Carotenoid Research: From Combating Malnutrition to Parasitic Weeds Control

5th Arab-American Frontiers of Science, Engineering and Medicine Symposium

جامعة الملك عبدالله
للعلوم والتقنية
King Abdullah University of
Science and Technology

Salim Al-Babili

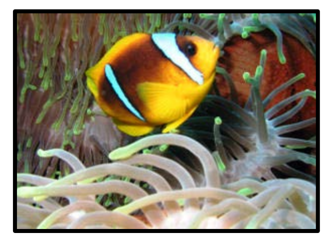
Rabat, Morocco

Nov 3, 2017

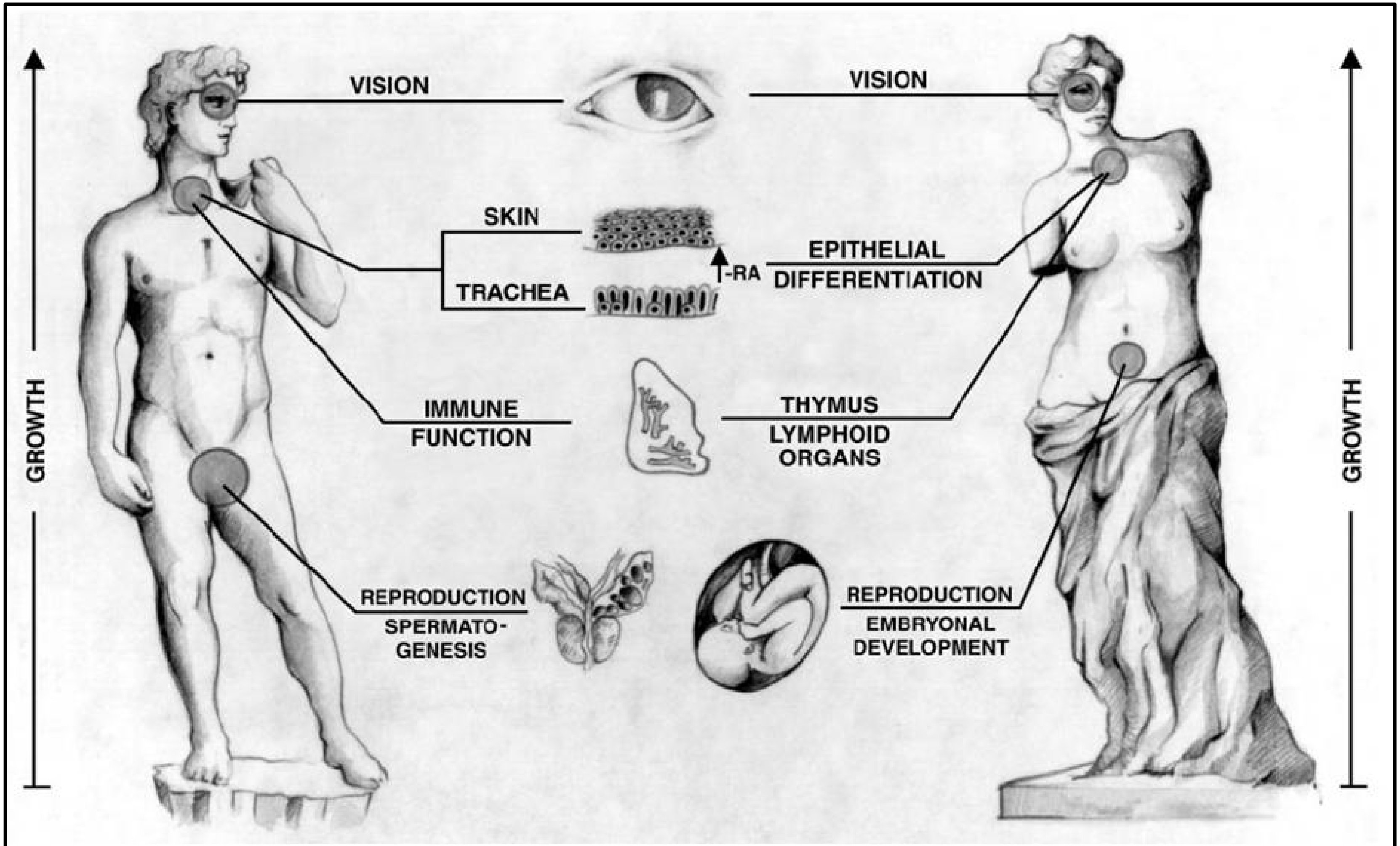
Carotenoids, Overview

- *Lipophilic pigments essential for photosynthesis*
- *Accumulated in flowers, fruits…*
- *No de novo synthesis in animals*
- *Required as precursors of vitamin A, β-carotene in carrots*
- *Precursors of a large number of signaling molecules and hormones*






Vitamin A, Functions



Physiological Reviews

Ross, S. A. *et al*. Physiol. Rev. 80: 1021-1054, 2000

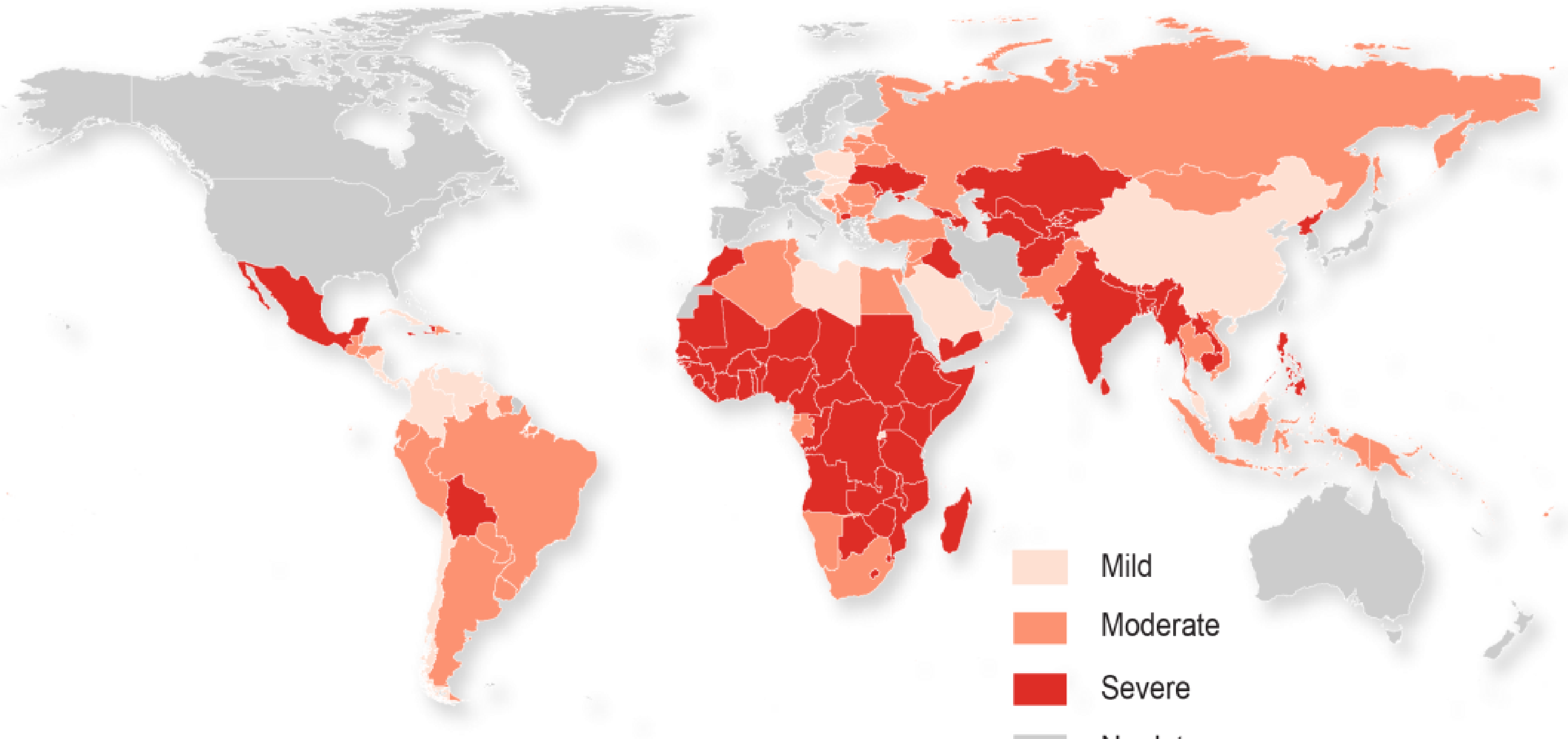
Vitamin A, Deficiency

- *A severe health problem*
- *Death during pregnancy*
- *Affecting 100-140 Mio. children*
- *Major reason for childhood mortality*
- *Blindness of 0.25-0.5 Mio. children every year*

Xerophthalmia

See:
http://www.who.int/vaccines-diseases/en/vitamina/advocacy

Global Prevalence of Vitamin A Deficiency



http://www.who.int/nutrition/topics/vad/en/index.html

Crops with Enhanced Provitamin A Content

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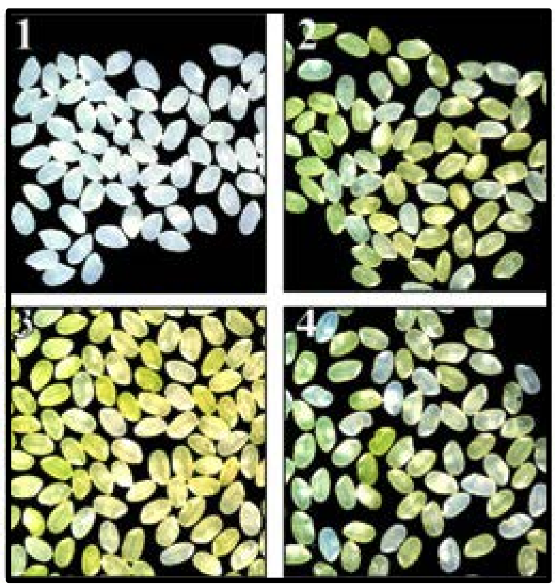
Engineering the Provitamin A (β-Carotene) Biosynthetic Pathway into (Carotenoid-Free) Rice Endosperm

Xudong Ye,[1*†] Salim Al-Babili,[2*] Andreas Klöti,[1‡] Jing Zhang,[1] Paola Lucca,[1] Peter Beyer,[2§] Ingo Potrykus[1§]

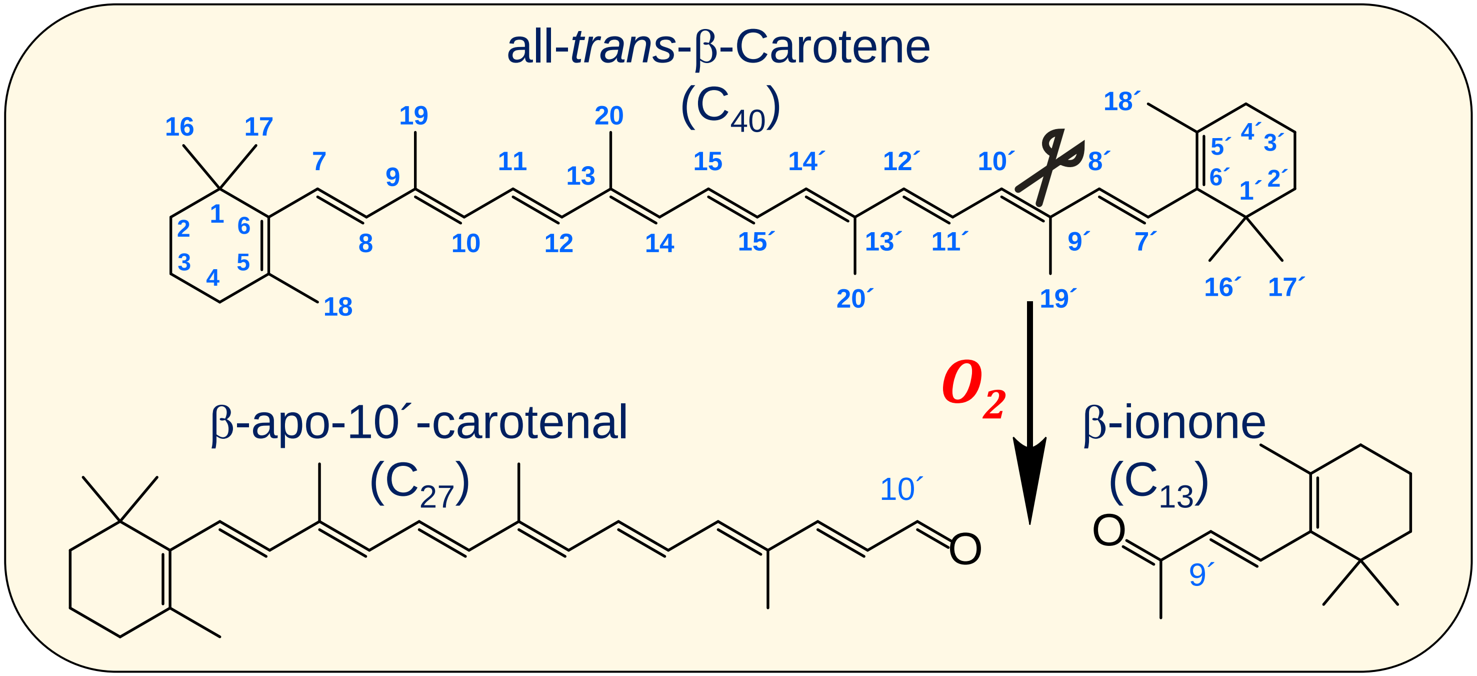
Science AAAS

*These authors contributed equally to this work.

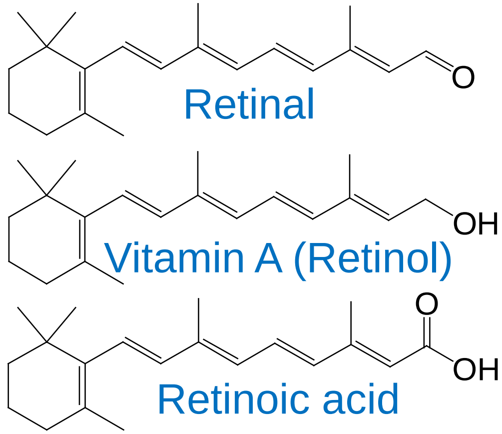


International Rice Research Institute (IRRI); www.irri.org

Carotenoids, Targets for Oxidative Cleavage



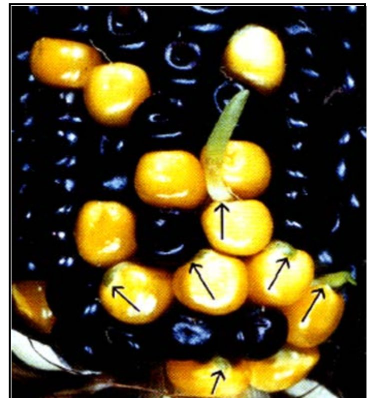
Important Compounds

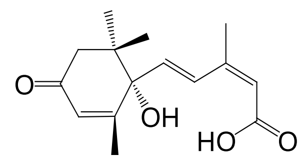


Retinal

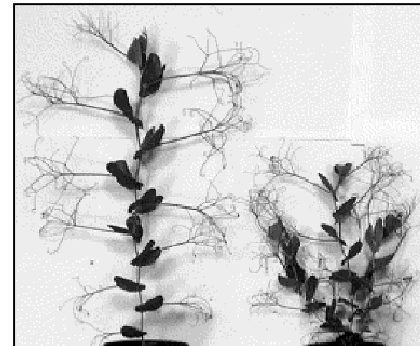
Vitamin A (Retinol)

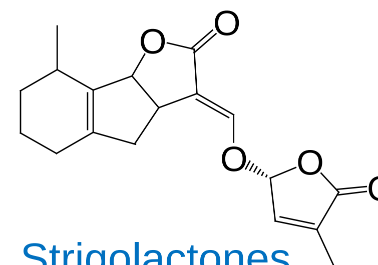
Retinoic acid



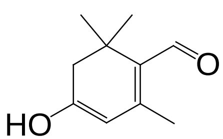


Abscisic acid

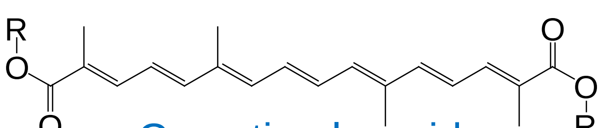




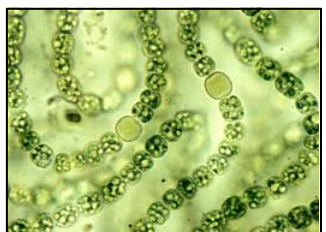
Strigolactones

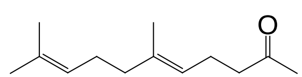


Safranal

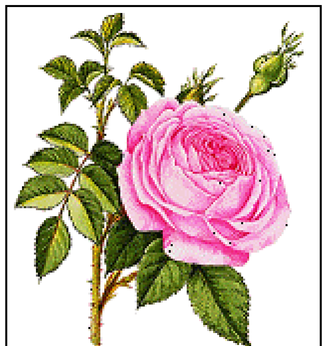


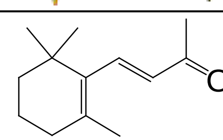
Crocetin glycosides



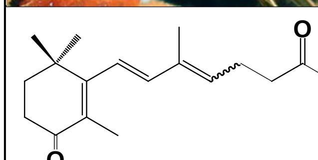


Geranylacetone





β-Ionone



Trisporoids

Strigolactones, Root Parasitic Plants

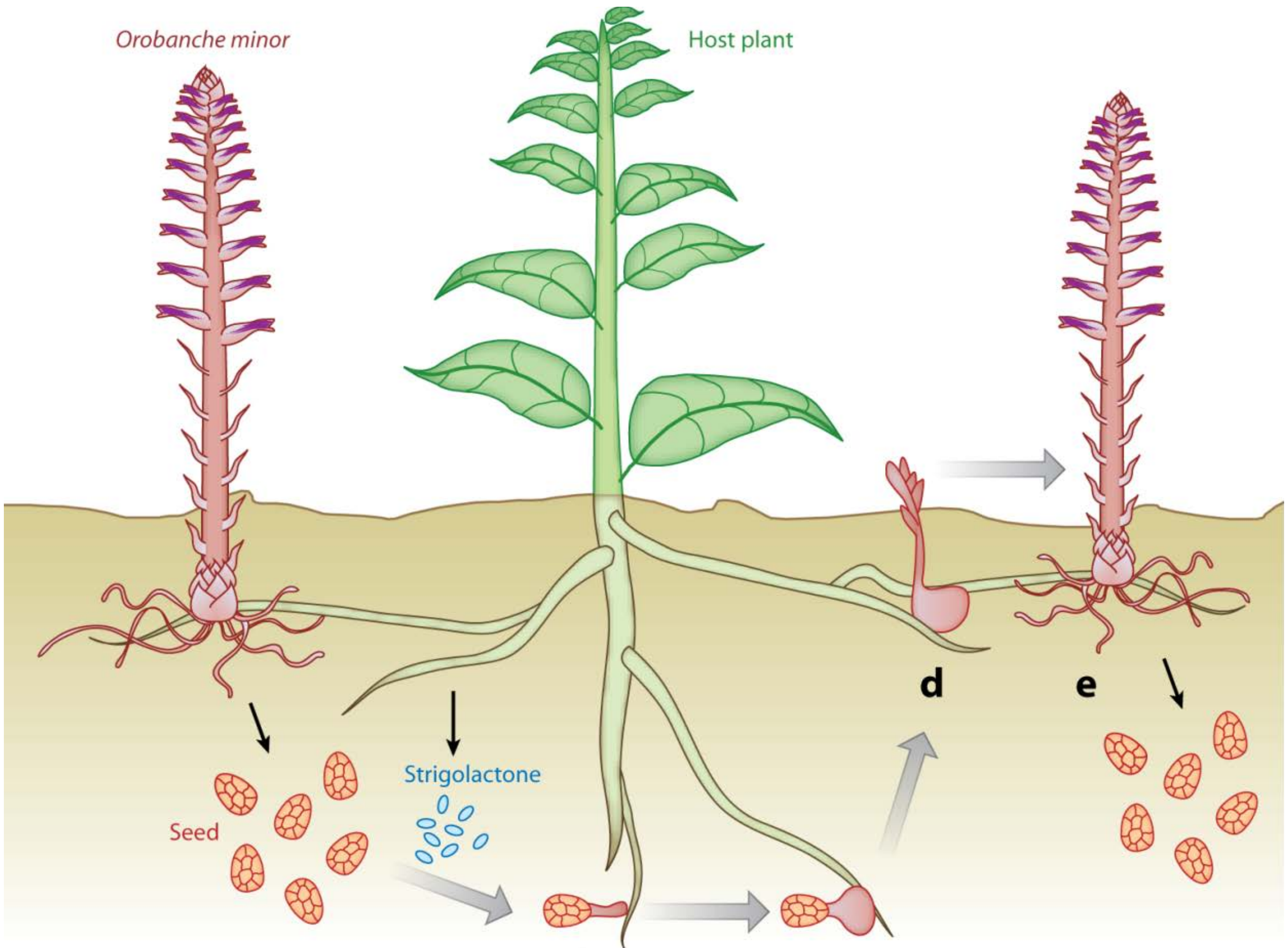
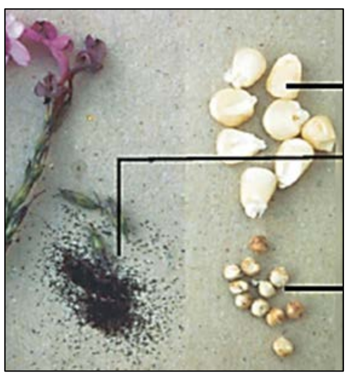


Figure from Xie, et al., (2010); Annu Rev Phytopathol.

Striga, Germination and Attachment

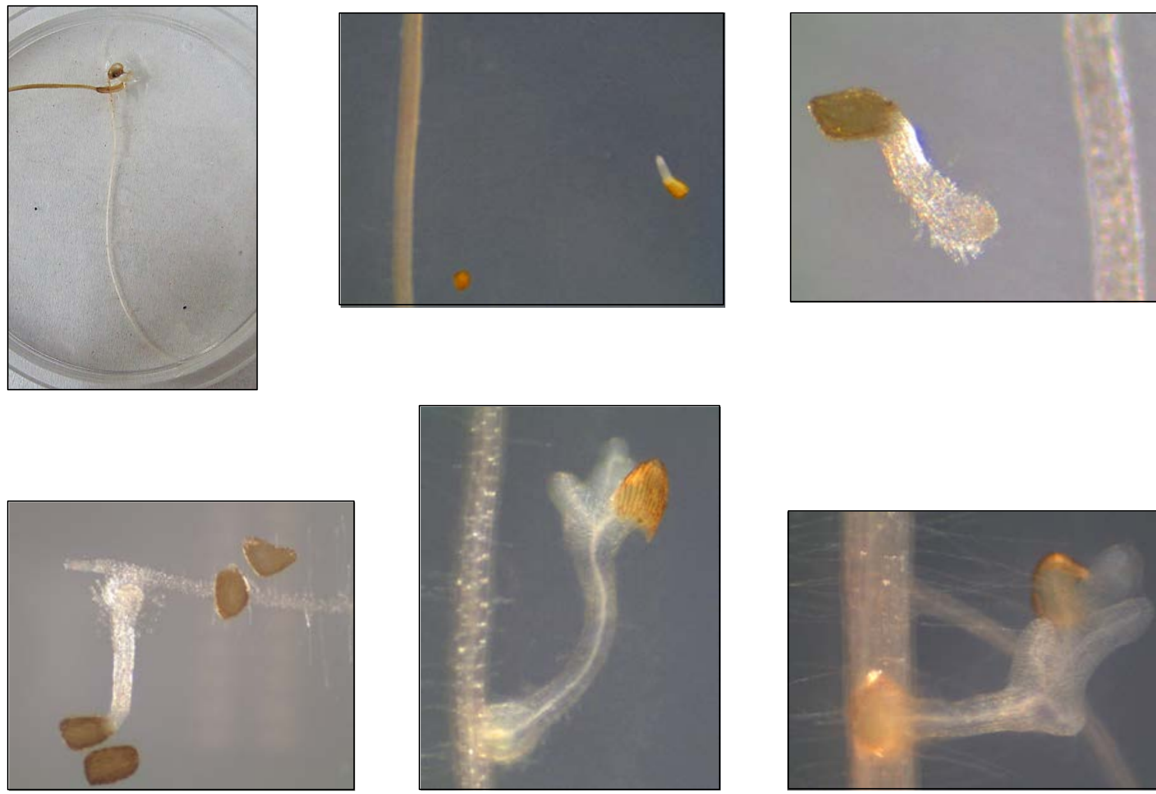
Striga hermonthica



Maize

Striga

Sorghum



Striga invading pearl millet roots on petri dish

(Taken by Dr. Boubacar Kountche)

Pearl millet, Striga infestation

Djibril Yonli, Burkina Faso

The Seven Major Biotic Threats to Food Security

FOOD SECURITY

NEWS

Armed and Dangerous

These fungi, weeds, and viruses are among the more serious biological threats to food security—so researchers are working hard on countermeasures

WHEAT STEM RUST

Pest: *Puccinia graminis* Ug99
Crop: Wheat
Whereabouts: Fifty years ago, stem rust led to the resistant wheat varieties that fueled the Green Revolution—leading many farmers to believe they were done with *Puccinia graminis*. But in 1998, a dangerous new strain named Ug99 appeared in Uganda (*Science*, 30 March 2007, p. 1786). By 2004, its spread prompted Green Revolution pioneer Norman Borlaug to launch a global research initiative to address the threat. Ug99 has since shown up in Yemen and Iran and threatens wheat crops throughout the Middle East and West Asia. The big fear: Ug99 could cause famine in Pakistan and India, where small farmers can't afford the fungicides used to control the disease.
Symptoms: The fungus infiltrates stems and plugs up vascular tissue. Of the three common rust diseases, stem rust is the worst because it causes the plant to fall over, so the entire harvest is lost.
Losses: Heavy infections can reduce yields by 40% or more. If it reaches India's Punjab region, losses could reach $3 billion per year; if it reaches the United States, the toll could be $10 billion annually.
Countermeasures: The International Maize and Wheat Improvement Center in Mexico has created 15 resistant wheat varieties, but Ug99 is infamous for quickly overcoming resistance.

POTATO BLIGHT

Pest: *Phytophthora infestans*
Crops: Potatoes; also tomatoes and other solanaceous crops
Whereabouts: This funguslike organism occurs wherever farmers grow potatoes.
Symptoms: Most notorious for causing the Irish potato-famine of 1845 to 1851, late blight still ranks as the world's most dangerous potato disease. Spread by spores or by planting infected tubers, it first appears as gray splotches on leaves. In high humidity and moderate temperatures, it can destroy a whole field in a week.
Losses: The International Potato Center in Peru reports that yield losses in developing countries are about $2.75 billion annually. Fungicide applications can total 10% of overall production costs.
Countermeasures: Fungicides work but can be harmful to human health and too costly for poor farmers.

BLACK SIGATOKA

Pest: *Mycosphaerella fijiensis*
Crops: Bananas, plantains
Whereabouts: This fungus, first detected in Fiji in 1964, is now found in 100 countries in the Americas, Africa and South Asia.
Symptoms: The fungus starts as small flecks on the undersides of the youngest leaves. They expand into brown streaks that can eventually destroy the leaf, decreasing photosynthesis. Fruit from diseased trees can ripen prematurely during shipping, causing further losses.
Losses: Yields reduced up to 50%.
Countermeasures: Commercial plantations frequently apply cocktails of fungicides, sometimes from airplanes, and remove leaves at a cost of 15% to 50% of the fruit's final retail price.

WITCHWEED

Pest: *Striga hermonthica*
Crops: Corn, sorghum, sugarcane, millet, native grasses
Whereabouts: *Striga* originated in Africa and has since become widespread in the tropics.
Symptoms: This parasitic plant attaches to the host's roots, where it siphons off nutrients and water, stunting the host's growth and causing it to wither. When *Striga* emerges aboveground, it makes a substance toxic to the host. One plant can produce 50,000 tiny seeds that stick to people and their tools or settle in the soil. Seeds can stay dormant for 15 years.
Losses: In sub-Saharan Africa, *Striga* infects 20 million to 40 million hectares, reducing yields by 20% to 100%. Losses total about $1 billion per year and affect 100 million people.
Countermeasures: Some *Striga*-tolerant maize can produce small ears despite being parasitized. But farmers must scramble to destroy plants before they produce seed and plant nonhost crops in affected soils. Another approach is to plant a legume called *Desmodium*, which secretes a chemical that kills *Striga*, but that requires using livestock to control the *Desmodium*. Researchers are looking into applying a fungus to kill the seeds.

RICE BLAST

Pest: *Magnaporthe oryzae*
Crops: Rice, 50 species of grasses and sedges
Whereabouts: Worldwide
Symptoms: Spores infect plants, particularly when humidity is high, often killing young plants. In older plants, the fungus can spread and prevent seed formation.
Losses: Destruction can be extremely fast but variable, with up to 100% loss in some paddies. Some analysts estimate that each year blast destroys harvests that could feed 60 million people, at a cost of some $66 billion.
Countermeasures: Rice blast is a formidable foe, persisting despite the best control efforts. Farmers can manage the disease by rotating crops, maintaining water levels (too little water promotes infection), and using fertilizers prudently. Resistant cultivars help, but no cultivar can withstand all races of the fungus, and blast tends to overcome resistance in two or three growing seasons. Farmers can also use fungicides.

SPECIALSECTION

ASIAN SOYBEAN RUST

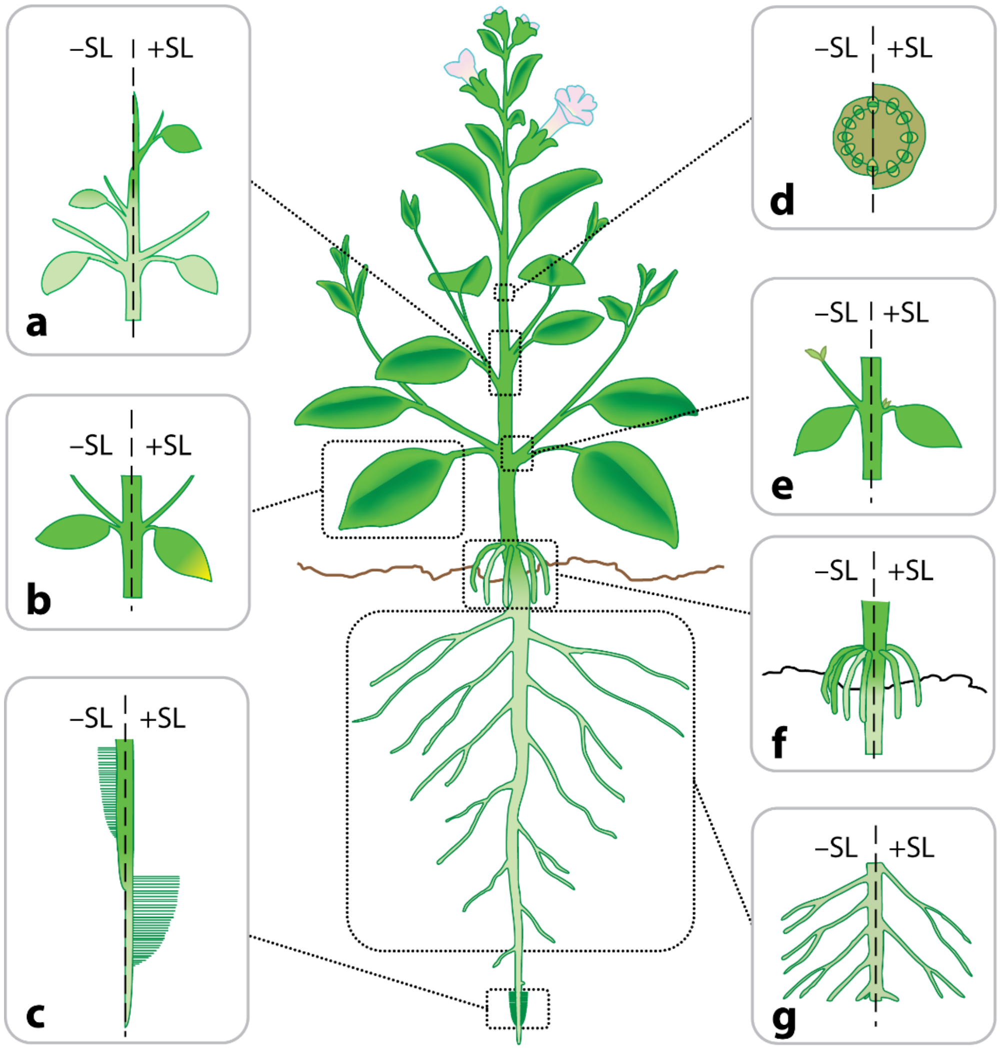
Pest: *Phakopsora pachyrhizi*
Crops: At least 31 legume species, notably soybeans
Whereabouts: Native to Asia, soybean rust spread to Australia in the 1980s and reached Africa a decade later. It hit South America in 2001, and Hurricane Ivan carried spores into the United States in 2004. It's now found throughout the Southeastern United States and Mexico (*Science*, 3 December 2004, p. 1672).
Symptoms: Infected plants develop small pustules on the undersides of leaves that spread throughout the plant. In the United States, the invasive vine kudzu is the primary host and vector for soybean rust.
Losses: Yields reduced 10% to 80%.
Countermeasures: Early detection and multiple applications of fungicide.

CASSAVA BROWN STREAK VIRUS

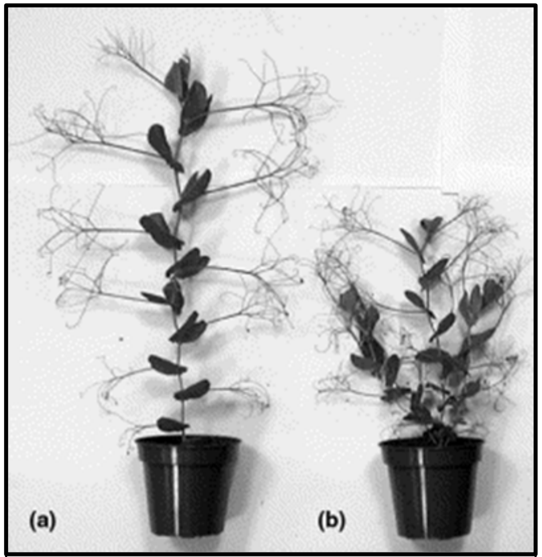
Pest: Virus
Crops: Cassava, also called yucca, manioc, and mandioca
Whereabouts: East and Central Africa
Symptoms: This virus is emerging as a major threat to a crop already under siege from cassava mosaic virus. Spread by whiteflies and by cuttings, brown streak virus is more insidious than the mosaic virus because the plant can look healthy even as the disease destroys the edible root. Once confined to lowlands in East Africa, it appeared in Uganda in 2004 and has become a threat throughout sub-Saharan Africa. Disease often appears where farmers have planted cassava varieties resistant to mosaic virus.
Losses: Yields drop by up to 100%. In 2003, economic losses totaled more than $100 million per year. This virus and cassava mosaic virus have been called Africa's biggest threat to food security.
Countermeasures: The International Institute of Tropical Agriculture, based in Nigeria, is developing tolerant varieties whose leaves become diseased but whose roots stay healthy. Early-warning monitoring programs and early harvesting can help reduce the impact of the diseases.

—ELIZABETH PENNISI

Strigolactones are Plant Hormones



Al-Babili S, Bouwmeester HJ. 2015.
Annu. Rev. Plant Biol. 66:161–86



Wt *rms1*
Morris et al., 2001

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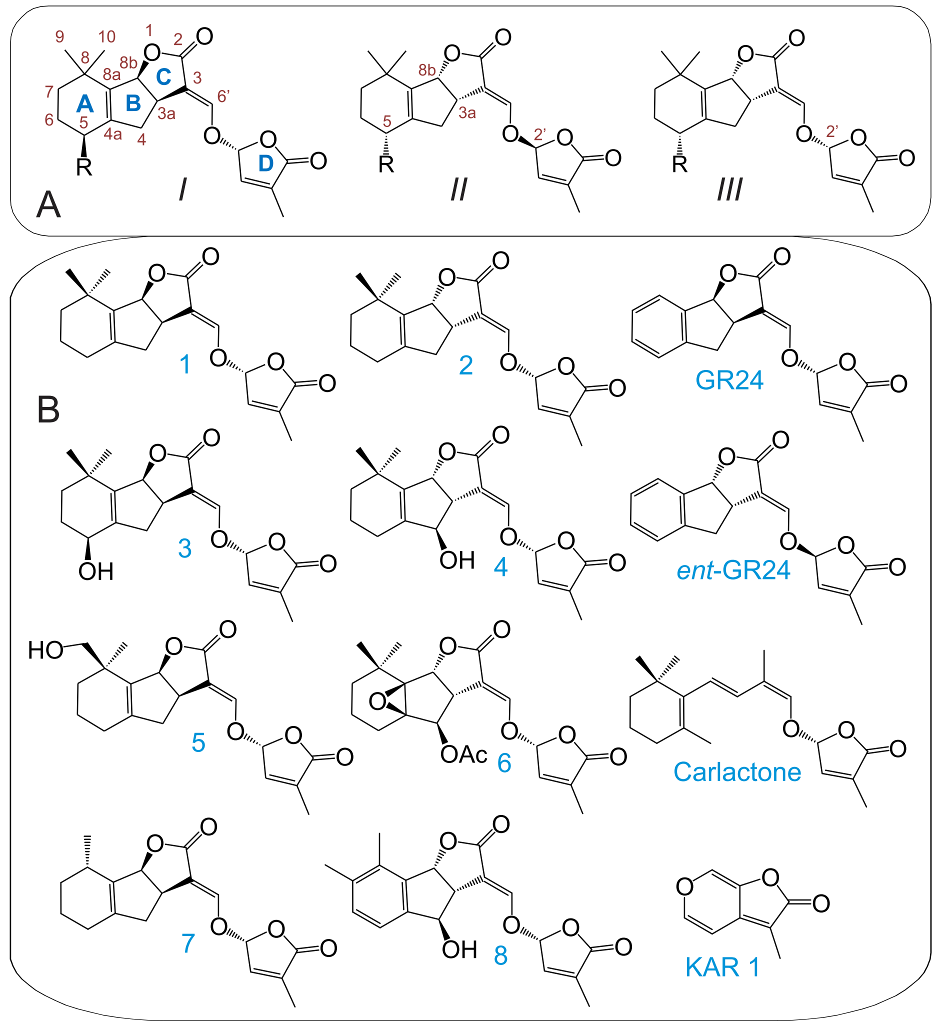
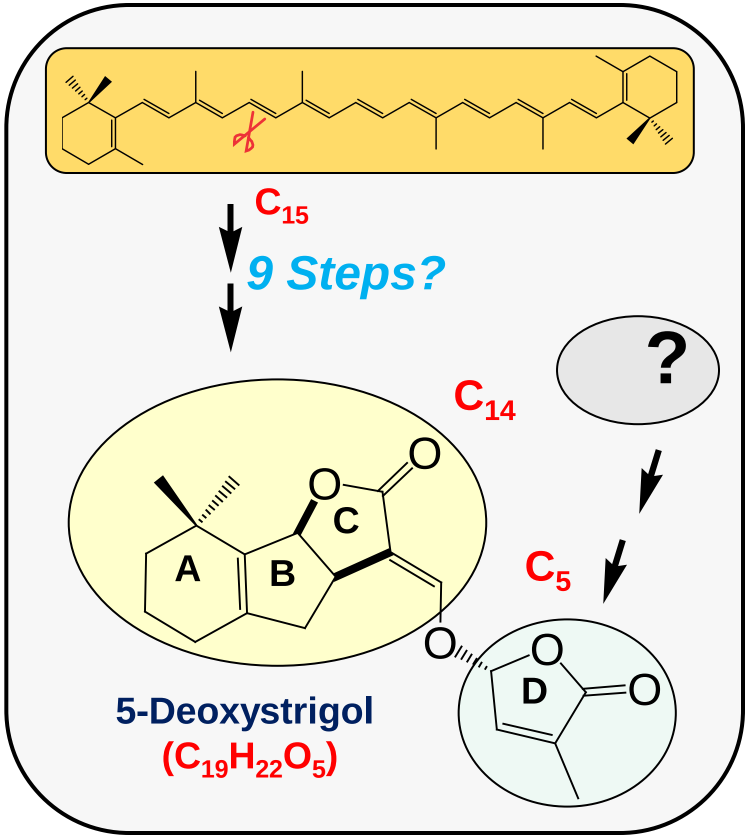
Strigolactones, Structure

- *Canonical strigolactones: ABC-ring connected to a mono-cylic lactone (D-ring) via an enol ether bridge*
- *Two sub-families with different stereochemistry (Strigol-like:1,3,5 and 7); (Orobanchol-like:2, 4, 6 and 8)*
- *Carlactone is representative of a third family lacking the BC-ring*



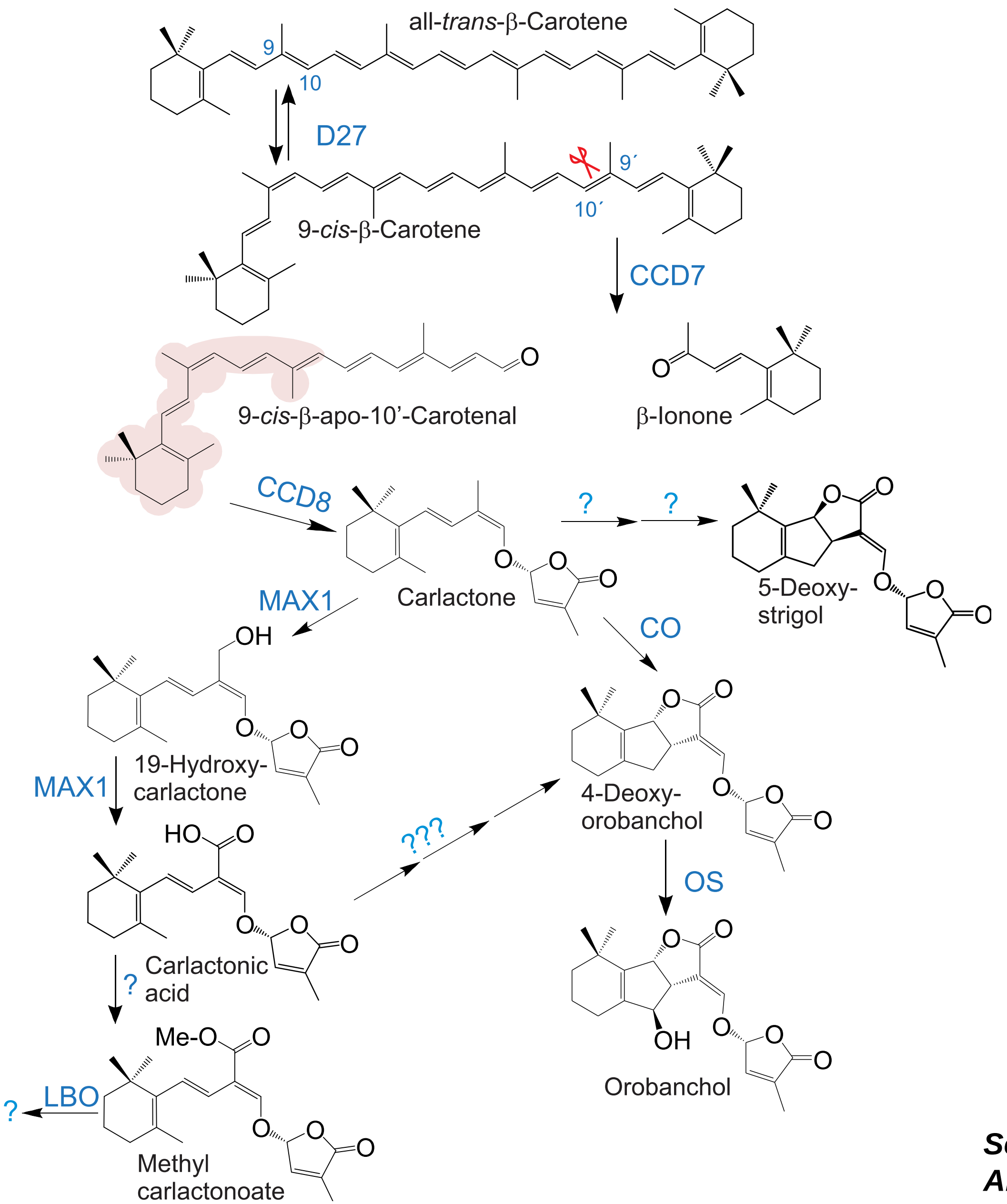
How to Make Strigolactones?

- *Carotenogenic origin suggested by carotenoid-deficient mutants and inhibitors studies **
- *Around 9 (theoretical) reactions required for the ABC-ring*
- *D-ring of an unknown origin*



**: Matusova et al., Plant Physiol. 139, 920 (2005)*

How to Make Strigolactones? Update



Alder et al., 2012, Science
Zhang et al., 2014, Nat. Chem. Biol.

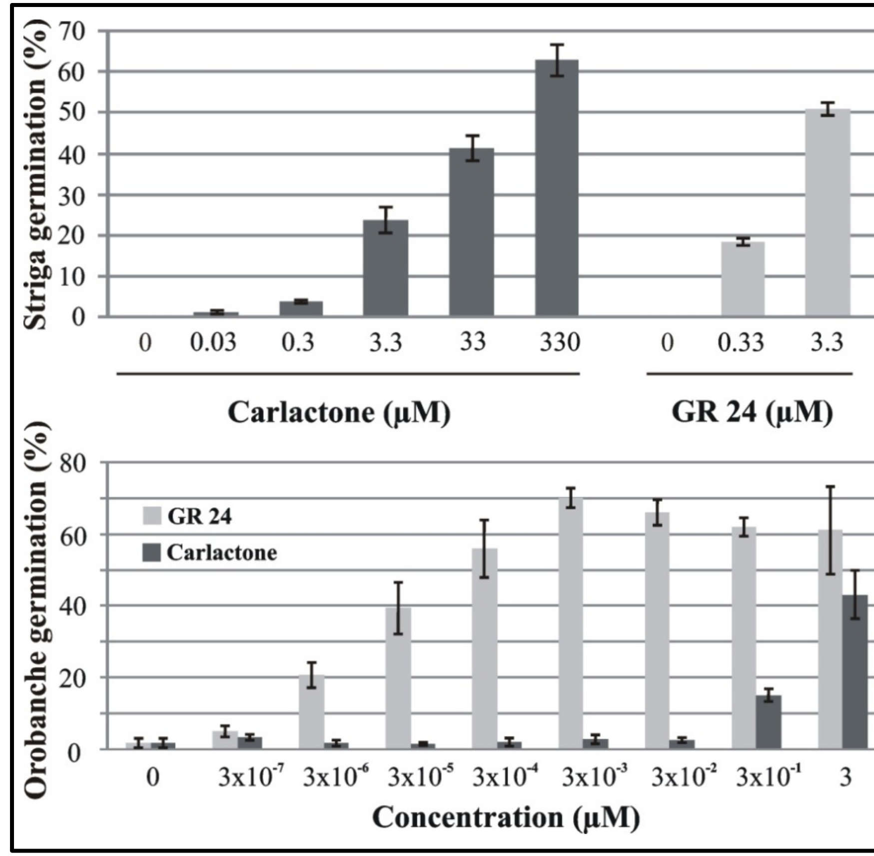
Seto et al., 2014, PNAS
Abe et al., 2014, PNAS
Brewer et al., 2016, PNAS

Carlactone, Inducing Seed Germination

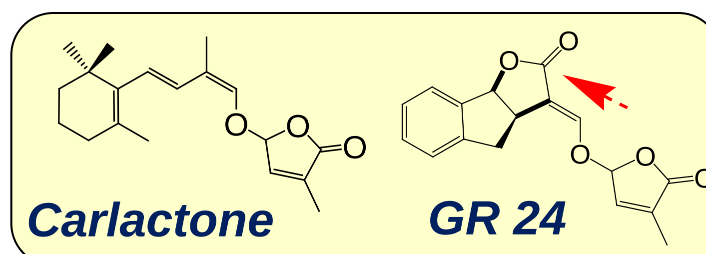
- ✓ *Carlactone induces seed germination of Striga and Orobanche*

Witchweeds (*Striga* sp)

Broomrapes (*Orobanche* sp)



Seeds Germination Assay



Alder *et al*., 335, 1348 (2012)

Science AAAS

Why Strigolactone Analogs?

- ✓ *SLs have a great application potential…..*
  - ✓ *In regulating plant's architecture,……*
  - ✓ *In combating root parasitic weeds by triggering „SUICIDAL GERMINATION“*

- ✓ *Natural SLs are produced at very low level.*

- ✓ *Chemical synthesis of natural SLs is quite challenging.*

- ✓ *Efficient and easy-to-synthesize SL analogs are needed.*

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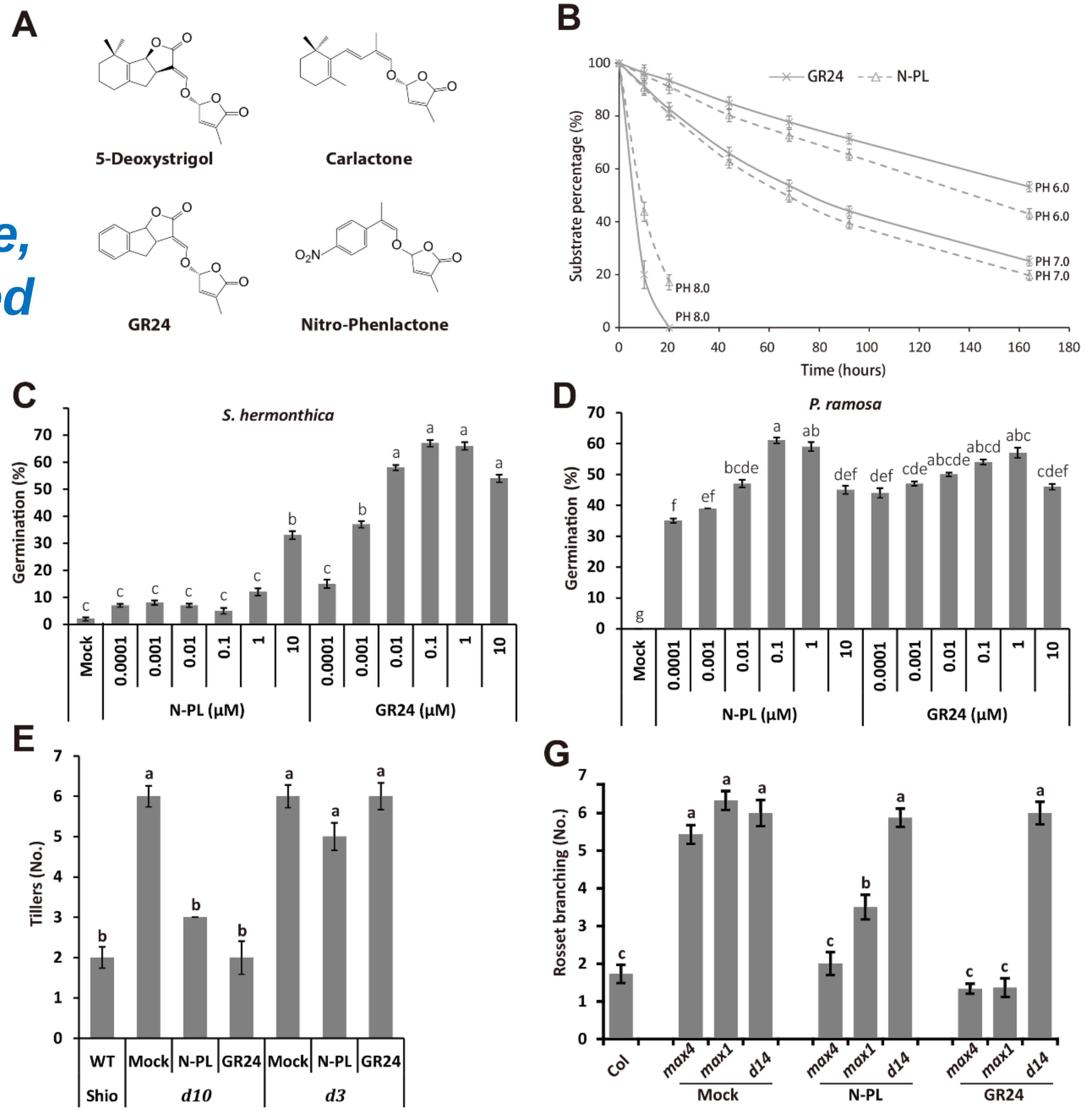
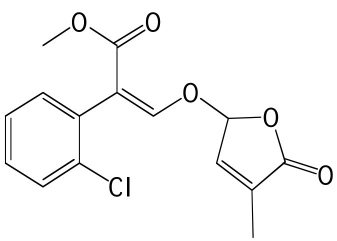
Nitro-Phenlactone, a Carlactone-based SL Analog



Jia *et al*., (2016) Mol. Plant

A New Family of SLs Analogoues (Methyl Phenlactonoates)



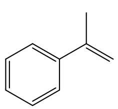
$_{3}FO_{5}$ $C_{15}H_{13}ClO_{5}$ $C_{15}H_{13}ClO_{5}$ $C_{15}H_{13}ClO_{5}$ $C_{15}H_{13}ClO_{5}$

$C_{19}H_{16}O_{5}$ $C_{14}H_{11}NO_{6}$ $C_{16}H_{16}O_{6}$ $C_{15}H_{14}O_{6}$ $C_{17}H_{14}O_{5}$

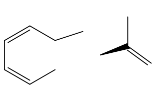
Jamil et al. In revision

Germinating Activty in Pot Experiments

Nijmegen-1 [(a)]



MP1



GR24

Stimulants applied at 0.5 μM concentration.

[(a)] Zwanenburg and Pospisil, 2013.

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Combating Striga in Pearl Millet

Two Contrasting Lines

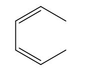
✓ *Evaluated in Striga sick-pot experiment…*

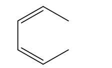
Developed at ICRISAT-Niger (Haussmann *et al*., unpublished data)

Mini-Field Trial at INERA, Burkina Faso

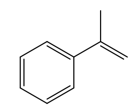
For more information, see Poster 13

Germinating Activty under Mini-Field Conditions

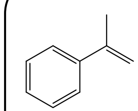




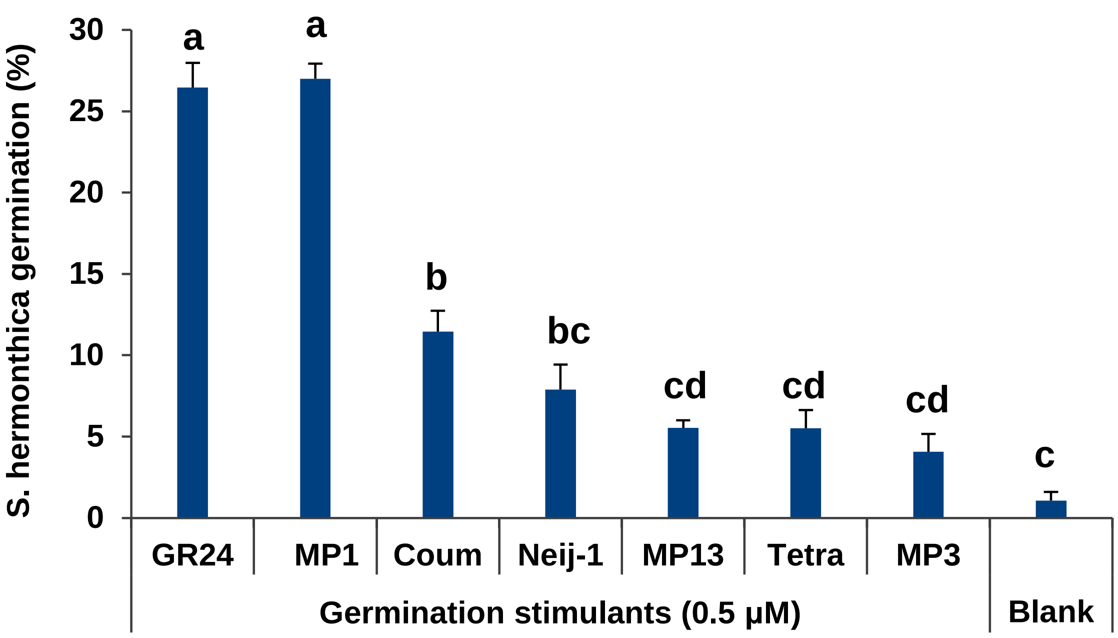
Tetra:
Tetralone derivative [a]



MP1



MP3



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Field trials in Burkina Faso

Thanks to:

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Lina Baz, Imran Haider, Xiujie Guo, Valentine Ntui,
Boubacar Kountche, Mohammad Jamil, Jianing Me
Kupeng Jia (KAUST)

Collaborations

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Maria Jesus Rodrigo, Gianfranco Diretto
Peter Beyer, Freiburg

Harro Bouwmeester, Wageningen

Koichi Yoneyama, Utsunomiya University

Khoki Akiyama, Osaka Prefecture University

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Ralf Reski, Freiburg

Prof. Tadao Asami, University of Tokyo

Prof. Binne Zwanenburg, Radboud University

Dr. Djibril Yonli, INERA, Burkina Faso

Pearl millet: The Miracle Grain

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- ✓ Pearl millet, *Cenchrus americanus*, is the sixth globally important staple food and fodder crop
- ✓ Mainly grown by subsistence farmers and smallholders
- ✓ Widely spread in the semi-arid regions of Africa, India and the Middle East
- ✓ Drought and heat resistant
- ✓ High nutritional value, containing iron, zinc and provitamin A
- ✓ Yields are severely affected by *Striga* infestation
- ✓ Research on pearl millet/*Striga* lags behind work done with other cereals (no validated resistance genes/QTLs)

Hash *et al.*, 2000; Ejeta, 2007; Haussmann *et al.*, 2004; Parker, 2012

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Combating Striga in Pearl Millet

We want to..

Evaluate the suicidal germination strategy in field application

- ✓ Develop low-cost and efficient SLs analogs
- ✓ Establish Protocols for Field application in Burkina Faso
  - ➢ Minibox trials
  - ➢ Field trials (artificially and naturally infested)

Identify resistance factors and provide targets for breeding

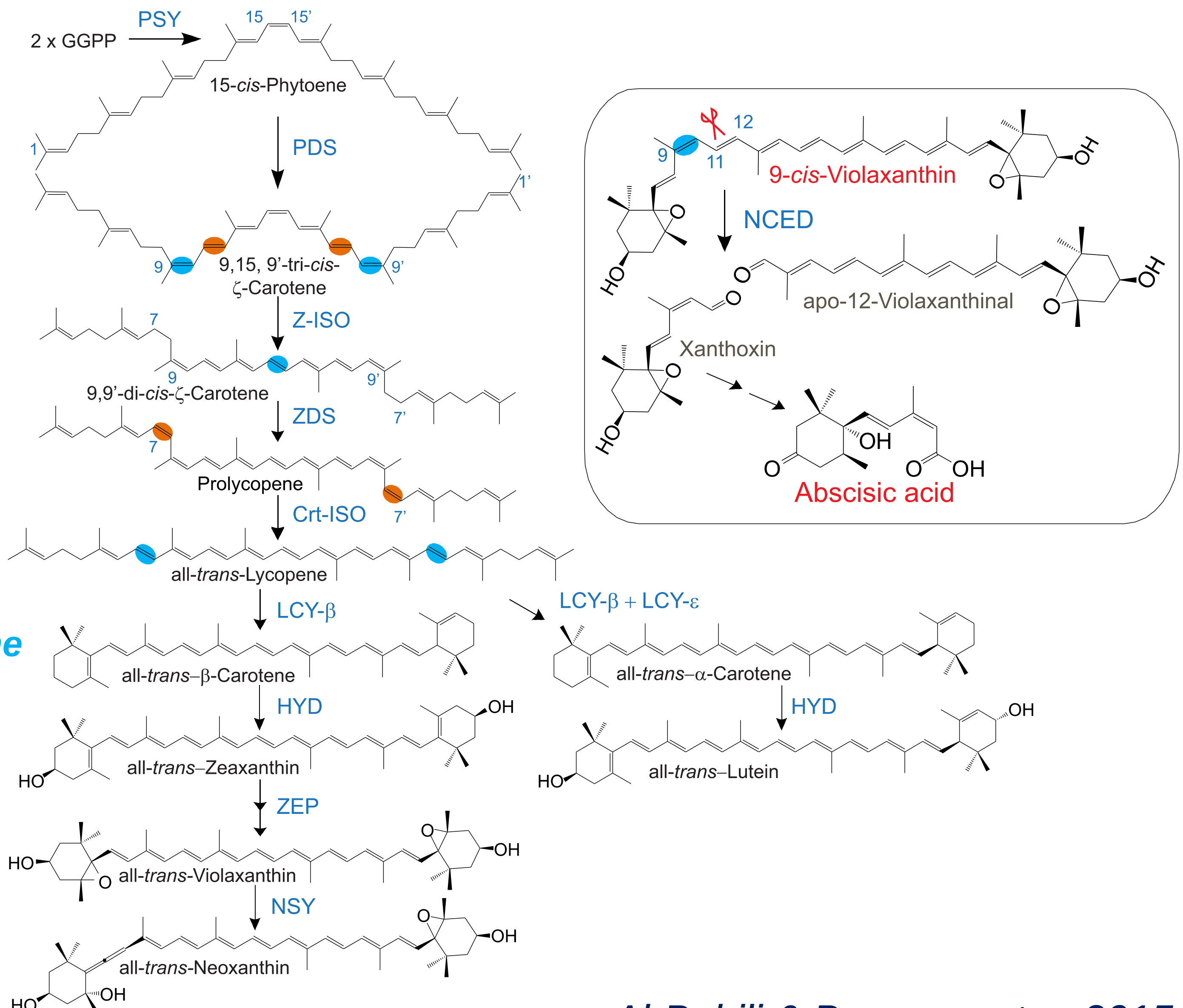
- ✓ Investigating SL biosynthesis and release
- ✓ Characterizing two contrasting lines

Consortium with:
Tada Asami, University of Tokyo
Binne Zwanenburg, Radboud University
Djibril Yonli, INERA, Burkina Faso

Plant Carotenoid Biosynthesis

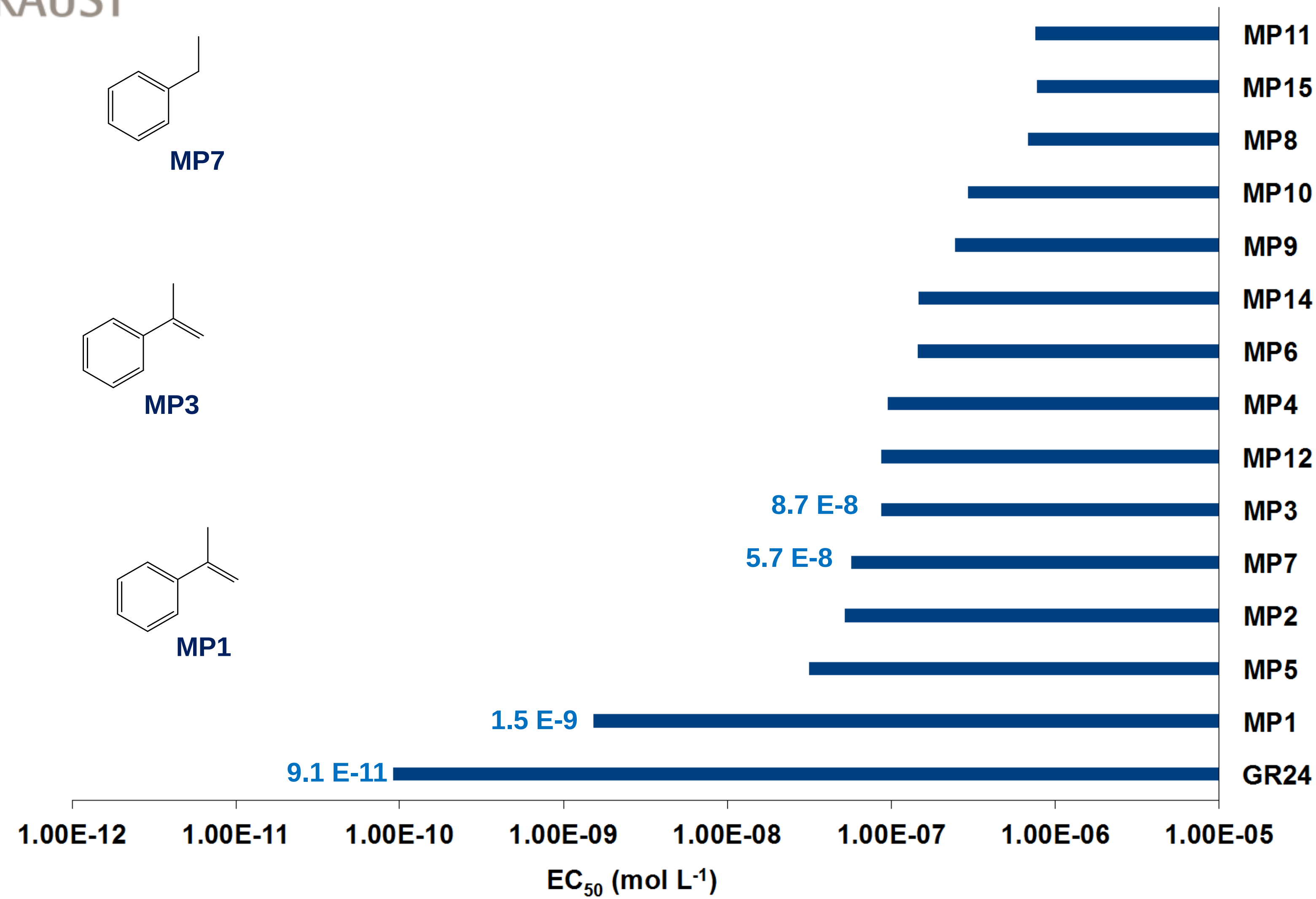
cis-Carotenoids Intermediates

Different β-Carotene cis-isomers



Al-Babili & Bouwmeester, 2015

S. hermonthica Germination Activity



EC50 (Half Maximal Effective Concentration), mean of n=3

Jamil et al. In preparation

How to Make Strigolactones?

- *Biosynthesis in plastids and cytoplasm*
- *Four biosynthetic enzymes*
- *CCD7, CCD8*
- *DWARF27 (D27), unknown catalytic properties*
- *A cytosolic cytochrome P_{450} (MAX1)*