant showed a decrease, resulting in a significant difference between the two genotypes at 28 DAT.

For *PIN5C*, *PIN1B* and *LAX1*, no statistically significant differences in expression were observed between the two genotypes at 11 and 28 DAT (Figure 4.7).

Auxin-related genes in nodal meristem

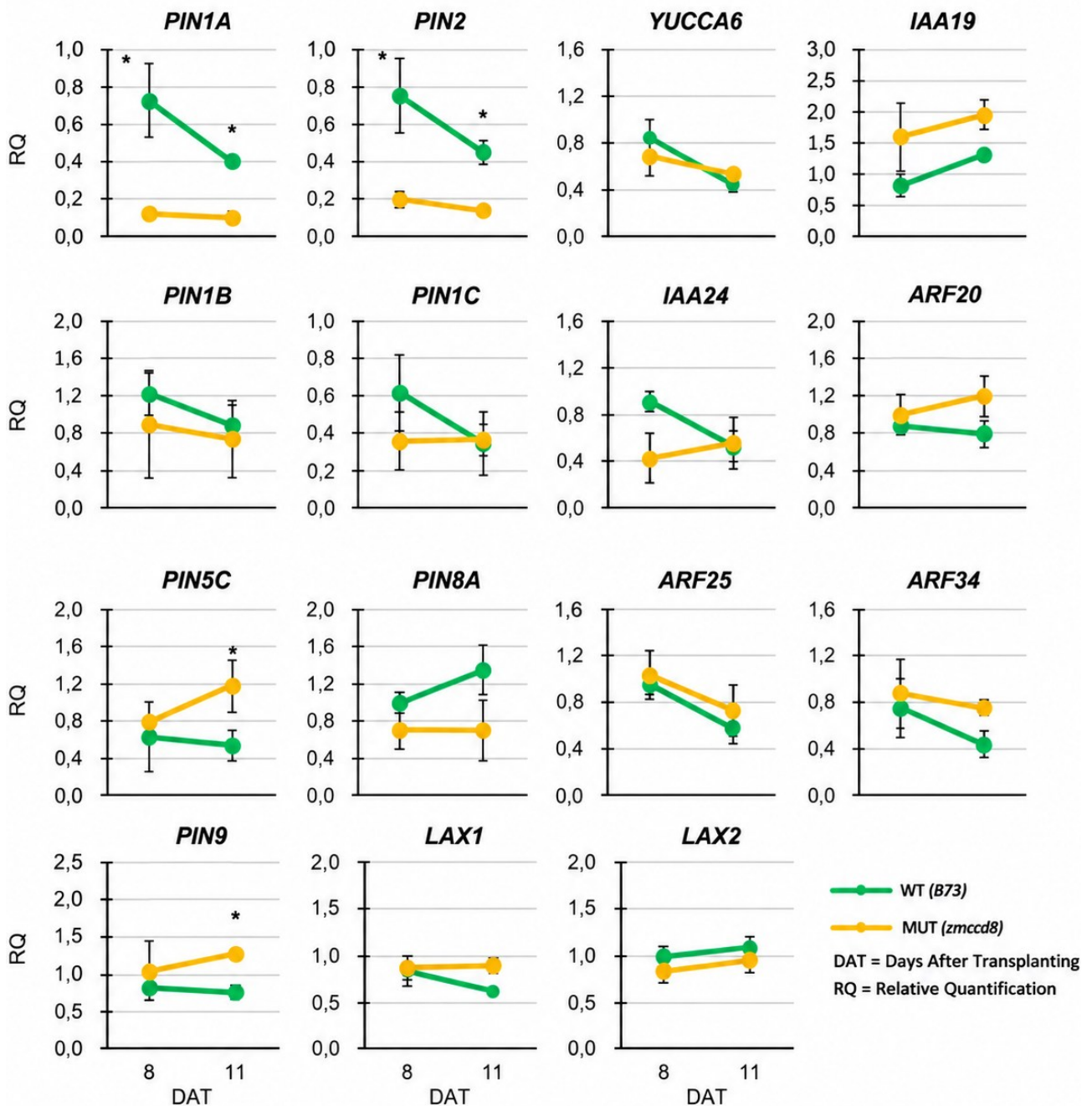


Figure 4.6: Relative expression levels of genes involved in auxin metabolism, transport and response in the nodal meristem of WT (B73) and *zmccd8* plants at 8 and 11 days after transplanting (DAT). Gene expression levels are expressed as relative quantification (RQ). Error bars represent standard error. Asterisks indicate statistically significant differences between genotypes (* $P < 0.05$).

Auxin-related genes in root tissues

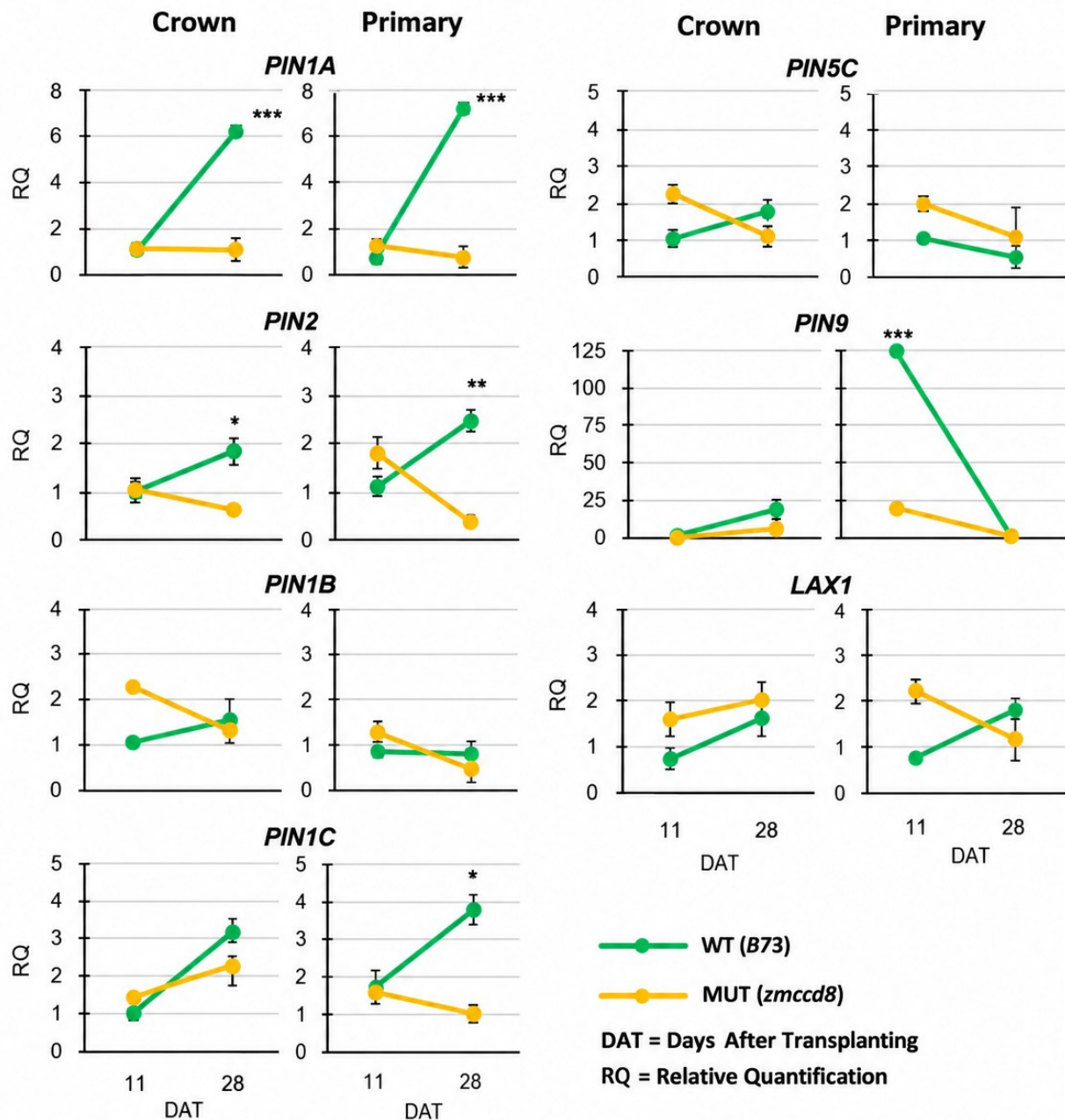


Figure 4.7: Relative expression of auxin-related genes in crown roots and primary root of WT (B73) and *zmccd8* plants at 11 and 28 days after transplanting (DAT). Gene expression levels are expressed as relative quantification (RQ). Error bars represent standard error. Asterisks indicate statistically significant differences between genotypes (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

4.3.2. Genes involved in lateral root formation and emergence

4.3.2.1. Lateral root-related genes in nodal meristem

Also for the genes associated with lateral root development and emergence in the nodal meristem, differences in expression were observed between the WT (B73) and *zmccd8* mutant genotypes at the two sampling time points considered, 8 and 11 DAT (Figure 4.8). For *ARGOS7*, the WT showed higher expression levels than the mutant at 8 DAT, followed by a decrease in both genotypes at 11 DAT. *RSL4* showed higher expression levels in the WT than in the mutant at both sampling times, with a decrease in expression in the WT between 8 and 11 DAT, whereas expression levels in the mutant remained relatively stable; the difference between the two genotypes was statistically significant at 8 DAT. Finally, *LBD7* showed a marked increase in expression in both genotypes between 8 and 11 DAT, particularly evident in the WT, which reached values close to 4 RQ compared with approximately 2 RQ in the mutant; the difference between the two genotypes was statistically significant at 11 DAT (Figure 4.8). For *RTCS*, *RTCL*, *RAL1* and *RAP1*, no statistically significant differences in expression were observed between the two genotypes at the time points considered.

4.3.2.2. Lateral root-related genes in root tissues

The genes analysed, including *RSL4*, *RAL1*, *RAP1* and *LBD7*, showed variations in expression between the two time points and between the two root types, with some differences in the expression patterns between genotypes. However, these variations did not result in statistically significant differences between the two genotypes or between crown roots and the primary root (Figure 4.9).

Lateral root-related genes in nodal meristem

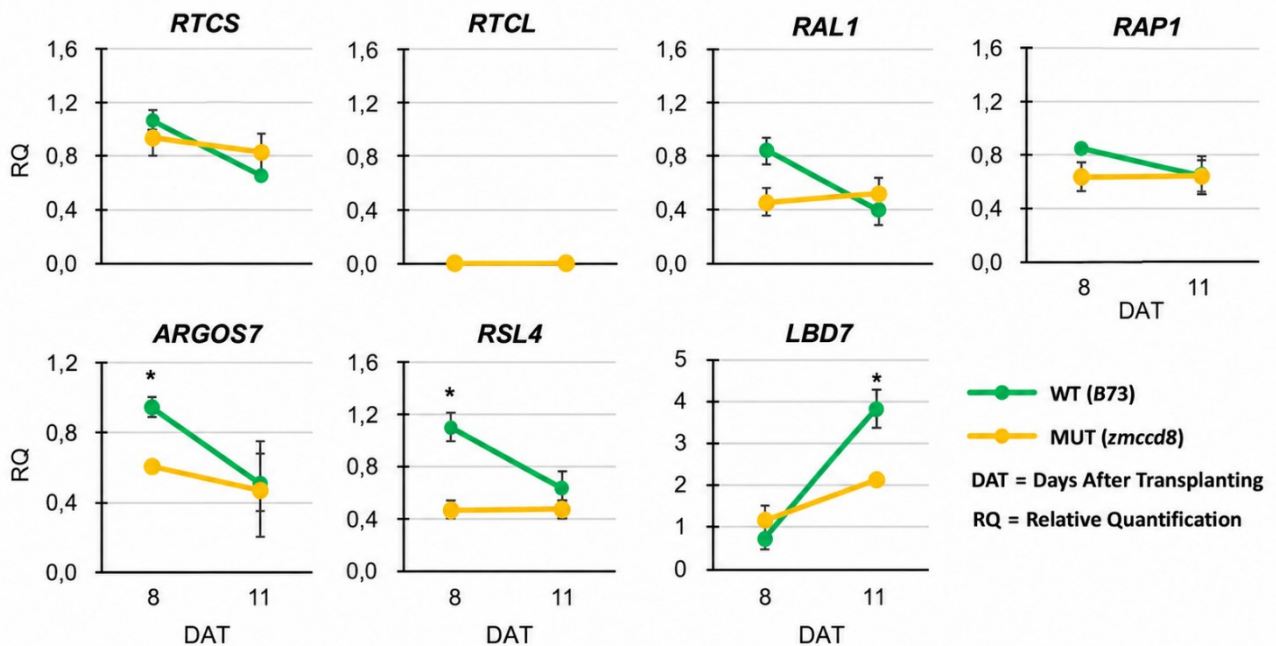


Figure 4.8: Relative expression levels of genes associated with lateral root development and emergence in the nodal meristem of WT (B73) and *zmccd8* plants at 8 and 11 days after transplanting (DAT). Gene expression levels are expressed as relative quantification (RQ). Error bars represent standard error. Asterisks indicate statistically significant differences between genotypes (* $P < 0.05$).

Lateral root-related genes in root tissues

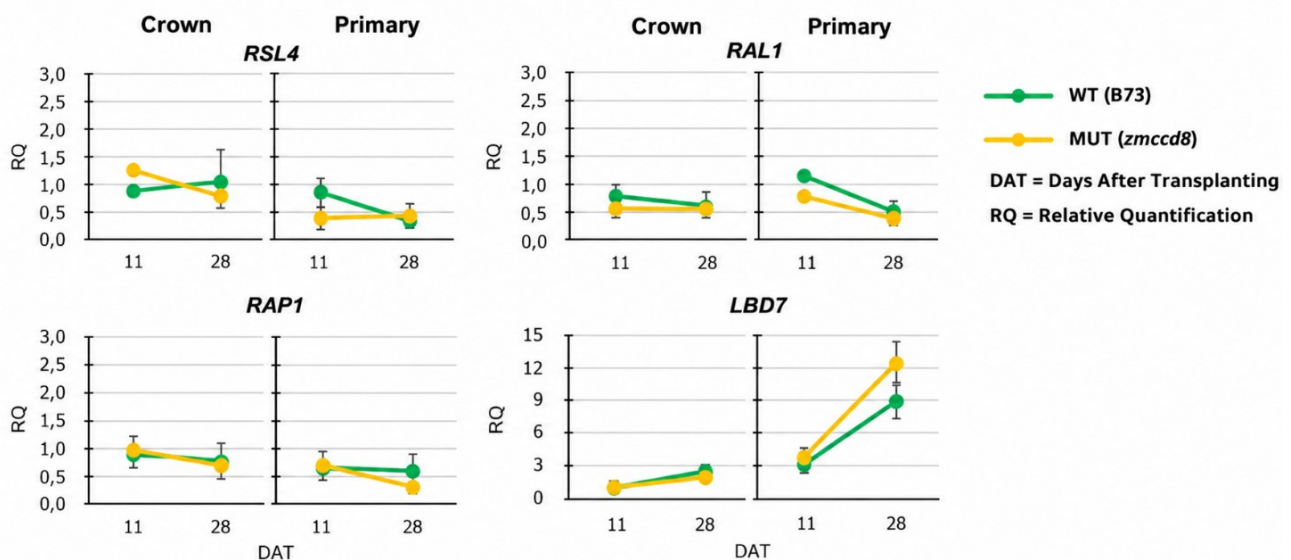


Figure 4.9: Relative expression levels of genes associated with lateral root development and emergence in crown roots and primary root of WT (B73) and *zmccd8* plants at 11 and 28 days after transplanting (DAT). Gene expression levels are expressed as relative quantification (RQ). Error bars represent standard error.

4.3.3. Genes involved in ethylene biosynthesis and response

4.3.3.1. Ethylene-related genes in nodal meristem

The genes associated with ethylene biosynthesis and the response to this hormone showed different expression profiles in the nodal meristem between 8 and 11 DAT (Figure 4.10). Among the genes involved in ethylene biosynthesis, *ACS7* showed higher expression levels in the WT than in the mutant at 8 DAT, whereas at 11 DAT expression decreased in the WT, reaching values similar to those observed in the mutant; the difference between the two genotypes at 8 DAT was statistically significant (Figure 4.10). A similar pattern was observed for *ACO2*, for which the WT showed higher expression levels than the mutant at 8 DAT, followed by a decrease at 11 DAT, when the expression levels of the two genotypes were more similar. Regarding the genes associated with the ethylene response, *EREB54* showed a decrease in expression in the WT between 8 and 11 DAT, whereas an increase was observed in the mutant, resulting in higher expression levels in the mutant than in the WT at 11 DAT. *EREB174* showed a marked decrease in expression in the WT between 8 and 11 DAT, whereas expression levels in the mutant remained close to zero at both time points; the differences between the two genotypes were statistically significant at both 8 and 11 DAT. Finally, *EREB209* showed significantly higher expression levels in the WT than in the mutant at 8 DAT; subsequently, expression in the WT decreased, whereas a slight increase was observed in the mutant, resulting in similar expression levels between the two genotypes at 11 DAT. For *SAMS4* and *EREB54*, no statistically significant differences in expression were observed between the two genotypes at the time points considered.

4.3.3.2. Ethylene-related genes in root tissues

Also in this case, no statistically significant differences in expression levels were observed between the WT and *zmccd8* mutant genotypes in either the crown roots or the primary root at the two time points considered, 11 and 28 DAT (Figure 4.11). The genes analysed, such as *ACS27*, *ACO2*, *EREB54* and *EREB209*, showed variations in expression between 11 and 28 DAT and, in some cases, different expression patterns between the two root types. However, these differences were not statistically significant and didn't result in significant differences between the two genotypes or between crown roots and the primary root. For example, *EREB54* showed a decrease in expression between 11 and 28 DAT in both genotypes and in both root tissues, reaching values close to 0 at 28 DAT.

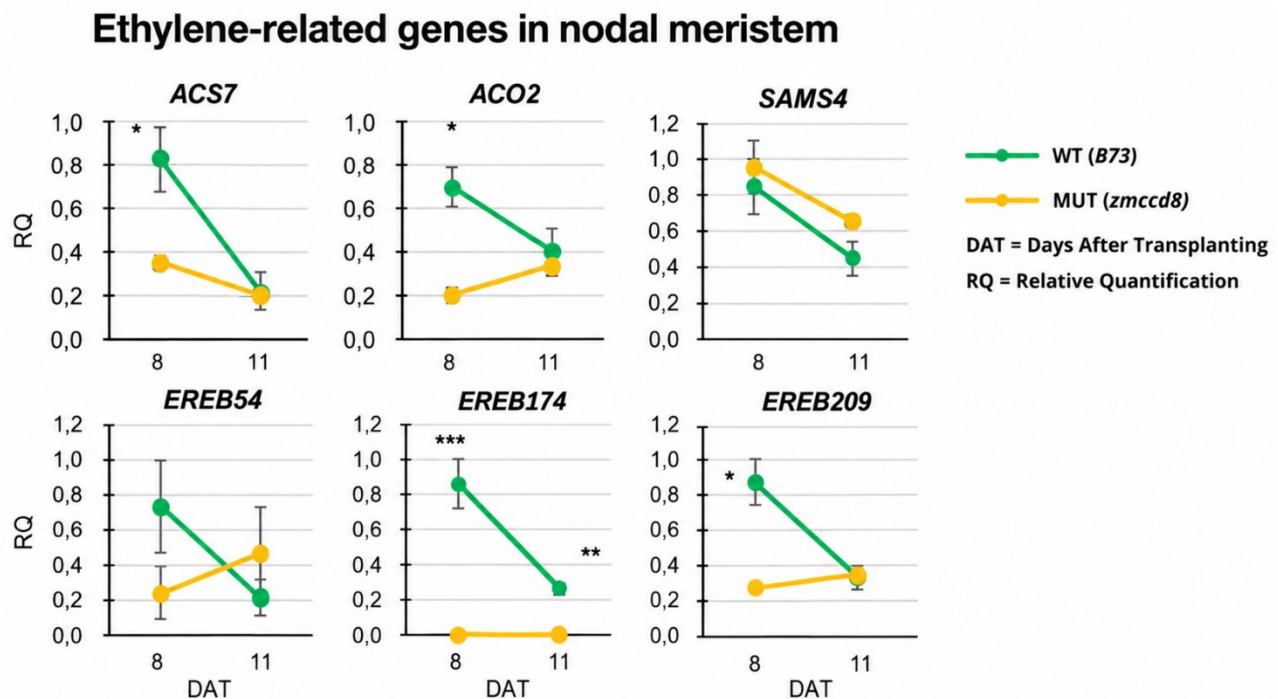


Figure 4.10: Relative expression levels of genes associated with ethylene biosynthesis and response in the nodal meristem of WT (B73) and *zmccd8* plants at 8 and 11 DAT. Gene expression levels are expressed as relative quantification (RQ). Error bars represent standard error. Asterisks indicate statistically significant differences between genotypes (* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

Ethylene-related genes in root tissues

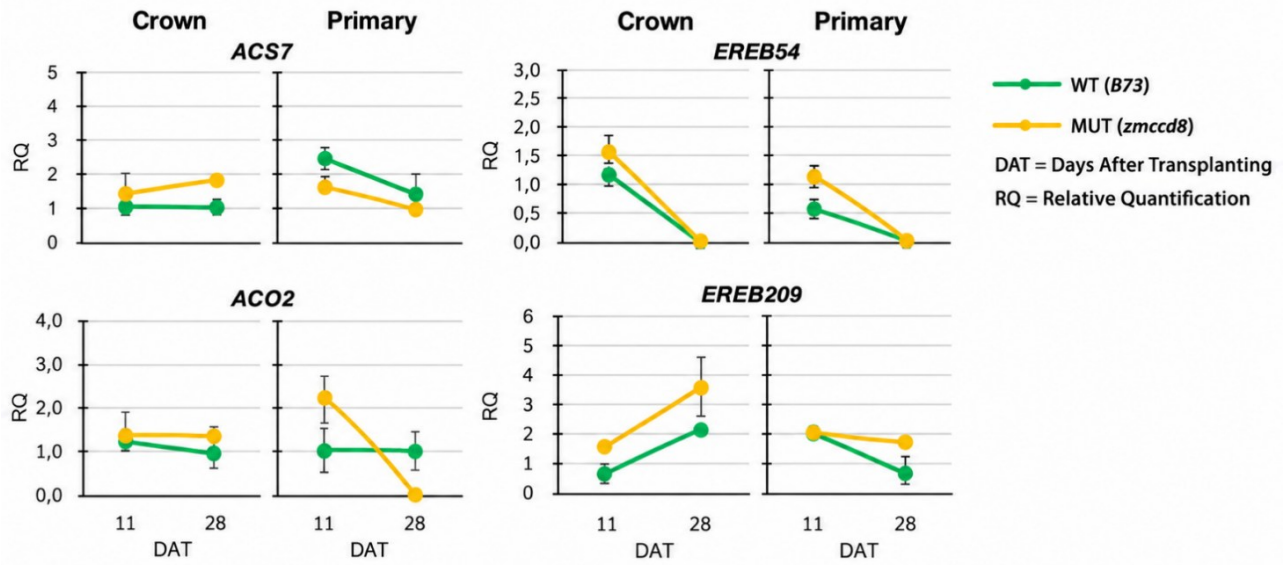


Figure 4.11: Relative expression levels of genes involved in ethylene biosynthesis and response in crown roots and primary root of WT (B73) and *zmccd8* plants at 11 and 28 DAT. Gene expression levels are expressed as relative quantification (RQ). Error bars represent standard error.

5.DISCUSSION AND CONCLUSIONS

The aim of the present study was to investigate the potential role of strigolactones (SLs) in maize (*Zea mays* L.) root development and plasticity, with a focus on early development and crown root formation. The B73 genotype, used as the wild type (WT), was compared with the *zmccd8* mutant, characterized by impaired strigolactone biosynthesis due to the alteration of the *ZmCCD8* gene (see Section 1.5.6). The integration of phenotypic, physiological, and molecular analyses revealed a progressive divergence between the two genotypes, with the WT showing greater shoot and root system growth, particularly at later developmental stages. Except at the first time point, the mutant showed a markedly reduced overall root biomass compared with the WT, consistently with previous observations in the *zmccd8* mutant (Quaggiotti et al., 2024; Buzzicotti et al., 2026). Guan et al. (2012) highlighted the key involvement of *ZmCCD8* in SL biosynthesis and described alterations in shoot architecture in the mutant associated with reduced development of adventitious roots. Similar phenotypic alterations were subsequently reported by Ravazzolo et al. (2024), who described an overall reduction in mutant growth compared with the WT, and by Buzzicotti et al. (2026), who reported a less developed root system in the *zmccd8* mutant, associated with reduced adventitious root formation.

An interesting aspect emerging from the analysis of the root system is the differential response of its components. Although the mutant showed reduced biomass across the different root classes analysed, the crown roots appeared to be particularly affected, as indicated by their lower biomass and number compared with the WT, especially at later developmental stages. This finding is particularly relevant considering the role of crown roots in the development of the post-embryonic root system of maize. Their formation contributes to the progressive transition from a root system initially composed predominantly of

embryonic components, such as the primary and seminal roots, to a more developed post-embryonic root system and is regulated by the complex interaction of numerous genetic and hormonal factors (Guan et al., 2012; Singh et al., 2022). The marked reduction in crown roots observed in the mutant therefore suggests an association between impaired SL biosynthesis and altered development of the post-embryonic component of the root system. This finding is consistent with previous observations in the *zmccd8* mutant by Guan et al. (2012) and with more recent evidence of altered root development reported by Buzzicotti et al. (2026). The present study reinforces these observations by providing a temporal and quantitative characterization of the different root components, showing that the divergence between the WT and mutant becomes particularly evident in the crown roots as development progresses. Overall, the reduction in crown roots therefore represents the most relevant phenotypic result of the present study and suggests that SLs may contribute to the regulation of post-embryonic root development. The reduced production of adventitious roots may also be associated with lower resource acquisition, potentially contributing to the reduced shoot biomass observed in the mutant.

The physiological analyses performed using DUALEX also revealed different dynamics of the indices related to chlorophyll, anthocyanins, and flavonoids between the two genotypes and depending on leaf age. These results suggest that impaired SL biosynthesis is also associated with differences in the dynamics of physiological parameters of the shoot, indicating that the observed differences extend beyond root development (Ravazzolo et al., 2024; Buzzicotti et al., 2026).

The pronounced response of crown roots observed in the mutant is also of interest in light of the complex hormonal regulation underlying shoot-borne root development. Among the major regulators involved is auxin, whose spatial distribution contributes to root formation and development and also depends on the activity of *PIN* family transporters.

In maize, several members of the *ZmPIN* family display distinct expression patterns across different tissues and developmental stages, consistent with their involvement in auxin transport (Forestan et al., 2012). The expression analysis revealed differences in several PIN genes between the two genotypes, with some changes occurring during the early stages of development and others becoming more evident at later stages.

These findings are of interest in light of evidence indicating an interaction between strigolactones and auxin in the regulation of root development. Ravazzolo et al. (2021b) provided evidence of an interaction between SLs and auxin in the regulation of maize root development, suggesting a coordinated involvement of these pathways in the control of root growth. The differences observed in the expression of some *PIN* genes may therefore reflect alterations in processes associated with auxin homeostasis and transport in the mutant. However, since auxin levels and auxin transport were not directly determined in the present study, the results don't allow us to conclude that impaired SL biosynthesis directly causes alterations in auxin homeostasis or transport in the tested tissues, but rather they point to a cooperative action between the two hormones. Such interplay is consistent with the well-known SL-auxin crosstalk governing multiple aspects of maize development (Manoli et al., 2016; Ravazzolo et al., 2021b)

The analysis of genes involved in root development revealed genotype-dependent differences in the expression of several regulatory components. The lack of significant changes in *RTCS* and *RTCL*, despite their documented role in shoot-borne root development, suggests that no detectable differences in their transcript levels were associated with impaired SL biosynthesis under the conditions analysed. In contrast, the differences in expression of *ARGOS7*, *RSL4* and *LBD7* point to a possible involvement of other regulatory components in the altered crown root development observed in the mutant (Taramino et al., 2007; Xu et al., 2015). It is also noteworthy that some expression

differences were already detected at the early stages analysed, whereas phenotypic differences in root biomass became progressively more pronounced at later stages. This temporal pattern may suggest that early transcriptional differences precede the more pronounced phenotypic divergence observed at later developmental stages, although further analyses would be required to establish such a relationship.

Another relevant finding emerging from the present study concerns the ethylene pathway. In the nodal meristem, *ACS7* and *ACO2*, which are involved in ethylene biosynthesis, showed differences in expression during the early stages analysed, while among genes associated with ethylene responses, differences were observed for *EREB174* and *EREB209*. These findings are of interest in light of recent evidence indicating that SLs seem to be involved in the regulation of ethylene-related responses in maize under nitrogen deficiency (Buzzicotti et al., 2026). In root tissues, by contrast, no statistically significant differences were observed in the analysed genes associated with ethylene biosynthesis and response. These results suggest a possible association between impaired SL biosynthesis and differential transcriptional regulation of the ethylene pathway during the early stages of nodal meristem development. However, these expression changes alone are not sufficient to establish a direct role of ethylene in determining the crown root phenotype. This interpretation is consistent with the broader framework of interactions among phytohormones involved in root development (Sun et al., 2022).

When the results concerning auxin, genes involved in root development, and ethylene are considered together, a picture emerges in which impaired SL biosynthesis is associated with modifications in multiple components of hormonal and transcriptional regulation. This finding is consistent with the current view of strigolactones as components of a network of hormonal interactions rather than regulators of a single linear pathway. Previous evidence indicates that SLs can interact with auxin and other phytohormones in the regulation of growth and

root development (Ravazzolo et al., 2021b; Sun et al., 2022). In the present study, the occurrence of some expression differences already at the early stages analysed, followed by the subsequent divergence of the root phenotype, suggests a possible integration of these signals during the development of the post-embryonic root component.

Overall, the results obtained highlight an association between impaired SL biosynthesis and altered root system development in maize, with particularly pronounced effects on the crown roots. The temporal and quantitative characterization of the different root components represents one of the main points of interest of the present study, as it shows that the reduction in root system development in the mutant is not uniform but is particularly pronounced in the post-embryonic component. The changes observed in the expression of genes involved in hormonal regulation and root development further suggest the possible coordinated involvement of multiple regulatory pathways in modulating developmental plasticity in maize.

Understanding the mechanisms underlying crown root development is of particular interest in the context of increasing environmental variability, since root system plasticity contributes to the ability of plants to modulate growth and resource acquisition according to environmental conditions (Maqbool et al., 2022; Jiang & Whalen, 2025). In this scenario, investigating the specific role of SLs can provide fundamental insights into hormone-mediated root plasticity. To further advance this understanding, high-resolution RNA-seq profiling of the nodal meristem, the site of crown root initiation and emergence, would represent a valuable approach to uncover key transcriptional networks and downstream SL-responsive target genes governing crown root morphogenesis.

6. REFERENCES

- Abbas, S., Javed, M. T., Ali, Q., Azeem, M., & Ali, S. (2021). Nutrient deficiency stress and relation with plant growth and development. In S. Fahad, O. Sönmez, S. Saud, D. Wang, C. Wu, M. Adnan, M. Arif, & Amanullah (Eds.), *Engineering tolerance in crop plants against abiotic stress* (pp. 239–262). CRC Press. <https://doi.org/10.1201/9781003160717-12>
- Ahmed, M. A., Zarebanadkouki, M., Kaestner, A., & Carminati, A. (2018). Measurements of water uptake along maize roots: The key function of lateral roots. *Plant and Soil*, 424, 59–74. <https://doi.org/10.1007/s11104-017-3425-9>
- Akiyama, K., Matsuzaki, K., & Hayashi, H. (2005). Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. *Nature*, 435(7043), 824–827. <https://doi.org/10.1038/nature03608>
- Al-Babili, S., & Bouwmeester, H. J. (2015). Strigolactones, a novel carotenoid-derived plant hormone. *Annual Review of Plant Biology*, 66, 161–186. <https://doi.org/10.1146/annurev-arplant-043014-114759>
- Alder, A., Jamil, M., Marzorati, M., Bruno, M., Vermathen, M., Bigler, P., Ghisla, S., Bouwmeester, H. J., Beyer, P., & Al-Babili, S. (2012). The path from β -carotene to carlactone, a strigolactone-like plant hormone. *Science*, 335(6074), 1348–1351. <https://doi.org/10.1126/science.1218094>
- Amoah, J. N., Keitel, C., & Kaiser, B. N. (2025). Nitrogen deficiency impacts growth and modulates carbon metabolism in maize. *Planta*, 262, 94. <https://doi.org/10.1007/s00425-025-04814-x>
- Arite, T., Kameoka, H., & Kyozuka, J. (2012). Strigolactone positively controls crown root elongation in rice. *Journal of Plant Growth Regulation*, 31(2), 165–172. <https://doi.org/10.1007/s00344-011-9228-6>
- Asseng, S., Ewert, F., Martre, P., Rötter, R. P., Lobell, D. B., Cammarano, D., Kimball, B. A., Ottman, M. J., Wall, G. W., White, J. W., Reynolds, M. P., Alderman, P. D., Prasad, P. V. V., Aggarwal, P. K., Anothai, J., Basso, B., Biernath, C., Challinor, A. J., De Sanctis, G., ... Zhu, Y. (2015). Rising temperatures reduce global wheat production. *Nature Climate Change*, 5(2), 143–147. <https://doi.org/10.1038/nclimate2470>

- Atkinson, N. J., & Urwin, P. E. (2012). The interaction of plant biotic and abiotic stresses. *Journal of Experimental Botany*, 63(10), 3523–3543. <https://doi.org/10.1093/jxb/ers100>
- Bais, H. P., Weir, T. L., Perry, L. G., Gilroy, S., & Vivanco, J. M. (2006). The role of root exudates in rhizosphere interactions with plants and other organisms. *Annual Review of Plant Biology*, 57, 233–266. <https://doi.org/10.1146/annurev.arplant.57.032905.105159>
- Ban, X., Qin, L., Yan, J., Wu, J., Li, Q., Su, X., Hao, Y., Hu, Q., Kou, L., Yan, Z., Xin, P., Zhang, Y., Dong, L., Bouwmeester, H. J., Yu, H., Yu, Q., Huang, S., Lin, T., Xie, Q., ... Wang, B. (2025). Manipulation of a strigolactone transporter in tomato confers resistance to the parasitic weed broomrape. *The Innovation*, 6(3), 100815. <https://doi.org/10.1016/j.xinn.2025.100815>
- Barberon, M. (2017). The endodermis as a checkpoint for nutrients. *New Phytologist*, 213(4), 1604–1610. <https://doi.org/10.1111/nph.14140>
- Barbier, F. F., Dun, E. A., Kerr, S. C., Chabikwa, T. G., & Beveridge, C. A. (2023a). Strigolactones and the regulation of plant development and stress responses. *Plant Physiology*, 192(1), 28–44. <https://doi.org/10.1093/plphys/kiac559>
- Barbier, F. F., Fichtner, F., & Beveridge, C. A. (2023b). The strigolactone pathway plays a crucial role in integrating metabolic and nutritional signals in plants. *Nature Plants*, 9, 1191–1200. <https://doi.org/10.1038/s41477-023-01453-6>
- Bartlett, M. E., & Thompson, B. (2014). Meristem identity and phyllotaxis in inflorescence development. *Frontiers in Plant Science*, 5, 508. <https://doi.org/10.3389/fpls.2014.00508>
- Bárzana, G., & Carvajal, M. (2020). Genetic regulation of water and nutrient transport in water stress tolerance in roots. *Journal of Biotechnology*, 324, 134–142. <https://doi.org/10.1016/j.jbiotec.2020.10.003>
- Beeckman, T., & Eshel, A. (Eds.). (2024). *Plant roots: The hidden half* (5th ed.). CRC Press. <https://doi.org/10.1201/b23126>
- Berendsen, R. L., Pieterse, C. M. J., & Bakker, P. A. H. M. (2012). The rhizosphere microbiome and plant health. *Trends in Plant Science*, 17(8), 478–486. <https://doi.org/10.1016/j.tplants.2012.04.001>

- Beveridge, C. A., Ross, J. J., & Murfet, I. C. (1994). Branching mutant rms-2 in pea: Grafting studies and endogenous indole-3-acetic acid levels. *Plant Physiology*, 104(3), 953–959. <https://doi.org/10.1104/pp.104.3.953>
- Beveridge, C. A., Ross, J. J., & Murfet, I. C. (1996). Branching in pea (action of genes Rms3 and Rms4). *Plant Physiology*, 110(3), 859–865. <https://doi.org/10.1104/pp.110.3.859>
- Bommert, P., & Jackson, D. (2015). Plant stem cell organization and specification in maize. *Current Opinion in Plant Biology*, 23, 61–69. <https://doi.org/10.1016/j.pbi.2014.11.004>
- Booker, J., Auldrige, M., Wills, S., McCarty, D., Klee, H., & Leyser, O. (2004). MAX3/CCD7 is a carotenoid cleavage dioxygenase required for the synthesis of a novel plant signaling molecule. *Current Biology*, 14(14), 1232–1238. <https://doi.org/10.1016/j.cub.2004.06.022>
- Boyer, J. S., & Westgate, M. E. (2004). Grain yields with limited water. *Journal of Experimental Botany*, 55(407), 2385–2394. <https://doi.org/10.1093/jxb/erh219>
- Bray, E. A., Bailey-Serres, J., & Weretilnyk, E. (2000). Responses to abiotic stresses. In B. Buchanan, W. Gruissem, & R. L. Jones (Eds.), *Biochemistry & molecular biology of plants* (pp. 1158–1203). American Society of Plant Physiologists.
- Buzzicotti, L., Camilletti, C., Ravazzolo, L., Wirtz, M., Hell, R., & Quaggiotti, S. (2026). Nitrogen deficiency outperforms water stress in triggering strigolactone-dependent responses in maize. *Physiologia Plantarum*, 178(4), e70987. <https://doi.org/10.1111/ppl.70987>
- Canadian Food Inspection Agency. (2021, May 7). The biology of Zea mays L. (maize) (Biology document BIO2020-01). <https://inspection.canada.ca/en/plant-health/plant-varieties/novel-traits/applicants/directive-94-08/biology-documents/zea-mays-maize>
- Ćavar, S., Zwanenburg, B., & Tarkowski, P. (2015). Strigolactones: Occurrence, structure, and biological activity in the rhizosphere. *Phytochemistry Reviews*, 14(4), 691–711. <https://doi.org/10.1007/s11101-014-9370-4>
- Charnikhova, T. V., Gaus, K., Lumbroso, A., Sanders, M., Vincken, J.-P., de Mesmaeker, A., Ruyter-Spira, C. P., Screpanti, C., & Bouwmeester, H. J. (2017). Zealactones: Novel natural strigolactones from maize. *Phytochemistry*, 137, 123–131. <https://doi.org/10.1016/j.phytochem.2017.02.010>

Charnikhova, T. V., Gaus, K., Lumbroso, A., Sanders, M., Vincken, J.-P., de Mesmaeker, A., Ruyter-Spira, C. P., Screpanti, C., & Bouwmeester, H. J. (2018). Zeapyranolactone—A novel strigolactone from maize. *Phytochemistry Letters*, 24, 172–178. <https://doi.org/10.1016/j.phytol.2018.01.003>

Colmer, T. D. (2003). Long-distance transport of gases in plants: A perspective on internal aeration and radial oxygen loss from roots. *Plant, Cell & Environment*, 26(1), 17–36. <https://doi.org/10.1046/j.1365-3040.2003.00846.x>

Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria. (2025, January 24). Giornata del Mais 2025: L'impatto di ricerca e innovazione per l'adattamento ai nuovi scenari climatici. <https://www.crea.gov.it/-/giornata-del-mais-2025-l-impatto-di-ricerca-e-innovazione-per-l-adattamento-ai-nuovi-scenari-climatici>

Cook, C. E., Whichard, L. P., Turner, B., Wall, M. E., & Egley, G. H. (1966). Germination of witchweed (*Striga lutea* Lour.): Isolation and properties of a potent stimulant. *Science*, 154(3753), 1189–1190. <https://doi.org/10.1126/science.154.3753.1189>

Den Herder, G., Van Isterdael, G., Beeckman, T., & De Smet, I. (2010). The roots of a new green revolution. *Trends in Plant Science*, 15(11), 600–607. <https://doi.org/10.1016/j.tplants.2010.08.009>

Dodd, I. C. (2005). Root-to-shoot signalling: Assessing the roles of 'up' in the up and down world of long-distance signalling in planta. *Plant and Soil*, 274(1–2), 251–270. <https://doi.org/10.1007/s11104-004-0966-0>

Doebley, J., & Iltis, H. H. (1980). Taxonomy of Zea. I. A subgeneric classification with key to taxa. *American Journal of Botany*, 67(6), 982–993. <https://doi.org/10.1002/j.1537-2197.1980.tb07717.x>

Doebley, J. F. (2004). The genetics of maize evolution. *Annual Review of Genetics*, 38, 37–59. <https://doi.org/10.1146/annurev.genet.38.072902.092425>

Doebley, J. F., Gaut, B. S., & Smith, B. D. (2006). The molecular genetics of crop domestication. *Cell*, 127(7), 1309–1321. <https://doi.org/10.1016/j.cell.2006.12.006>

Dong, J., Fu, H., Wang, Z., Zhang, L., Liu, Z., Hu, Y., Shen, F., & Wang, W. (2025). Mechanisms of strigolactone-regulated abiotic stress responses in plants. *Plants*, 14(16), 2582. <https://doi.org/10.3390/plants14162582>

- Duvick, D. N. (2001). Biotechnology in the 1930s: The development of hybrid maize. *Nature Reviews Genetics*, 2(1), 69–74. <https://doi.org/10.1038/35047587>
- Duvick, D. N. (2005). The contribution of breeding to yield advances in maize (*Zea mays* L.). *Advances in Agronomy*, 86, 83–145. [https://doi.org/10.1016/S0065-2113\(05\)86002-X](https://doi.org/10.1016/S0065-2113(05)86002-X)
- East, E. M. (1908). Inbreeding in corn. Report of the Connecticut Agricultural Experiment Station, 419–428.
- Erdelská, O., & Vidovencová, Z. (1993). Development of the maize root system. *Biologia Plantarum*, 35(2), 161–168
- Fahad, S., Bajwa, A. A., Nazir, U., Anjum, S. A., Farooq, A., Zohaib, A., Sadia, S., Nasim, W., Adkins, S., Saud, S., Ihsan, M. Z., Alharby, H., Wu, C., Wang, D., & Huang, J. (2017). Crop production under drought and heat stress: Plant responses and management options. *Frontiers in Plant Science*, 8, 1147. <https://doi.org/10.3389/fpls.2017.01147>
- Fahn, A. (1990). *Plant Anatomy* (4th ed.). Pergamon Press.
- Farooq, M., Hussain, M., Wahid, A., & Siddique, K. H. M. (2012). Drought stress in plants: An overview. In R. Aroca (Ed.), *Plant Responses to Drought Stress* (pp. 1–33). Springer. https://doi.org/10.1007/978-3-642-32653-0_1
- Farooq, M., Hussain, M., Wakeel, A., & Siddique, K. H. M. (2015). Salt stress in maize: Effects, resistance mechanisms, and management. *Agronomy for Sustainable Development*, 35(2), 461–481. <https://doi.org/10.1007/s13593-015-0287-0>
- Fitzpatrick, C. R., Salas-González, I., Conway, J. M., Finkel, O. M., Gilbert, S., Russ, D., Teixeira, P. J. P. L., & Dangl, J. L. (2020). The plant microbiome: From ecology to reductionism and beyond. *Nature Reviews Microbiology*, 18(10), 607–621. <https://doi.org/10.1038/s41579-020-0405-0>
- Flint-Garcia, S. A., Bodnar, A. L., & Scott, M. P. (2009). Wide variability in kernel composition, seed characteristics, and zein profiles among diverse maize inbreds, landraces, and teosinte. *Theoretical and Applied Genetics*, 119(6), 1129–1142. <https://doi.org/10.1007/s00122-009-1115-1>
- Food and Agriculture Organization of the United Nations. (2022). World food and agriculture – Statistical yearbook 2022. <https://doi.org/10.4060/cc2211en>

- Food and Agriculture Organization of the United Nations. (2026). Crop production, yield, harvested area (Global, National - Annual) [Data set]. FAOSTAT. <https://data.fao.org/catalog/iso/d24a448b-3b62-4c09-8c1d-4a39bb599876>
- Forestan, C., Farinati, S., & Varotto, S. (2012). The maize PIN gene family of auxin transporters. *Frontiers in Plant Science*, 3, 16. <https://doi.org/10.3389/fpls.2012.00016>
- Foyer, C. H., & Noctor, G. (2005). Oxidant and antioxidant signaling in plants: A re-evaluation of the concept of oxidative stress in a physiological context. *Plant, Cell & Environment*, 28(8), 1056–1071. <https://doi.org/10.1111/j.1365-3040.2005.01327.x>
- George, T. S., Bulgarelli, D., Carminati, A., Chen, Y., Jones, D., Kuzyakov, Y., Schnepf, A., Wissuwa, M., & Roose, T. (2024). *Bottom-up perspective: The role of roots and rhizosphere in climate change adaptation and mitigation in agroecosystems*. *Plant and Soil*, 500(1–2), 297–323. <https://doi.org/10.1007/s11104-024-06626-6>
- Giehl, R. F. H., Laginha, A. M., Duan, F., Rentsch, D., Yuan, L., & von Wirén, N. (2017). A critical role of AMT2;1 in root-to-shoot translocation of ammonium in Arabidopsis. *Molecular Plant*, 10(11), 1449–1460. <https://doi.org/10.1016/j.molp.2017.10.001>
- Gomez-Roldan, V., Feras, S., Brewer, P. B., Puech-Pagès, V., Dun, E. A., Pillot, J.-P., Letisse, F., Matusova, R., Danoun, S., Portais, J.-C., Bouwmeester, H., Bécard, G., Beveridge, C. A., Rameau, C., & Rochange, S. F. (2008). Strigolactone inhibition of shoot branching. *Nature*, 455(7210), 189–194. <https://doi.org/10.1038/nature07271>
- Guan, J. C., Koch, K. E., Suzuki, M., Wu, S., Latshaw, S., Petruff, T., Goulet, C., Klee, H. J., McCarty, D. R., & Tan, B. C. (2012). Diverse roles of strigolactone signaling in maize architecture and the uncoupling of a branching-specific subnetwork. *Plant Physiology*, 160(3), 1303–1317. <https://doi.org/10.1104/pp.112.204503>
- Guan, J.-C., Li, C., Flint-Garcia, S., Suzuki, M., Wu, S., Saunders, J. W., Dong, L., Bouwmeester, H. J., McCarty, D. R., & Koch, K. E. (2023). Maize domestication phenotypes reveal strigolactone networks coordinating grain size evolution with kernel-bearing cupule architecture. *The Plant Cell*, 35(3), 1013–1037. <https://doi.org/10.1093/plcell/koac370>
- Guoyong, L., & Hall, J. W. (2019). Crop yield sensitivity of global major agricultural countries to droughts and the projected changes in the future: A modelling study. *Science of the Total Environment*, 654, 811–821. <https://doi.org/10.1016/j.scitotenv.2018.10.434>

- Ha, C. V., Leyva-González, M. A., Osakabe, Y., Tran, U. T., Nishiyama, R., Watanabe, Y., Tanaka, M., Seki, M., Yamaguchi, S., Dong, N. V., Yamaguchi-Shinozaki, K., Shinozaki, K., Herrera-Estrella, L., & Tran, L.-S. P. (2014). Positive regulatory role of strigolactone in plant responses to drought and salt stress. *Proceedings of the National Academy of Sciences of the United States of America*, 111(2), 851–856. <https://doi.org/10.1073/pnas.1322135111>
- Haider, I., Andreo-Jimenez, B., Bruno, M., Bimbo, A., Floková, K., Abuauf, H., Ntui, V. O., Guo, X., Charnikhova, T., Al-Babili, S., & Bouwmeester, H. J. (2018). The interaction of strigolactones with abscisic acid during the drought response in rice. *Journal of Experimental Botany*, 69(10), 2403–2414. <https://doi.org/10.1093/jxb/ery089>
- Hasanuzzaman, M., Bhuyan, M. H. M. B., Parvin, K., Bhuiyan, T. F., Anee, T. I., Nahar, K., Hossen, M. S., Zulfikar, F., Alam, M. M., & Fujita, M. (2020). Regulation of ROS metabolism in plants under environmental stress: A review of recent experimental evidence. *International Journal of Molecular Sciences*, 21(22), 8695. <https://doi.org/10.3390/ijms21228695>
- Hawkes, C. V., DeAngelis, K. M., & Firestone, M. K. (2007). Root interactions with soil microbial communities and processes. In Z. G. Cardon & J. L. Whitbeck (Eds.), *The rhizosphere: An ecological perspective* (pp. 1–29). Elsevier Academic Press. <https://doi.org/10.1016/B978-012088775-0/50003-3>
- Hawkesford, M. J., Cakmak, I., Coskun, D., de Kok, L. J., Lambers, H., Schjoerring, J. K., & White, P. J. (2023). Functions of macronutrients. In Z. Rengel, I. Cakmak, & P. J. White (Eds.), *Marschner's Mineral Nutrition of Plants* (4th ed., pp. 201–281). Elsevier. <https://doi.org/10.1016/B978-0-12-819773-8.00019-8>
- Hirsch, C. N., Hirsch, C. D., Brohammer, A. B., Bowman, M. J., Soifer, I., Barad, O., Shem-Tov, D., Baruch, K., Lu, F., Hernandez, A. G., Fields, C. J., Wright, C. L., Koehler, K., Springer, N. M., Buckler, E., Buell, C. R., de Leon, N., Kaeppler, S. M., Childs, K. L., & Mikel, M. A. (2016). Draft assembly of elite inbred line PH207 provides insights into genomic and transcriptome diversity in maize. *The Plant Cell*, 28(11), 2700–2714. <https://doi.org/10.1105/tpc.16.00353>
- Hoang, N. V., Choe, G., Zheng, Y., Aliaga Fandino, A. C., Sung, I., Hur, J., Kamran, M., Park, C., Kim, H., Ahn, H., Kim, S., Fei, Z., & Lee, J.-Y. (2020). Identification of conserved gene-regulatory networks that integrate environmental sensing and growth in the root cambium. *Current Biology*, 30(15), 2887–2900.e7. <https://doi.org/10.1016/j.cub.2020.05.046>

Hochholdinger, F. (2009). The maize root system: Morphology, anatomy, and genetics. In J. L. Bennetzen & S. Hake (Eds.), *Handbook of Maize: Its Biology* (pp. 145–160). Springer.

Hochholdinger, F., Park, W. J., Sauer, M., & Woll, K. (2004). From weeds to crops: Genetic analysis of root development in cereals. *Trends in Plant Science*, 9(1), 42–48. <https://doi.org/10.1016/j.tplants.2003.11.003>

Hochholdinger, F., & Tuberosa, R. (2009). Genetic and genomic dissection of maize root development and architecture. *Current Opinion in Plant Biology*, 12(2), 172–177. <https://doi.org/10.1016/j.pbi.2008.12.002>

Hostetler, A. N., Khangura, R. S., Dilkes, B. P., & Sparks, E. E. (2021). Bracing for sustainable agriculture: The development and function of brace roots in members of Poaceae. *Current Opinion in Plant Biology*, 59, 101985. <https://doi.org/10.1016/j.pbi.2020.101985>

Hostetler, A. N., Erndwein, L., Reneau, J. W., Stager, A., Tanner, H. G., Cook, D., & Sparks, E. E. (2022). Multiple brace root phenotypes promote anchorage and limit root lodging in maize. *Plant, Cell & Environment*, 45(5), 1573–1583. <https://doi.org/10.1111/pce.14289>

Huang, G., Kilic, A., Karady, M., Zhang, J., Mehra, P., Song, X., Pandey, B. K., Shi, H., Mou, B., Lynch, J. P., & Yu, P. (2022). Ethylene inhibits rice root elongation in compacted soil via ABA- and auxin-mediated mechanisms. *Proceedings of the National Academy of Sciences*, 119(30), e2201072119. <https://doi.org/10.1073/pnas.2201072119>

Iltis, H. H. (2000). Homeotic sexual translocations and the origin of maize (*Zea mays*, Poaceae): A new look at an old problem. *Economic Botany*, 54(1), 7–42.

Inukai, Y., Sakamoto, T., Ueguchi-Tanaka, M., Shibata, Y., Gomi, K., Umemura, I., Hasegawa, Y., Ashikari, M., Kitano, H., & Matsuoka, M. (2005). Crown rootless1, which is essential for crown root formation in rice, is a target of an AUXIN RESPONSE FACTOR in auxin signaling. *The Plant Cell*, 17(5), 1387–1396. <https://doi.org/10.1105/tpc.105.030981>

Itoh, J., Nonomura, K., & Ikeda, K. (2005). Rice plant development: From zygote to spikelet. *Plant and Cell Physiology*, 46(1), 23–47. <https://doi.org/10.1093/pcp/pci501>

Jia, K.-P., Baz, L., & Al-Babili, S. (2018). From carotenoids to strigolactones. *Journal of Experimental Botany*, 69(9), 2189–2204. <https://doi.org/10.1093/jxb/erx476>

- Jha, U. C., Nayyar, H., Jha, R., Khurshid, M., Zhou, M., Mantri, N., & Siddique, K. H. M. (2020). Long non-coding RNAs: Emerging players regulating plant abiotic stress response and adaptation. *BMC Plant Biology*, 20, 466. <https://doi.org/10.1186/s12870-020-02595-x>
- Jiang, Y., & Whalen, J. K. (2025). Plasticity of maize (*Zea mays* L.) roots in water-deficient and nitrogen-limited soil. *Crop Science*, 65(3), e70084. <https://doi.org/10.1002/csc2.70084>
- Kang, J., Voothuluru, P., Hoyos-Miernyk, E., Alexander, D., Oliver, M. J., & Sharp, R. E. (2022). Antioxidant metabolism underlies different metabolic strategies for primary root growth maintenance under water stress in cotton and maize. *Antioxidants*, 11(5), 820. <https://doi.org/10.3390/antiox11050820>
- Kant, S., Bi, Y. M., & Rothstein, S. J. (2011). Understanding plant response to nitrogen limitation for the improvement of crop nitrogen use efficiency. *Journal of Experimental Botany*, 62(4), 1499–1509. <https://doi.org/10.1093/jxb/erq297>
- Kapulnik, Y., & Koltai, H. (2014). Strigolactone involvement in root development, response to abiotic stress, and interactions with the biotic soil environment. *Plant Physiology*, 166(2), 560–569. <https://doi.org/10.1104/pp.114.244939>
- Kapulnik, Y., Resnick, N., Mayzlish-Gati, E., Kaplan, Y., Wininger, S., Hershenhorn, J., & Koltai, H. (2011). Strigolactones interact with ethylene and auxin in regulating root-hair elongation in *Arabidopsis*. *Journal of Experimental Botany*, 62(8), 2915–2924. <https://doi.org/10.1093/jxb/erq464>
- Karlova, R., Boer, D., Hayes, S., & Testerink, C. (2021). Root plasticity under abiotic stress. *Plant Physiology*, 187(3), 1057–1070. <https://doi.org/10.1093/plphys/kiab392>
- Kellogg, E. A. (2000). The grasses: A case study in macroevolution. *Annual Review of Ecology and Systematics*, 31, 217–238. <https://doi.org/10.1146/annurev.ecolsys.31.1.217>
- Kitomi, Y., Ito, H., Hobo, T., Aya, K., Kitano, H., & Inukai, Y. (2011). The auxin-regulated AP2/ERF gene CROWN ROOTLESS5 is involved in crown root initiation in rice through the control of cytokinin signaling. *The Plant Cell*, 23(9), 3276–3291. <https://doi.org/10.1105/tpc.111.084475>
- Kodama, K., Rich, M. K., Yoda, A., Shimazaki, S., Xie, X., Akiyama, K., Mizuno, Y., Komatsu, A., Luo, Y., Suzuki, H., Kameoka, H., Libourel, C., Keller, J., Sakakibara, K., Nishiyama, T., Nakagawa, T., Mashiguchi, K., Uchida, K., Yoneyama, K., ... Kyoizuka, J. (2022). An

ancestral function of strigolactones as symbiotic rhizosphere signals. *Nature Communications*, 13, 3974. <https://doi.org/10.1038/s41467-022-31708-3>

Koevoets, I. T., Venema, J. H., Elzenga, J. T. M., & Testerink, C. (2016). Roots withstanding their environment: Exploiting root system architecture responses to abiotic stress to improve crop tolerance. *Frontiers in Plant Science*, 7, 1335. <https://doi.org/10.3389/fpls.2016.01335>

Kretschmar, T., Kohlen, W., Sasse, J., Borghi, L., Schlegel, M., Bachelier, J.-B., Reinhardt, D., Bours, R., Bouwmeester, H. J., & Martinoia, E. (2012). A petunia ABC protein controls strigolactone-dependent symbiotic signalling and branching. *Nature*, 483(7389), 341–344. <https://doi.org/10.1038/nature10873>

Lesk, C., Rowhani, P., & Ramankutty, N. (2016). Influence of extreme weather disasters on global crop production. *Nature*, 529(7584), 84–87. <https://doi.org/10.1038/nature16467>

Li, C., Dong, L., Durairaj, J., Guan, J.-C., Yoshimura, M., Quinodoz, P., Horber, R., Gaus, K., Li, J., Setotaw, Y. B., Qi, J., De Groote, H., Wang, Y., Thiombiano, B., Floková, K., Walmsley, A., Charnikhova, T. V., Chojnacka, A., Correia de Lemos, S., ... Bouwmeester, H. J. (2023). Maize resistance to witchweed through changes in strigolactone biosynthesis. *Science*, 379(6627), 94–99. <https://doi.org/10.1126/science.abq4775>

Li, W., Nguyen, K. H., Chu, H. D., Watanabe, Y., Osakabe, Y., Sato, M., Toyooka, K., Seo, M., Tian, L., Tian, C., Yamaguchi, S., Tanaka, M., Seki, M., & Tran, L.-S. P. (2020). Comparative functional analyses of DWARF14 and KARRIKIN INSENSITIVE 2 in drought adaptation of *Arabidopsis thaliana*. *The Plant Journal*, 103(1), 111–127. <https://doi.org/10.1111/tpj.14712>

Ludwig, Y., Zhang, Y., & Hochholdinger, F. (2013). The maize (*Zea mays* L.) AUXIN/INDOLE-3-ACETIC ACID gene family: Phylogeny, synteny, and unique root-type and tissue-specific expression patterns during development. *PLoS ONE*, 8(11), e78859. <https://doi.org/10.1371/journal.pone.0078859>

Liu, X., Hu, Q., Yan, J., Sun, K., Liang, Y., Jia, M., Meng, X., Fang, S., Wang, Y., Jing, Y., Liu, G., Wu, D., Chu, C., Smith, S. M., Chu, J., Wang, Y., Li, J., & Wang, B. (2020). ζ -Carotene isomerase suppresses tillering in rice through the coordinated biosynthesis of strigolactone and abscisic acid. *Molecular Plant*, 13(12), 1784–1801. <https://doi.org/10.1016/j.molp.2020.10.001>

- Lüdemann, Y., Zhang, Y., & Hochholdinger, F. (2022). Estimating maize harvest index and nitrogen concentrations in grain and residue using globally available data. *Field Crops Research*, 284, 108578. <https://doi.org/10.1016/j.fcr.2022.108578>
- Lynch, J. P. (2013). Steep, cheap and deep: An ideotype to optimize water and nitrogen acquisition by maize root systems. *Annals of Botany*, 112(2), 347–357. <https://doi.org/10.1093/aob/mcs293>
- Lynch, J. P. (2019). Root phenotypes for improved nutrient capture: An underexploited opportunity for global agriculture. *New Phytologist*, 223(2), 548–564. <https://doi.org/10.1111/nph.15738>
- Manoli, A., Sturaro, A., Trevisan, S., Quaggiotti, S., & Nonis, A. (2012). Evaluation of candidate reference genes for qPCR in maize. *Journal of Plant Physiology*, 169(8), 807–815. <https://doi.org/10.1016/j.jplph.2012.01.019>
- Manoli, A., Trevisan, S., Voigt, B., Yokawa, K., Baluška, F., & Quaggiotti, S. (2016). Nitric oxide-mediated maize root apex responses to nitrate are regulated by auxin and strigolactones. *Frontiers in Plant Science*, 6, 1269. <https://doi.org/10.3389/fpls.2015.01269>
- Maqbool, S., Hassan, M. A., Xia, X., York, L. M., Rasheed, A., & He, Z. (2022). Root system architecture in cereals: Progress, challenges and perspective. *The Plant Journal*, 110(1), 23–42. <https://doi.org/10.1111/tpj.15669>
- Marocco, A., & Lorenzoni, C. (2007). Morfologia e fisiologia. In *Il mais: Botanica, storia e arte, alimentazione, paesaggio, coltivazione, ricerca, utilizzazione, mondo e mercato* (pp. 1–25). Bayer CropScience.
- Marschner, P. (Ed.). (2012). *Marschner's mineral nutrition of higher plants* (3rd ed.). Academic Press.
- Martin, E. M., & Harris, W. M. (1976). Adventitious root development from the coleoptilar node in *Zea mays* L. *American Journal of Botany*, 63(6), 890–897.
- Marzec, M. (2016). Perception and signaling of strigolactones. *Frontiers in Plant Science*, 7, 1260. <https://doi.org/10.3389/fpls.2016.01260>
- Marzec, M., & Melzer, M. (2018). Regulation of root development and architecture by strigolactones under optimal and nutrient deficiency conditions. *International Journal of Molecular Sciences*, 19(7), 1887. <https://doi.org/10.3390/ijms19071887>

- Matsuoka, Y., Vigouroux, Y., Goodman, M. M., Sanchez G., J., Buckler, E., & Doebley, J. (2002). A single domestication for maize shown by multilocus microsatellite genotyping. *Proceedings of the National Academy of Sciences of the United States of America*, 99(9), 6080–6084. <https://doi.org/10.1073/pnas.052125199>
- Matusova, R., Rani, K., Verstappen, F. W. A., Franssen, M. C. R., Beale, M. H., & Bouwmeester, H. J. (2005). The strigolactone germination stimulants of the plant-parasitic *Striga* and *Orobanch* spp. are derived from the carotenoid pathway. *Plant Physiology*, 139(2), 920–934. <https://doi.org/10.1104/pp.105.061382>
- Mayzlish-Gati, E., De Cuyper, C., Goormachtig, S., Beeckman, T., & Brewer, P. B. (2012). Strigolactones are involved in root response to low phosphate conditions in *Arabidopsis*. *Plant Physiology*, 160(3), 1329–1341. <https://doi.org/10.1104/pp.112.202358>
- Mehmood Jan, M., Muhammad, S., Jin, W., Zhong, W., Zhang, S., Lin, Y., Zhou, Y., Liu, J., Liu, H., Munir, R., Yue, Q., Afzal, M., & Wang, G. (2024). Modulating root system architecture: Cross-talk between auxin and phytohormones. *Frontiers in Plant Science*, 15, 1343928. <https://doi.org/10.3389/fpls.2024.1343928>
- Mishra, S., Kumar, A., Sharma, P., Singh, R., & Prasad, S. M. (2024). Plants under stress: Exploring physiological and molecular responses to nitrogen and phosphorus deficiency. *Plants*, 13, 3144. <https://doi.org/10.3390/plants13223144>
- Mittler, R. (2002). Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science*, 7(9), 405–410. [https://doi.org/10.1016/S1360-1385\(02\)02312-9](https://doi.org/10.1016/S1360-1385(02)02312-9)
- Mittler, R. (2006). Abiotic stress, the field environment and stress combination. *Trends in Plant Science*, 11(1), 15–19. <https://doi.org/10.1016/j.tplants.2005.11.002>
- Moore, C. E., Meacham-Hensold, K., Lemonnier, P., Slattery, R. A., Benjamin, C., Bernacchi, C. J., Lawson, T., & Cavanagh, A. P. (2021). The effect of increasing temperature on crop photosynthesis: From enzymes to ecosystems. *Journal of Experimental Botany*, 72(8), 2822–2844. <https://doi.org/10.1093/jxb/erab090>
- Munns, R., & Tester, M. (2008). Mechanisms of salinity tolerance. *Annual Review of Plant Biology*, 59, 651–681. <https://doi.org/10.1146/annurev.arplant.59.032607.092911>
- Muthreich, N., Majer, C., Beatty, M., Paschold, A., Schützenmeister, A., Fu, Y., Malik, W. A., Schnable, P. S., Piepho, H.-P., Sakai, H., & Hochholdinger, F. (2013). Comparative

transcriptome profiling of maize coleoptilar nodes during shoot-borne root initiation. *Plant Physiology*, 163(1), 419–430. <https://doi.org/10.1104/pp.113.221481>

Nacry, P., Bouguyon, E., & Gojon, A. (2013). Nitrogen acquisition by roots: Physiological and developmental mechanisms ensuring plant adaptation to a fluctuating resource. *Plant and Soil*, 370, 1–29. <https://doi.org/10.1007/s11104-013-1645-9>

Napoli, C. (1996). Highly branched phenotype of the *Petunia* dad1-1 mutant is reversed by grafting. *Plant Physiology*, 111(1), 27–37. <https://doi.org/10.1104/pp.111.1.27>

Ou, H., Xie, D., Yao, R., & Shan, X. (2026). Strigolactones: Biosynthesis, transport, perception, and signal transduction. *Molecular Plant*, 19(3), 515–537. <https://doi.org/10.1016/j.molp.2025.10.008>

Parker, C. (2012). Parasitic weeds: A world challenge. *Weed Science*, 60(2), 269–276. <https://doi.org/10.1614/WS-D-11-00068.1>

Piperno, D. R., Ranere, A. J., Holst, I., Iriarte, J., & Dickau, R. (2009). Starch grain and phytolith evidence for early ninth millennium B.P. maize from the Central Balsas River Valley, Mexico. *Proceedings of the National Academy of Sciences of the United States of America*, 106(13), 5019–5024. <https://doi.org/10.1073/pnas.0812525106>

Preston, J. C., Wang, H., Kursel, L. E., Doebley, J. F., & Kellogg, E. A. (2012). The role of duplicated teosinte glume architecture genes during maize domestication. *Genetics*, 191(3), 851–864. <https://doi.org/10.1534/genetics.112.140608>

Quaggiotti, S., Buzzicotti, L., Koch, K. E., Guan, J. C., Trevisan, S., Varotto, S., Ruperti, B., & Ravazzolo, L. (2024). Strigolactone roles in maize tolerance to low nitrogen involve shifts in acquisition and partitioning of protein, sulfur, and iron. *Plant and Soil*, 505, 41–64. <https://doi.org/10.1007/s11104-024-06561-6>

Ramzan, M. T., Nawab, A., Razaq, L., Mushtaq, N. T., Waqar, M., & Fizza, U. (2026). Insights into plant abiotic stress physiology through conventional and nonconventional approaches. *Discover Agriculture*, 4, 33. <https://doi.org/10.1007/s44279-026-00475-w>

Ravazzolo, L., Trevisan, S., Iori, S., Forestan, C., Malagoli, M., & Quaggiotti, S. (2021a). Nitrate Regulates Maize Root Transcriptome through Nitric Oxide Dependent and Independent Mechanisms. *International journal of molecular sciences*, 22(17), 9527. <https://doi.org/10.3390/ijms22179527>

- Ravazzolo, L., Boutet-Mercey, S., Perreau, F., Forestan, C., Varotto, S., Ruperti, B., & Quaggiotti, S. (2021b). Strigolactones and auxin cooperate to regulate maize root development and response to nitrate. *Plant and Cell Physiology*, 62(4), 610–623. <https://doi.org/10.1093/pcp/pcab014>
- Ravazzolo, L., Chichi, A., Meggio, F., Buzzicotti, L., Ruperti, B., Varotto, S., Malagoli, M., & Quaggiotti, S. (2024). A maize mutant impaired in SL biosynthesis (zmccd8) shows a lower growth, an altered response to nitrogen starvation, and a potential secondary effect on drought tolerance. *Stresses*, 4(4), 614–626. <https://doi.org/10.3390/stresses4040039>
- Ravazzolo, L., Trevisan, S., Manoli, A., Boutet-Mercey, S., Perreau, F., & Quaggiotti, S. (2019). The control of zealactone biosynthesis and exudation is involved in the response to nitrogen in maize root. *Plant and Cell Physiology*, 60(9), 2100–2112. <https://doi.org/10.1093/pcp/pcz108>
- Reneau, J. W., Mlodzinski, D. K., Williams, M. M., & Hostetler, A. N. (2020). Maize brace roots provide stalk anchorage. *Plant Direct*, 4(11), e00284. <https://doi.org/10.1002/pld3.284>
- Rogers, E. D., & Benfey, P. N. (2015). Regulation of plant root system architecture: Implications for crop advancement. *Current Opinion in Biotechnology*, 32, 93–98. <https://doi.org/10.1016/j.copbio.2014.11.015>
- Rowe, J. H., Topping, J. F., Liu, J., & Lindsey, K. (2016). Absciscic acid regulates root growth under osmotic stress conditions via an interacting hormonal network with cytokinin, ethylene and auxin. *New Phytologist*, 211(1), 225–239. <https://doi.org/10.1111/nph.13882>
- Sass, J. E. (1977). Morphology. In G. F. Sprague (Ed.), *Corn and Corn Improvement* (2nd ed., pp. 89–110). American Society of Agronomy.
- Sasse, J., Simon, S., Gübeli, C., Liu, G.-W., Cheng, X., Friml, J., Bouwmeester, H., Martinoia, E., & Borghi, L. (2015). Asymmetric localizations of the ABC transporter PaPDR1 trace paths of directional strigolactone transport. *Current Biology*, 25(5), 647–655. <https://doi.org/10.1016/j.cub.2015.01.015>
- Schnable, P. S., Ware, D., Fulton, R. S., Stein, J. C., Wei, F., Pasternak, S., Liang, C., Zhang, J., Fulton, L., Graves, T. A., Minx, P., Reily, A. D., Courtney, L., Kruchowski, S. S., Tomlinson, C., Strong, C., Delehaunty, K., Fronick, C., Courtney, B., ... Wilson, R. K. (2009). The B73

maize genome: Complexity, diversity, and dynamics. *Science*, 326(5956), 1112–1115.
<https://doi.org/10.1126/science.1178534>

Schroeder, J. I., Delhaize, E., Frommer, W. B., Guerinot, M. L., Harrison, M. J., Herrera-Estrella, L., Horie, T., Kochian, L. V., Munns, R., Nishizawa, N. K., Tsay, Y.-F., & Sanders, D. (2013). Using membrane transporters to improve crops for sustainable food production. *Nature*, 497(7447), 60–66. <https://doi.org/10.1038/nature11909>

Sebastian, J., Yee, M.-C., Goudinho Viana, W., Rellán-Álvarez, R., Feldman, M., Priest, H. D., Trontin, C., Lee, T., Jiang, H., Baxter, I., Mockler, T. C., Hochholdinger, F., Brutnell, T. P., & Dinneny, J. R. (2016). Grasses suppress shoot-borne roots to conserve water during drought. *Proceedings of the National Academy of Sciences of the United States of America*, 113(31), 8861–8866. <https://doi.org/10.1073/pnas.1604021113>

Setter, T. L., & Waters, I. (2003). Review of prospects for germplasm improvement for waterlogging tolerance in wheat, barley and oats. *Plant and Soil*, 253(1), 1–34.
<https://doi.org/10.1023/A:1024573305997>

Shafiq, F., Ahmad, N., Wang, X., Hussain, S., Ali, M., Mahmood, F., & Zhang, G. (2024). Advances in plant–soil nitrogen management strategies. *Frontiers in Plant Science*, 15, 1477389. <https://doi.org/10.3389/fpls.2024.1477389>

Shanker, A. K., Maheswari, M., Yadav, S. K., Desai, S., Bhanu, D., Attal, N. B., & Venkateswarlu, B. (2014). Drought stress responses in crops. *Functional & Integrative Genomics*, 14(1), 11–22. <https://doi.org/10.1007/s10142-013-0356-x>

Shi, J., Mei, C., Ge, F., Hu, Q., Ban, X., Xia, R., Xin, P., Cheng, S., Zhang, G., Nie, J., Zhang, S., Ma, X., Wang, Y., Chu, J., Chen, Y., Wang, B., Wu, W., Li, J., Xie, Q., & Yu, F. (2025). Resistance to *Striga* parasitism through reduction of strigolactone exudation. *Cell*, 188(7), 1955–1966.e13. <https://doi.org/10.1016/j.cell.2025.01.022>

Shiferaw, B., Prasanna, B. M., Hellin, J., & Bänziger, M. (2011). Crops that feed the world 6. Past successes and future challenges to the role played by maize in global food security. *Food Security*, 3(3), 307–327. <https://doi.org/10.1007/s12571-011-0140-5>

Shull, G. H. (1908). The composition of a field of maize. Report of the American Breeders' Association, 4, 296–301.

- Singh, A., Yadav, S., Khanday, I., & Sundaresan, V. (2022). Genetic and hormonal blueprint of shoot-borne adventitious root development in rice and maize. *Plant and Cell Physiology*, 63(12), 1806–1822. <https://doi.org/10.1093/pcp/pcac115>
- Smith, S. E., & De Smet, I. (2012). Root system architecture: Insights from Arabidopsis and cereal crops. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 367(1595), 1441–1452. <https://doi.org/10.1098/rstb.2011.0342>
- Smith, S. E., & Read, D. J. (2008). *Mycorrhizal Symbiosis* (3rd ed.). Academic Press.
- Soundappan, I., Bennett, T., Morffy, N., Liang, Y., Stanga, J. P., Abbas, A., Leyser, O., & Nelson, D. C. (2015). SMAX1-LIKE/D53 family members enable distinct MAX2-dependent responses to strigolactones and karrikins in Arabidopsis. *The Plant Cell*, 27(11), 3143–3159. <https://doi.org/10.1105/tpc.15.00562>
- Sparks, E. E. (2023). Maize plants and the brace roots that support them. *New Phytologist*, 237(1), 48–52. <https://doi.org/10.1111/nph.18489>
- Steffens, B., & Rasmussen, A. (2016). The physiology of adventitious roots. *Plant Physiology*, 170(2), 603–617. <https://doi.org/10.1104/pp.15.01360>
- Stitzer, M. C., & Ross-Ibarra, J. (2018). Maize domestication and gene interaction. *New Phytologist*, 220(2), 395–408. <https://doi.org/10.1111/nph.15275>
- Studer, A., Zhao, Q., Ross-Ibarra, J., & Doebley, J. (2011). Identification of a functional transposon insertion in the maize domestication gene tb1. *Nature Genetics*, 43(11), 1160–1163. <https://doi.org/10.1038/ng.942>
- Sun, H., Tao, J., Hou, M., Huang, S., Chen, S., Liang, Z., Xie, T., Wei, Y., Xie, X., Yoneyama, K., & Xu, G. (2015). A strigolactone signal is required for adventitious root formation in rice. *Annals of Botany*, 115(7), 1155–1162. <https://doi.org/10.1093/aob/mcv052>
- Sun, H., Tao, J., Hou, M., Huang, S., Chen, S., Liang, Z., Xie, X., Yoneyama, K., Zhang, Y., & Xu, G. (2022). Strigolactones interact with other phytohormones to modulate root development. *The Crop Journal*, 10(6), 1637–1651. <https://doi.org/10.1016/j.cj.2022.07.014>
- Suzuki, N., Rivero, R. M., Shulaev, V., Blumwald, E., & Mittler, R. (2014). Abiotic and biotic stress combinations. *New Phytologist*, 203(1), 32–43. <https://doi.org/10.1111/nph.12797>
- Taramino, G., Sauer, M., Stauffer, J. L., Multani, D., Niu, X., Sakai, H., & Hochholdinger, F. (2007). The maize (*Zea mays* L.) RTCS gene encodes a LOB domain protein that is a key

- regulator of embryonic seminal and post-embryonic shoot-borne root initiation. *The Plant Journal*, 50(4), 649–659. <https://doi.org/10.1111/j.1365-313X.2007.03075.x>
- Tardieu, F., Parent, B., Caldeira, C. F., & Welcker, C. (2014). Genetic and physiological controls of growth under water deficit. *Plant Physiology*, 164(4), 1628–1635. <https://doi.org/10.1104/pp.113.233353>
- Tigchelaar, M., Battisti, D. S., Naylor, R. L., & Ray, D. K. (2018). Future warming increases probability of globally synchronized maize production shocks. *Proceedings of the National Academy of Sciences*, 115(26), 6644–6649. <https://doi.org/10.1073/pnas.1718031115>
- Tollenaar, M., & Lee, E. A. (2002). Yield potential, yield stability and stress tolerance in maize. *Field Crops Research*, 75(2–3), 161–169. [https://doi.org/10.1016/S0378-4290\(02\)00024-2](https://doi.org/10.1016/S0378-4290(02)00024-2)
- Trevisan, S., Manoli, A., Ravazzolo, L., Botton, A., Pivato, M., Masi, A., & Quaggiotti, S. (2015). Nitrate sensing by the maize root apex transition zone: A merged transcriptomic and proteomic survey. *Journal of Experimental Botany*, 66(13), 3699–3715. <https://doi.org/10.1093/jxb/erv165>
- Turnbull, C. G. N., Booker, J. P., & Leyser, H. M. O. (2002). Micrografting techniques for testing long-distance signalling in Arabidopsis. *The Plant Journal*, 32(2), 255–262. <https://doi.org/10.1046/j.1365-313X.2002.01419.x>
- Umehara, M., Hanada, A., Yoshida, S., Akiyama, K., Arite, T., Takeda-Kamiya, N., Magome, H., Kamiya, Y., Shirasu, K., Yoneyama, K., Kyojuka, J., & Yamaguchi, S. (2008). Inhibition of shoot branching by new terpenoid plant hormones. *Nature*, 455(7210), 195–200. <https://doi.org/10.1038/nature07272>
- Umehara, M., Hanada, A., Magome, H., Takeda-Kamiya, N., & Yamaguchi, S. (2010). Contribution of strigolactones to the inhibition of tiller bud outgrowth under phosphate deficiency in rice. *Plant and Cell Physiology*, 51(7), 1118–1126. <https://doi.org/10.1093/pcp/pcq084>
- Villaécija-Aguilar, J. A., Hamon-Josse, M., Carbonnel, S., Kretschmar, W. W., Schmidt, C., Dawid, C., Bennett, T., & Gutjahr, C. (2019). SMAX1/2/3 regulate root and shoot development through the perception of karrikins and strigolactones in Arabidopsis. *PLoS Genetics*, 15(8), e1008327. <https://doi.org/10.1371/journal.pgen.1008327>

- Villordon, A. Q., Ginzberg, I., & Firon, N. (2014). Root architecture and root and tuber crop productivity. *Trends in Plant Science*, 19(7), 419–425. <https://doi.org/10.1016/j.tplants.2014.02.002>
- Vollbrecht, E., & Schmidt, R. J. (2009). Development of the inflorescences. In J. L. Bennetzen & S. Hake (Eds.), *Handbook of Maize: Its Biology* (pp. 13–40). Springer.
- Wahid, A., Gelani, S., Ashraf, M., & Foolad, M. R. (2007). Heat tolerance in plants: An overview. *Environmental and Experimental Botany*, 61(3), 199–223. <https://doi.org/10.1016/j.envexpbot.2007.05.011>
- Wakabayashi, T., Hamana, M., Mori, A., Akiyama, R., Ueno, K., Osakabe, K., Osakabe, Y., Suzuki, H., Takikawa, H., Mizutani, M., & Sugimoto, Y. (2019). Direct conversion of carlactonoic acid to orobanchol by cytochrome P450 CYP722C in strigolactone biosynthesis. *Science Advances*, 5(12), eaax9067. <https://doi.org/10.1126/sciadv.aax9067>
- Wang, Y., & Bouwmeester, H. J. (2018). Structural diversity in the strigolactones. *Journal of Experimental Botany*, 69(9), 2219–2230. <https://doi.org/10.1093/jxb/ery091>
- Wang, J. Y., Chen, G. T. E., Braguy, J., & Al-Babili, S. (2024). Distinguishing the functions of canonical strigolactones as rhizospheric signals. *Trends in Plant Science*, 29(8), 925–936. <https://doi.org/10.1016/j.tplants.2024.03.014>
- Waters, M. T., Gutjahr, C., Bennett, T., & Nelson, D. C. (2017). Strigolactone signaling and evolution. *Annual Review of Plant Biology*, 68, 291–322. <https://doi.org/10.1146/annurev-arplant-042916-040925>
- Werner, T., Nehnevajova, E., Köllmer, I., Novák, O., Strnad, M., Krämer, U., & Schmülling, T. (2010). Root-specific reduction of cytokinin causes enhanced root growth, drought tolerance, and leaf mineral enrichment in *Arabidopsis* and tobacco. *The Plant Cell*, 22(12), 3905–3920. <https://doi.org/10.1105/tpc.109.072694>
- Wills, D. M., Fang, Z., York, A. M., Holland, J. B., & Doebley, J. F. (2018). Defining the role of the MADS-box gene, *Zea agamous-like1*, in maize domestication. *Journal of Heredity*, 109(3), 333–338. <https://doi.org/10.1093/jhered/esx073>
- Wu, X., Skirpan, A., & McSteen, P. (2009). Suppressor of sessile spikelets1 functions in the ramosa pathway controlling meristem determinacy in maize. *Plant Physiology*, 150(1), 360–370. <https://doi.org/10.1104/pp.109.137539>

- Xie, X., Wang, G., Yang, L., Cheng, T., Gao, J., Wu, Y., & Xia, Q. (2015). Cloning and characterization of a novel *Nicotiana tabacum* ABC transporter involved in shoot branching. *Physiologia Plantarum*, 153(2), 299–306. <https://doi.org/10.1111/ppl.12267>
- Xie, X., Kisugi, T., Yoneyama, K., Nomura, T., Akiyama, K., Uchida, K., Yokota, T., McErlean, C. S. P., & Yoneyama, K. (2017). Methyl zealactonoate, a novel germination stimulant for root parasitic weeds produced by maize. *Journal of Pesticide Science*, 42(2), 58–61. <https://doi.org/10.1584/jpestics.D16-103>
- Xu, C., Tai, H., Saleem, M., Ludwig, Y., Majer, C., Berendzen, K. W., Nagel, K. A., Wojciechowski, T., Meeley, R. B., Taramino, G., & Hochholdinger, F. (2015). Cooperative action of RTCS and RTCL controls shoot-borne root formation in maize. *Plant Physiology*, 169(4), 270–284. <https://doi.org/10.1104/pp.15.00907>
- Xu, M., Zhu, L., Shou, H., & Wu, P. (2005). A PIN1 family gene, *OsPIN1*, involved in auxin-dependent adventitious root emergence and tillering in rice. *Plant and Cell Physiology*, 46(10), 1674–1681. <https://doi.org/10.1093/pcp/pci183>
- Yoneyama, K. (2020). Recent progress in the chemistry and biochemistry of strigolactones. *Journal of Pesticide Science*, 45(2), 45–53. <https://doi.org/10.1584/jpestics.D19-084>
- Yoneyama, K., & Brewer, P. B. (2021). Strigolactones, how are they synthesized to regulate plant growth and development? *Current Opinion in Plant Biology*, 63, 102072. <https://doi.org/10.1016/j.pbi.2021.102072>
- Yu, P., Hochholdinger, F., & Li, C. (2019). Plasticity of lateral root branching in maize. *Frontiers in Plant Science*, 10, 363. <https://doi.org/10.3389/fpls.2019.00363>
- Zandalinas, S. I., Sengupta, S., Fritschi, F. B., Azad, R. K., Nechushtai, R., & Mittler, R. (2021). The impact of multifactorial stress combination on plant growth and survival. *New Phytologist*, 230(3), 1034–1048. <https://doi.org/10.1111/nph.17232>
- Zhang, H., Zhu, J., Gong, Z., & Zhu, J.-K. (2022). Abiotic stress responses in plants. *Nature Reviews Genetics*, 23(2), 104–119. <https://doi.org/10.1038/s41576-021-00413-0>
- Zhang, Y., Paschold, A., Marcon, C., Liu, S., Tai, H., Nestler, J., Yeh, C.-T., Opitz, N., Lanz, C., Schnable, P. S., & Hochholdinger, F. (2014). The Aux/IAA gene *rum1* involved in seminal and lateral root formation controls vascular patterning in maize (*Zea mays* L.) primary roots. *Journal of Experimental Botany*, 65(17), 4919–4930. <https://doi.org/10.1093/jxb/eru249>

- Zhang, Y., Marcon, C., Tai, H., von Behrens, I., Ludwig, Y., Hey, S., Berendzen, K. W., & Hochholding, F. (2016). Conserved and unique features of the homeologous maize Aux/IAA proteins ROOTLESS WITH UNDETECTABLE MERISTEM 1 and RUM1-like 1. *Journal of Experimental Botany*, 67(4), 1137–1147. <https://doi.org/10.1093/jxb/erv519>
- Zhang, Y., Li, X., Wang, J., Liu, Z., Chen, H., Zhao, L., & Xu, G. (2024). Effects of nitrogen deficiency on photosynthesis, metabolism, and stress adaptation in plants. *International Journal of Molecular Sciences*, 25, 10469. <https://doi.org/10.3390/ijms251810469>
- Zhao, C., Liu, B., Piao, S., Wang, X., Lobell, D. B., Huang, Y., Huang, M., Yao, Y., Bassu, S., Ciais, P., Durand, J.-L., Elliott, J., Ewert, F., Janssens, I. A., Li, T., Lin, E., Liu, Q., Martre, P., Müller, C., Asseng, S. (2017). Temperature increase reduces global yields of major crops in four independent estimates. *Proceedings of the National Academy of Sciences of the United States of America*, 114(35), 9326–9331. <https://doi.org/10.1073/pnas.1701762114>
- Zhao, Y., Hu, Y., Dai, M., Huang, L., & Zhou, D.-X. (2009). The WUSCHEL-related homeobox gene WOX11 is required to activate shoot-borne crown root development in rice. *The Plant Cell*, 21(3), 736–748. <https://doi.org/10.1105/tpc.108.061655>
- Zhao, Y., Cheng, S., Song, Y., Huang, Y., Zhou, S., Liu, X., & Zhou, D.-X. (2015). The interaction between rice ERF3 and WOX11 promotes crown root development by regulating gene expression involved in cytokinin signaling. *The Plant Cell*, 27(9), 2469–2483. <https://doi.org/10.1105/tpc.15.00227>
- Zhu, J., Ingram, P. A., Benfey, P. N., & Elich, T. (2011). From lab to field, new approaches to phenotyping root system architecture. *Current Opinion in Plant Biology*, 14(3), 310–317. <https://doi.org/10.1016/j.pbi.2011.03.020>
- Zwanenburg, B., Pospíšil, T., & Čavar Zeljković, S. (2016). Strigolactones: New plant hormones in action. *Planta*, 243(6), 1311–1326. <https://doi.org/10.1007/s00425-015-2455-5>