Strigolactones affect responses to osmotic stress and fruit ripening in crop plants

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The discovery of strigolactones

Strigolactones (SLs) were first discovered as germination stimulators of parasitic weeds (1960s)

Later on, a role for SLs was discovered as branching elicitors for arbuscular mycorrhizal fungi (2005)
SLs mediate biotic interaction in the rhizosphere

Branching factors for AM fungi (positive role)

Germination stimulants for obligate parasites (negative role)

Root exudates (secondary metabolites)

Strigolactones

AM fungus Symbiotic interaction

Orobanche or Striga Parasitic interaction

Hyphal branching signals

Germination stimulants
In 2008, reverse genetics studies in mutants of Arabidopsis (max), rice (d/htd), pea (rms) and petunia (dad) demonstrated an endogenous function for SL, acting as (mainly) root-produced hormones translocated to the shoot where they repress shoot branching.
SLs control a suite of morphological traits that go well beyond lateral shoot formation.
SL are carotenoid-derived tricyclic lactones; a fourth ring is connected via an enol-ether bridge.
The SL biosynthetic pathway

All-trans-β-carotene

9-cis-β-carotene

9-cis-β-apo-10'-carotenal

Carlactone

5-deoxystrigol or Its stereo-isomers

Various SLs

MAX1s and?

Plastid
In rice and Arabidopsis, the SL receptor D14 is a hydrolase that cleaves SL upon binding.

Shoot branching, senescence, secondary thickening, root development, and abiotic stress.
D14-like proteins act as receptors of SLs in parasitic plants and possibly of unknown SL-like ligands in Arabidopsis

ShHTLs (D14-like) function as SL receptors mediating seed germination in Striga

In fire-succession species, D14-like/KAI2 mediates response to karrikins, smoke-borne compounds that stimulate seed germination and share structural resemblance to SLs
SL mediates adaptation of plant architecture to phosphate starvation
Drought induces reduction of SL levels in Lotus roots but not in shoots

In *Lotus japonicus* WT plants, drought does not affect SL levels in shoots but concentration in roots **decreases** and so does the transcript concentration of biosynthetic genes.

*Liu 2015, Planta 241:1435*
Ljccd7, a Lotus japonicus CCD7 (MAX3) mutant with reduced SL biosynthesis and concentration

Liu 2013, JXB 64:1967
Ljccd7 is hypersensitive to osmotic (drought) stress

The Lotus SL biosynthetic mutant Ljccd7 undergoes higher stress than WT when exposed to drought

Ljccd7 closes stomata slower than WT at any stress level

Liu 2015, Planta 241:1435
Experiments with Lotus mutants confirm results obtained with Arabidopsis mutants

Arabidopsis max3 (ccd7) and max4 (ccd8) biosynthetic mutants, and max2 (d14) signaling mutants are hypersensitive to drought

Ha 2013, PNAS 111:251

A second paper confirms drought hypersensitivity for max2 but not max3-max4 mutants

BU 2013, Plant Phys 164:424
Root- and shoot-specific SL control of drought stress sensitivity investigated in tomato

We used the M82 indeterminate tomato genotype and its SL-depleted mutant Slccd7...

Visentin 2016, New Phytol 212:954

Vogel 2010, Plant J 61:300
Grafting reveals that root-specific SL downregulation actually reduces sensitivity to drought stress

While WT and SL- (Slccd7) genotypes and their homografts have the expected stomatal and drought tolerance phenotype, the WT/SL- (root SL-depleted) heterograft has lower stomatal conductance than WT/WT (as would be expected for drought-induced SL repression)

Visentin 2016, New Phytol 212:954
In WT/SL- heterografts, drought stress downregulates SL biosynthesis in the root but upregulates biosynthesis in the shoot.

Drought decreases CCD7 expression in the root of both WT/WT and WT/SL- plants, while in shoot expression increases in both graft combinations.

Visentin 2016, New Phytol 212:954
SL depletion in roots affects free ABA concentration

In *Ljccd7*, root and shoot free ABA concentration increases upon osmotic stress comparable as in WT

In tomato, lower endogenous SL induces higher free ABA in the root but lower in the shoot

*Visentin 2016, New Phytol 212:954*
Endogenous and exogenous SL levels control stomatal sensitivity to ABA

In *Ljccd7*, stomatal conductance is higher than in WT at any level of leaf ABA

*Liu 2015, Planta 241:1435*

*Ljccd7* closes stomata slower than WT in presence of exogenous ABA

Stomata of tomato WT/SL−, or WT/WT plants treated with exogenous SL, are ABA hypersensitive

*Visentin 2016, New Phytol 212:954*
A model for SL modulation under drought stress

Park 2016 lant Cell 28:2528
miR156: a controller of phase transition in plants

Wu 2009 Cell 138:750

MiR156 genes encode a family of conserved miRNAs that target SQUAMOSA binding-like (SPL) proteins

miR156 controls maintenance of vegetative phase and morphology regulation in Arabidopsis through interaction within a “miRNA cascade”
miR156 also controls responses to drought stress

Cui et al. 2014
Plant J 80:1108

miR156 is induced by drought and is needed for tolerance to osmotic and salt stress in Arabidopsis (Cui et al. 2014 Plant J 80:1108)
Overexpression of miR156 in tomato (var. MicroTom) causes stomatal closure

In tomato, stomatal conductance of miR156-oe plants is lower than in WT plants
miR156 is induced by drought only when endogenous SL is available

In SL- tomato mutants, miR156 is not accumulated under stress in shoot or root
Exogenous SL is sufficient to increase miR156 in the absence of stress

Treatment with the GR24 artificial SL increases miR156 concentration in non-stressed plants
SL are needed for drought-induced MIR156 transcription in shoots

miR156 transcript (pri-miRNAs) are detected in shoot only; mature miRNA accumulation is controlled not only by expression, but also by maturation and transport processes.
SL are needed for drought-induced MIR156 transcription in shoots

miR156 transcript (pri-miRNAs) are detected in shoot only; mature miRNA accumulation is controlled not only by expression, but also by maturation and transport processes.
The ABCG25 ABA membrane transporter

ABC25 is an ABA membrane transporter expressed in the vasculature

Overexpressors close stomata suggesting that ABCG25 mediates efflux of ABA from vasculature cells to guard cells

Kuromori 2010 PNAS 107:2361
ABC25 localizes to the plasma membrane upon ABA treatment and is internalized upon stress

A GFP:ABC25 fusion localizes at the PM and in the prevacuolar compartment

ABA treatment induces increased localization to the PM of GFP:ABC25

Park 2016 Plant Cell 28:2528
Exogenously applied ABA and GR24 increase ABCG25 expression and PM localization of sGFP:ABCG25

![Graph showing ratio of GFP signal (Cytoplasm/PM) with control, NT 4h, ABA 4h, and GR24 4h]

- t test: p-value<0.01 - n=20

![Microscopic image with indicated cytosol and plasma membrane]
SLs affect reproductive development

Petunia CCD8 mutants show delayed flowering and smaller flowers than WT  

Snowden 2005 Plant Cell 17:746
Fruit ripening processes are controlled by ABA

In grapevine, ripening and anthocyanin accumulation are activated by exogenous ABA in whole berries and berry cells.
Exploring ABA-SL interactions in ripening grape berries

We treated grape berries (incubated or in clusters) as follows: no treatment, GR24, ABA, GR24+ABA (clockwise) – SL treatment reduced ABA-induced anthocyanin accumulation
GR24 negatively affects ABA concentration in ABA-treated grape berries
GR24 induces ABA degradation

In Phelipanche seeds, a decrease in ABA was reported following application of exogenous SL

Liu 2015, Planta 241:1435

In grape berries, GR24 activates the ABA-oxidase HYD1
Control of ABA transport maybe a recurrent SL-dependent mechanism to control ABA responses?

GR24 reduces the ABA-induced activation of ABCG25
Conclusions: a complex control of drought stress responses and reproductive development by SL

- Besides controlling shoot and root branching, SLs have pervasive effects on plant physiology, including tolerance to stress and flower/fruit development.
- An increase in shoot SL levels are required for plants to develop full responses to drought stress.
- In fruit, SLs can induce ABA degradation quenching responses to ABA.
- miR156 is part of the SL-signal transduction pathway.
- ABA relocalization due to activity of ABA transporters may be a common pattern of SL modulation of ABA effects.
Application of SLs in agriculture

- SLs activate seed germination in parasitic plants and can synchronize germination in crop plants
- SL application to the shoot increases tolerance to drought stress
- SL application accelerates flowering in tomato and could affect ripening processes
- SL-enriched biostimulants have effects similar as exogenous SL (GR24)
Credits

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