Brassinosteroids: A Plant Steroid hormones

ASPEE College of Horticulture and Forestry

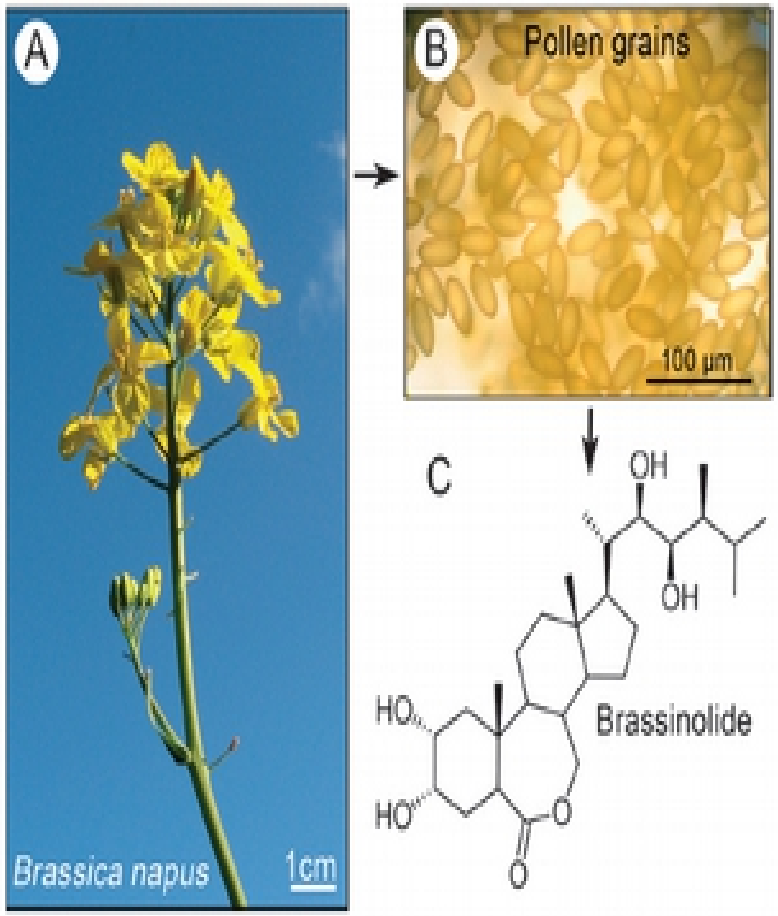
Discovery :

- Recognition that certain pollen extracts cause growth promotion.

- J. W. Mitchell et. al. (1970) screened pollen extracts from nearly 60 species and about half of them caused growth of bean seedling.

- The greatest growth stimulating activity was found in organic solvent extract of pollen from the rape (Brassica napus L.) so the named brassins.

Nine years later, Grove et al. (1979) isolated from 227 kg of bee collected rape pollen was processed and purify 10 mg of the most bioactive crystalline material which was identified as (22R,23R,24S)-2a,3a,22,23-tetrahydroxy- 24-methyl-B-homo-7-oxa-5a-cholestan-6-one and called **BRASSINOLIDE (BL)**

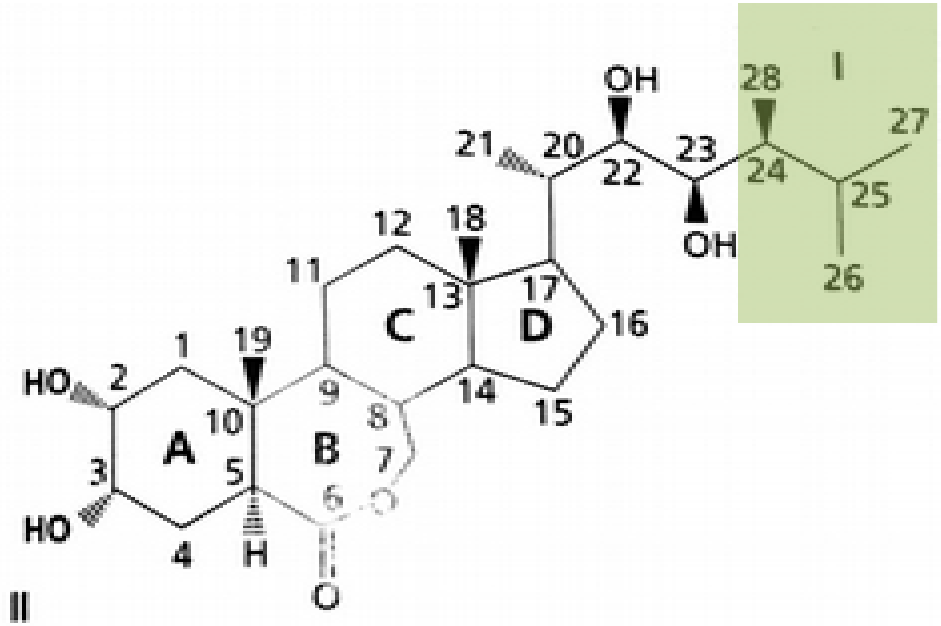


- 1982, Another steroidal substance with growth promoting nature was isolated from insect galls of chestnut (Castenea crenata), named as castasterone. (Precursor of BR)

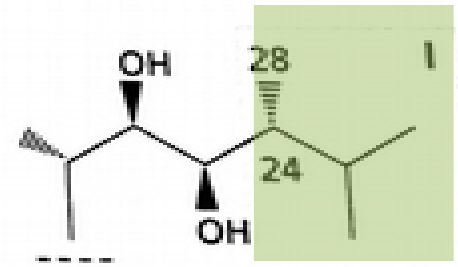
STRUCTURE :

-About 60 related compounds are identified.

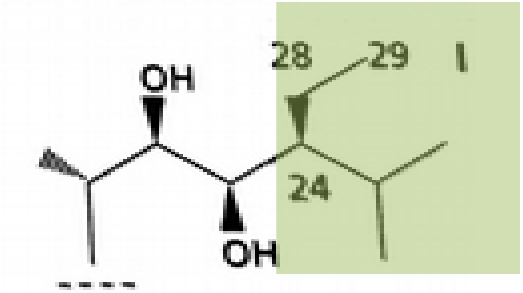
- The direct precursor of BL, castasterone (CS), has weak BR activity.



Brassinolode (BL)



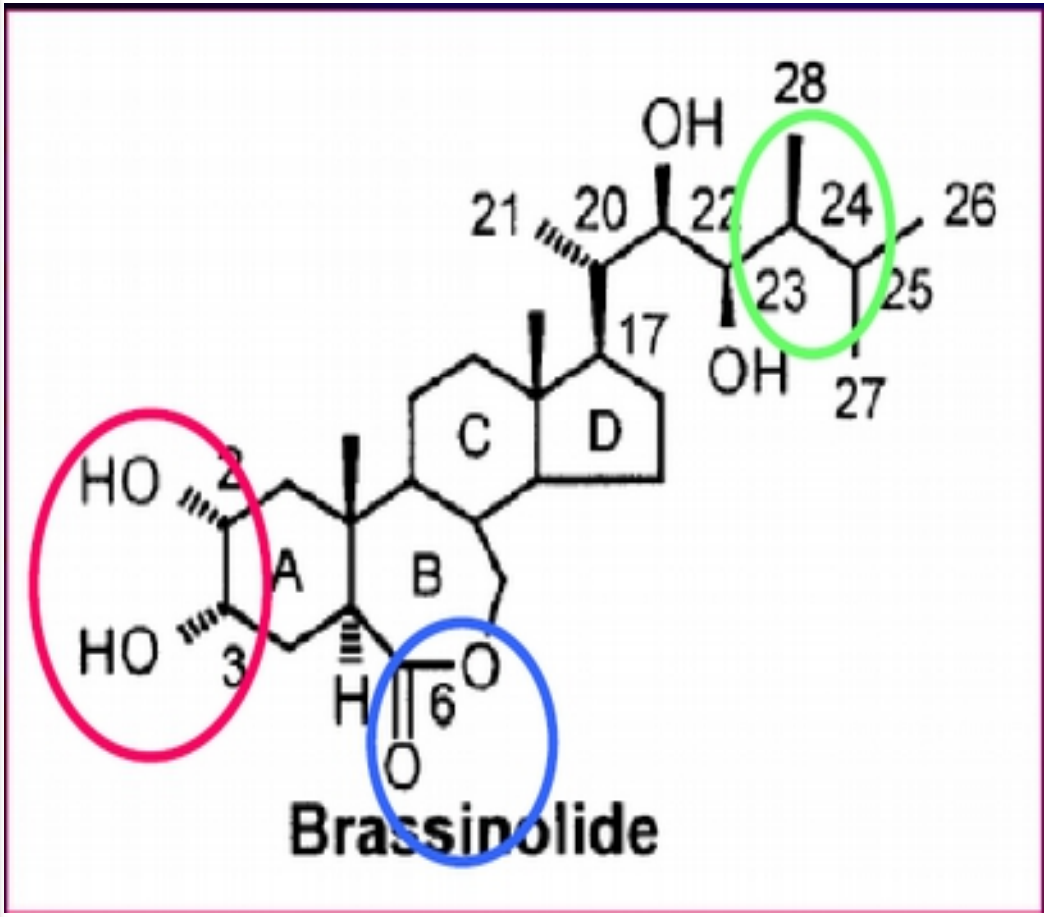
24-epibrassinolide



28-homobrassinolide

24-epiBL synthesized more cheaply, so very often used in experiments, however, only 10% active as BL

Structure and activity relationship:

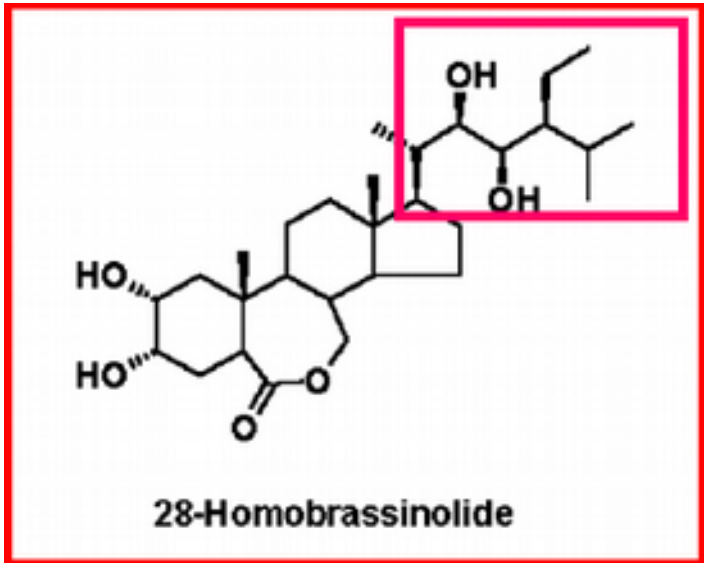


A Ring: Absence of single hydroxyl group at either C-2 or C-3 results in a significant loss of function.

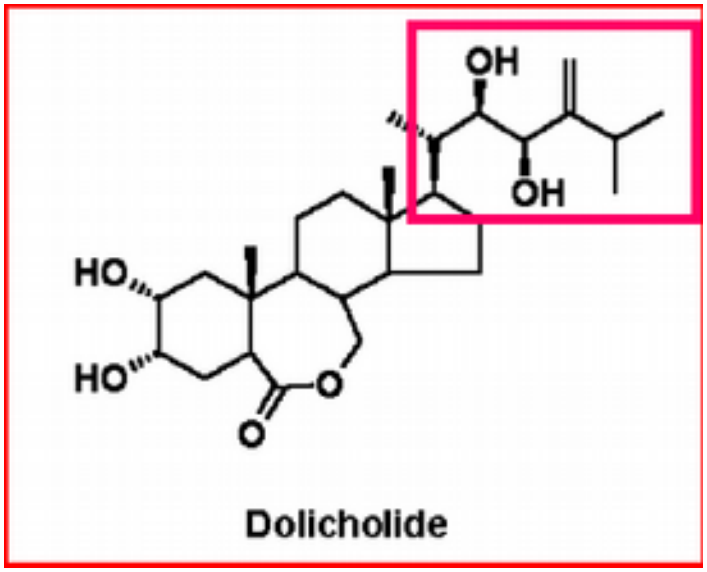
Limited modification in B ring don't eliminate activity.

The hydroxyls on the steroid side chain at C-22 and C-23 are both essential for activity.

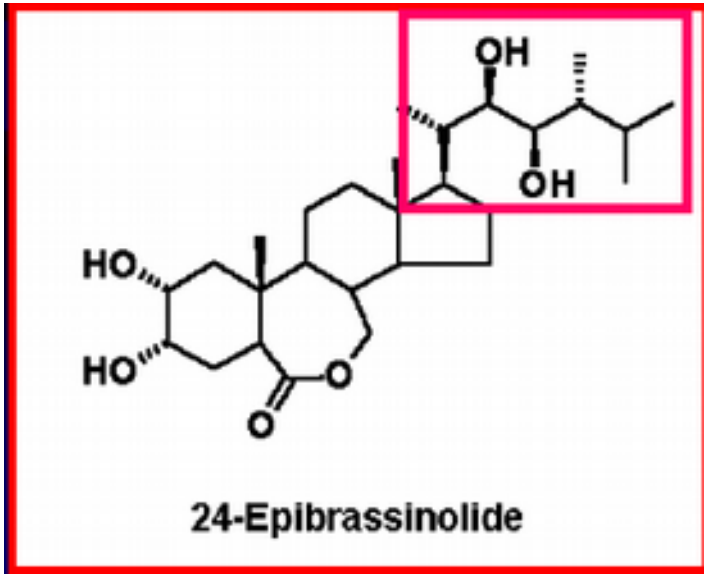
Variations are present in C-24 and C-28 but do not significantly affect activity.



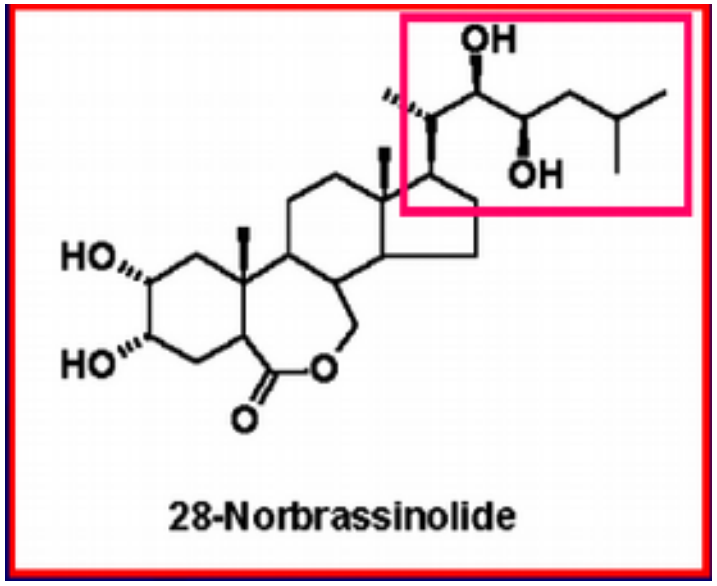
28-Homobrassinolide



Dolicholide



24-Epibrassinolide

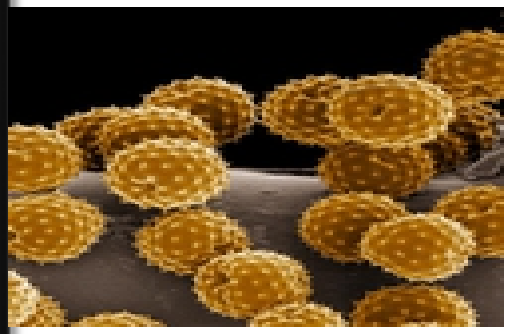


28-Norbrassinolide

Distribution of Brassinosteroids :

Plant part	Plant species
Pollen	*Helianthus annuus, Alnus glutinosa, Brassica napus, Robinia pseudo-acacia, Vicia faba, Fagopyrum esculentum, Citrus unshiu, Citrus sinensis, Cupresus arizonica, Pinus thunbergii, Cryptimeria japonica*
Seed	*Gypsophili perfoliata, Beta vulgaris, Pharbitis purpurea, Brassica campestris, Raphanus sativus, Cassia tora, Lablab purpreus, Orinthopus sativus, Phaseolus vulgaris, Pisum sativum, Vicia faba, Cannabinus sativa, Apium graveolens*
Shoot	*Arabidopsis thaliana, Ornithopus sativus, Pisum sativum, Lycopersicon esculentum*
Leaf	*Castanea crenata, Distylium recemosus, Thea sinensis*
Others	
Cultured cell	*Catharanthus roseus*
Panicle	*Rheum rhabarum*
Cambial region	*Cryptomeria japonica*
Gall	*Castanea crenata*
Strobilus	*Equisetum arvense*
Thallus	*Hydrodictyon reticulatum*

Content of BR in plants:



Pollen 5-1000 ng g FW -1

Seeds: 0.3-1600 ng g FW -1

Seeds of wheat
Brassinolide 0.127 ng g FW -1
Castasterone 0.159 ng g FW -1
24-epicastasterone 0.535 ng g FW -1

Fruits: 0.2-3.5 ng g FW -1

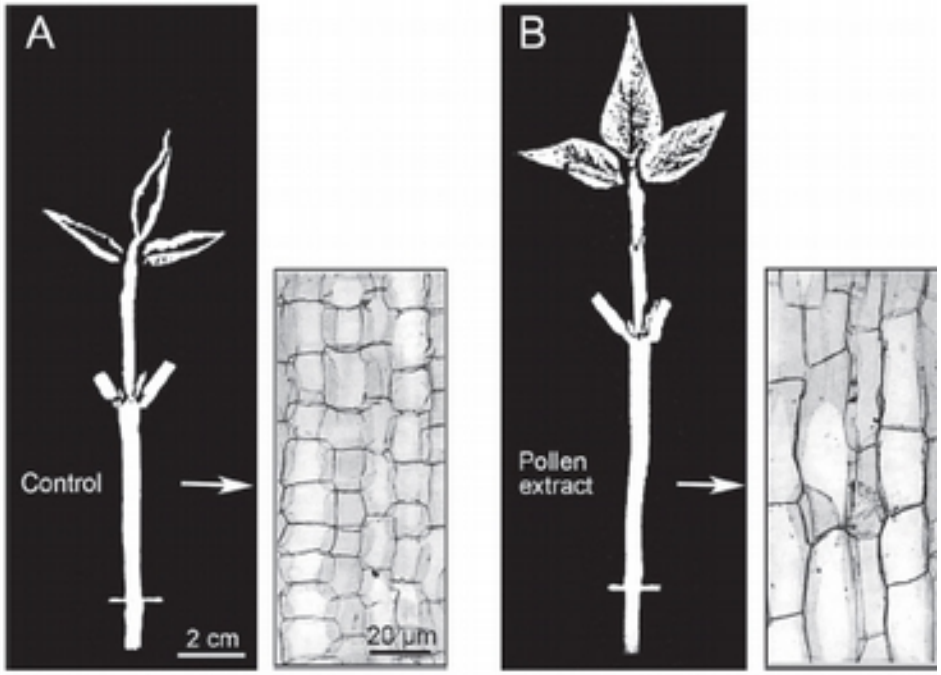
(Symons et al. 2008 & Janeczko et al. 2010)

Bioassay:

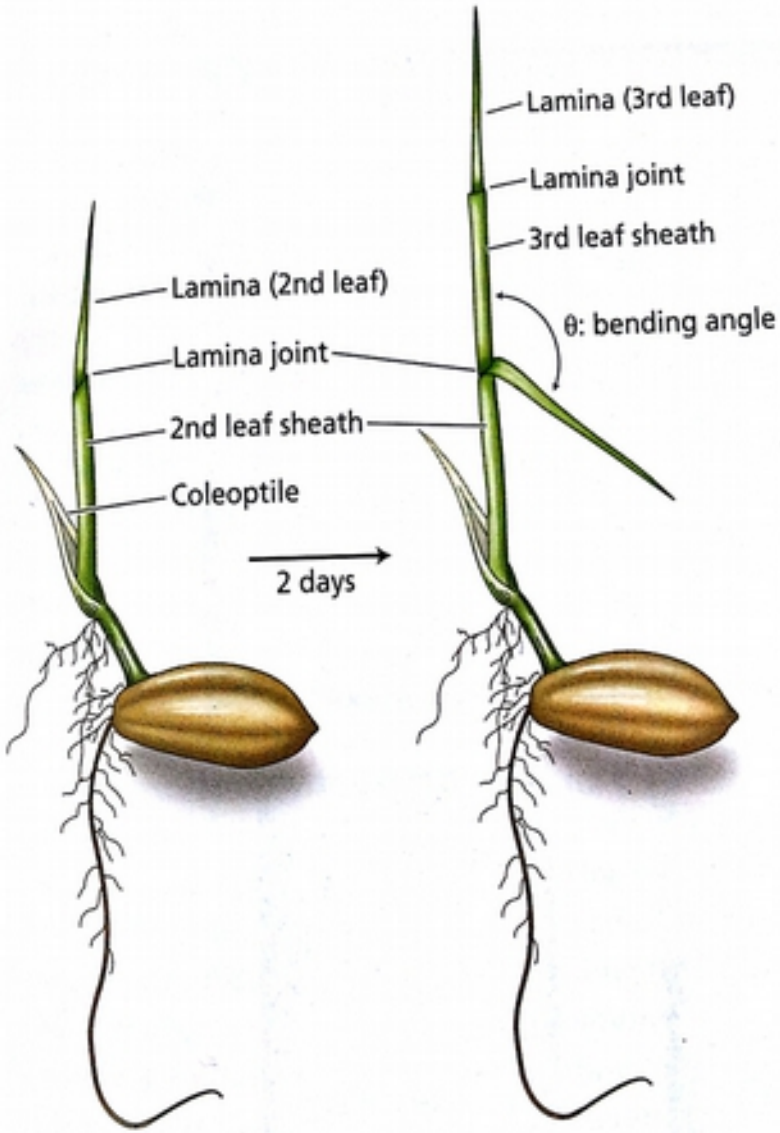
Increasing brassinosteroid concentration →

Excised sections from bean plants were floated on solutions containing increased conc. of BRs.

BR induce elongation, growth, higher conc. Result in stem thickening, bending and splitting.

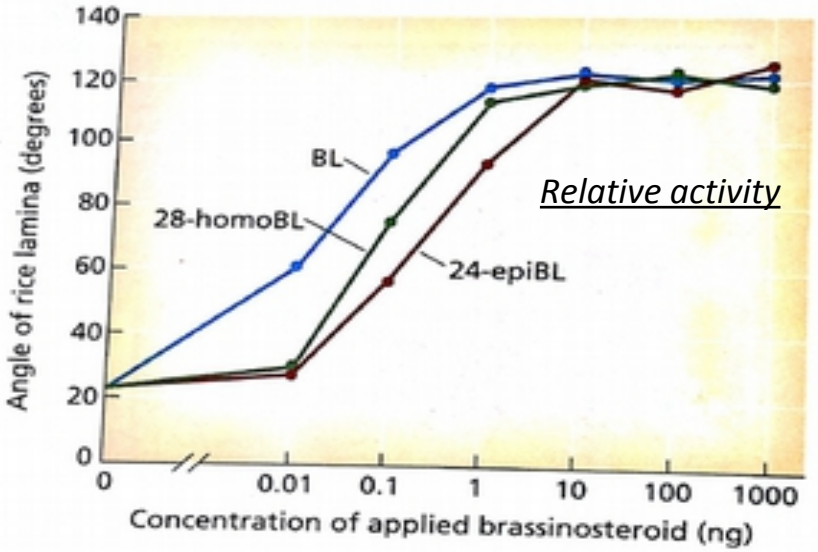


The discovery of growth-promoting substances from pollen extracts of maize (Zea mays) plants using the bean (Phaseolus vulgaris) second internode bioassay. Control experiment (A) and effect of pollen extract on stem- and cell elongation in light-grown pinto beans (B) (adapted from Mitchell and Whitehead, 1941. Response of vegetative parts of plants following application of extracts of pollen from Zea mays. Botanical Gazette 102, 770-791



Dwarf rice lamina inclination bioassay for BRs.

A small droplets of sample dissolved in ethanol is applied to the joint between the lamina and the leaf sheath. After incubation for 2 days in high humidity, the bending angle between the lamina and the leaf sheath is measured. The angle is proportional to the amount of BR in the sample.

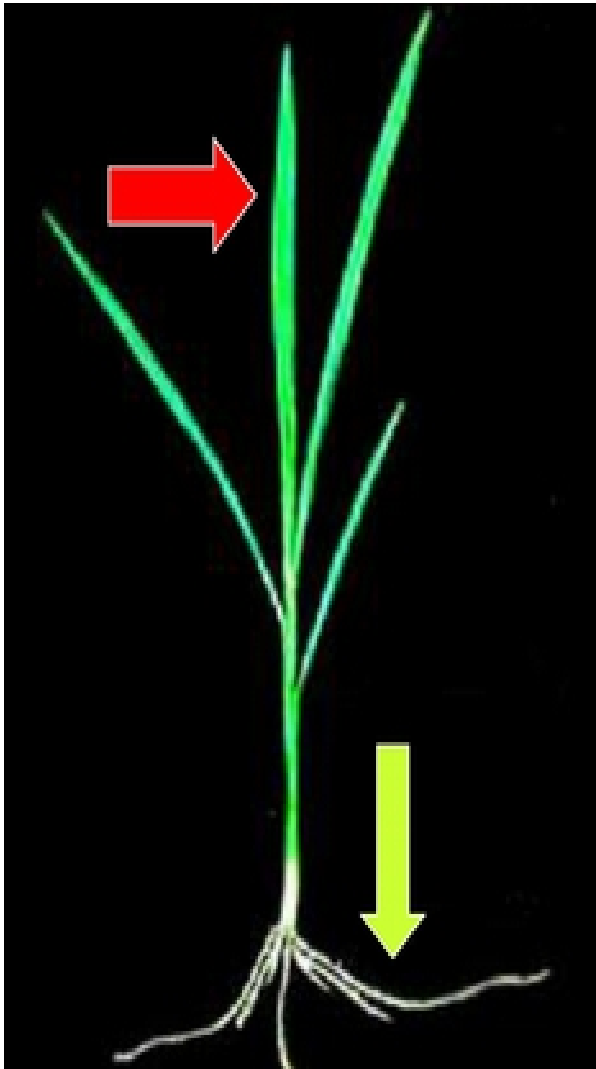


TRANSPORT OF BRS

Widespread distribution of endogenous BR throughout the plant raises the question of whether BR are synthesized in a localized region of the plant and then transported to distant sites of action.

Do BR undergo long-distance transport?

Studies of root uptake and transport of exogenous BR.



ROOT application:

Experiment with radioactive BR made by Yokota et al. (1992) showed that after application of [3H] BR to roots of rice – about 10% of the radioactivity was taken up by the plant and found in the shoot.

The content of 24-epibrassinolide [ng g FW -1] in the aerial part of 10-d-old wheat seedlings after application of 24-epibrassinolide by drenching of 3-d-old plants.

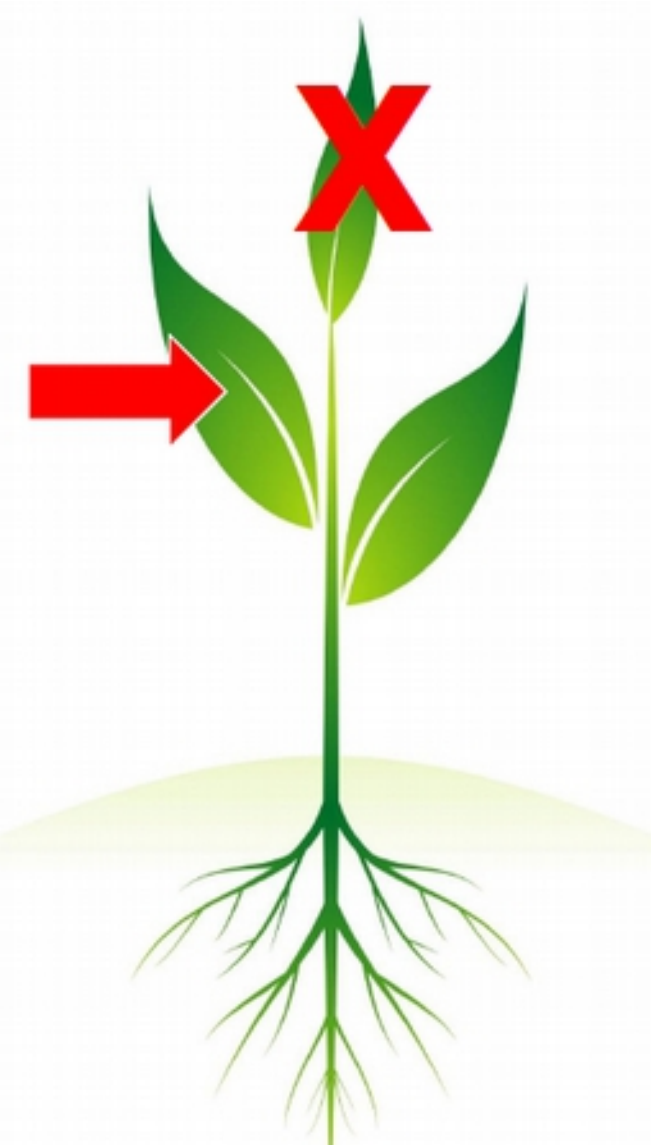
Data marked with the same letter are not significantly different according to the Duncan test at $P < 0.05$.

	Applied: 24-epiBL [μM]	**Detected:** 24-epiBL
Drenching	0.0	0.258 a
	0.1	0.696 b
	2.0	1.109 b

Janeczko & Swaczynová (2010)

FOLIAR APPLICATION

Radioactive BR applied to mature leaves or apical bud of pea did not move beyond the application site (Symons and Reid 2004).



This is consist with data Janeczko & Swaczynová (2010) 24-epibrassinolide applied to second leaf of wheat was not detected in third newly developed leaf.

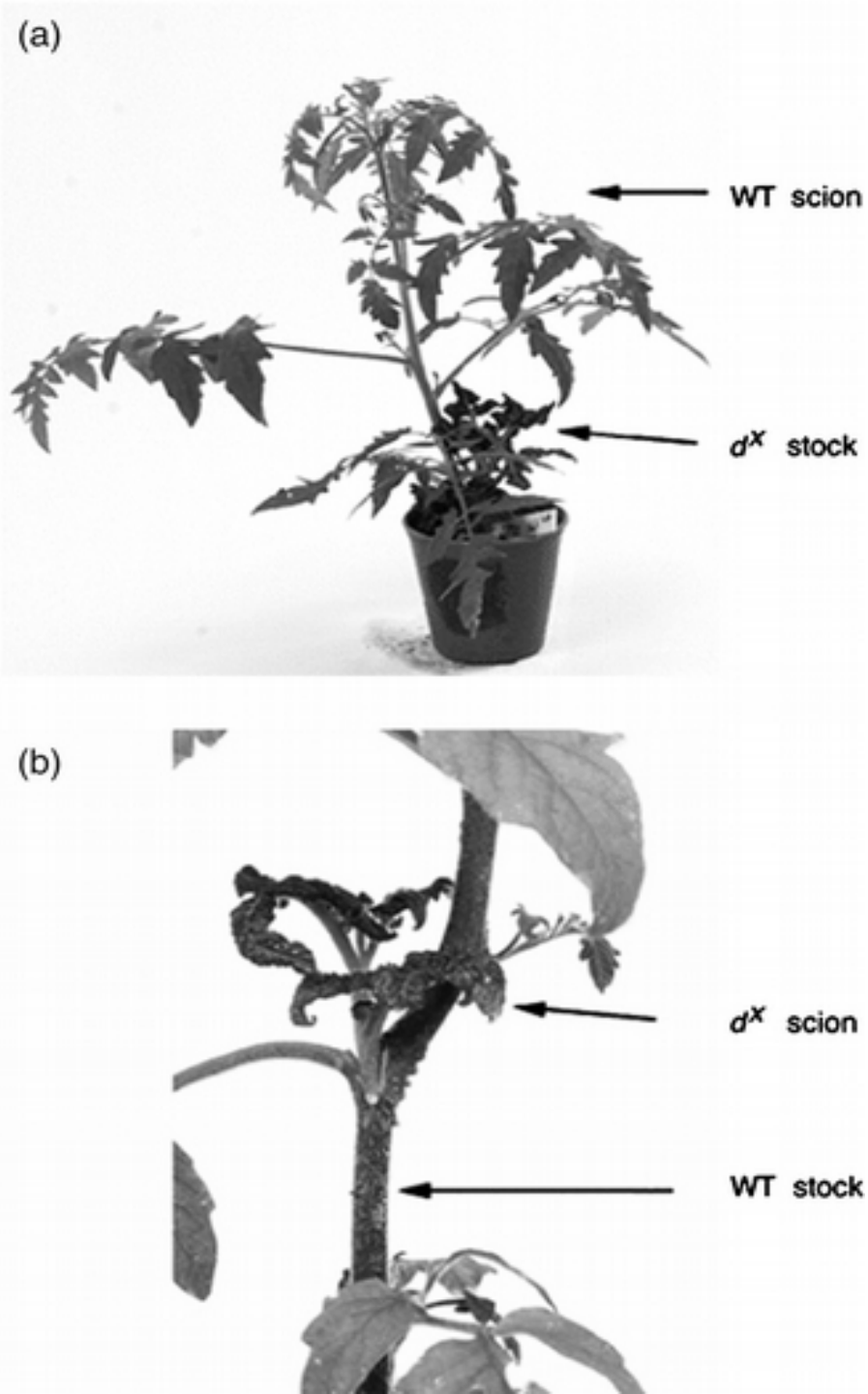
24-epibrassinolide applied via spraying are also not accumulated in formed seeds (Janeczko et al. 2010)

These results suggest that exogenous BRs are readily translocated from root to the shoot, but are poorly translocated out of leaves.

ENDOGENOUS BRS DO NOT SEEM TO UNDERGO ROOT TO SHOOT TRANSLOCATION.

Lkb pea mutant – dwarf Phenotype,
Blocked of synthesis of campesterol from 24-methylenecholesterol

Symons G M et al. J. Exp. Bot. 2007;59:17-24



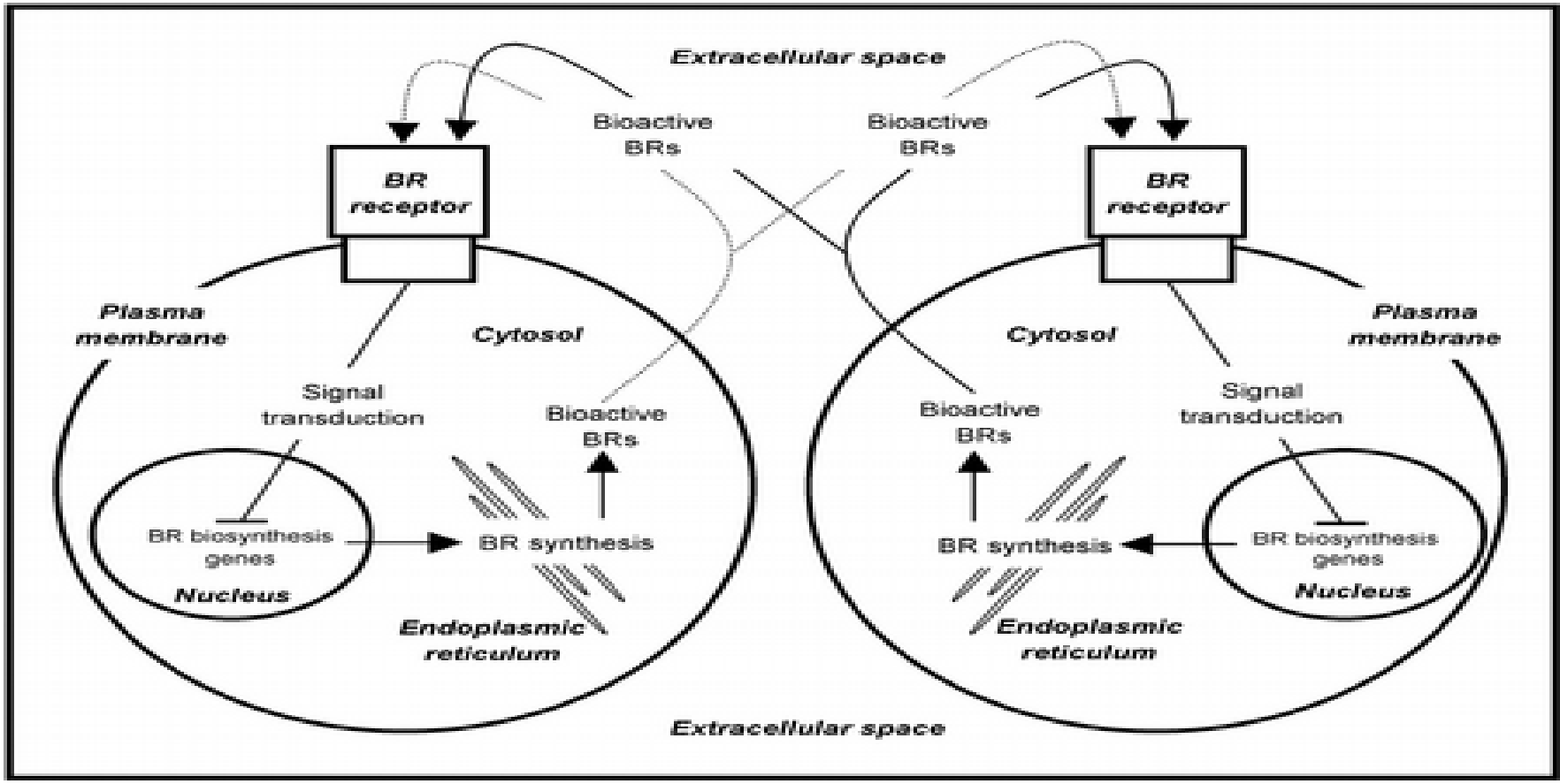
Grafting of brassinosteroid-deficient mutant , dx and WT tomato plants.

Both the experiment indicate lack of acropetal and basipetal movement

BRs are present in all plant organs, although different intermediates predominant in different organ.

These results suggest that
(1)Endogenous BRs act locally at or near their sites of synthesis and
(2) Each organ synthesizes and responds to its own active BRs.

A proposed model of short-distance BR transport within and between neighbouring cells.

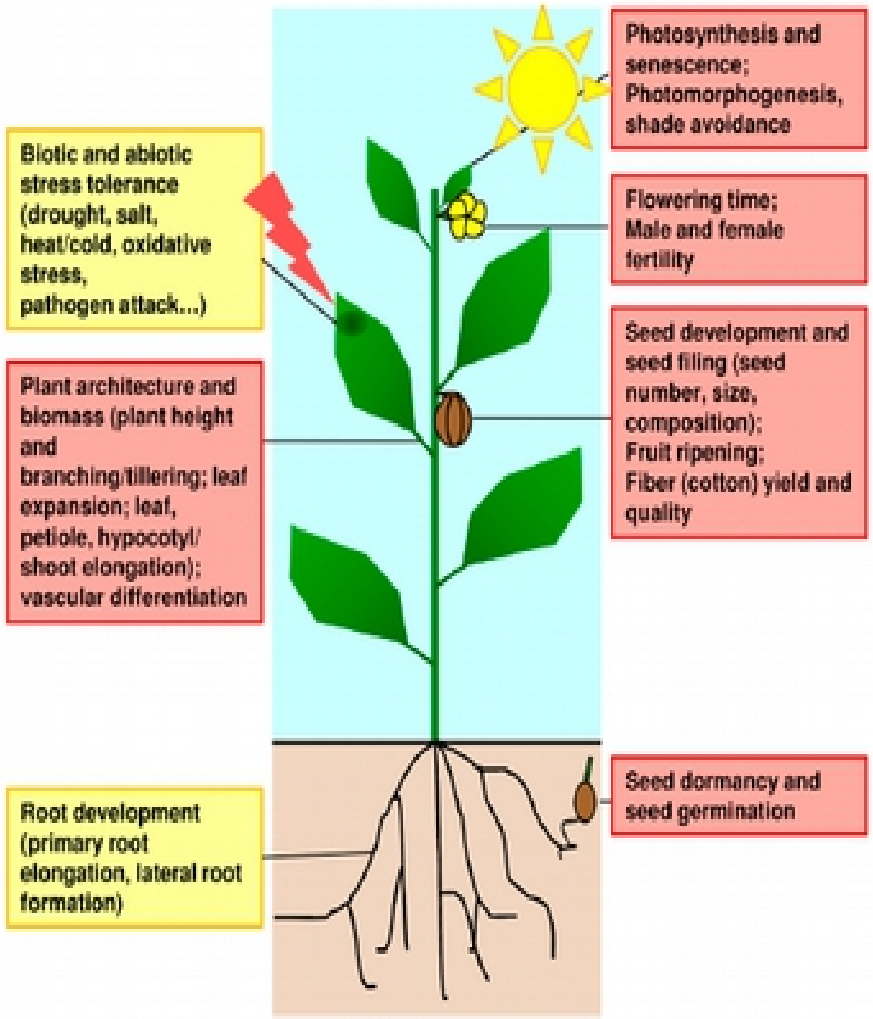


Since BRs are synthesized within the cell, it appears that they are transported, actively or passively, out of the cell, to interact subsequently with the BR receptor at the surface of the same, or of a neighbouring, cell . This would allow similar cells within that tissue effectively to communicate with each other regarding their BR status, to act together to regulate BR levels, and all to respond in a co-ordinated manner to the BR signal.

Symons G M et al. J. Exp. Bot. 2007;59:17-24

Brassinosteroids: Effect on Growth and Development:

- *Fiber development in cotton*
- *Development of lateral roots*
- *Maintenance of apical dominance*
- *Vascular differentiation*
- *Pollen tube growth*
- *Plant defensce*
- *Seed germination*
- *Leaf senescence*



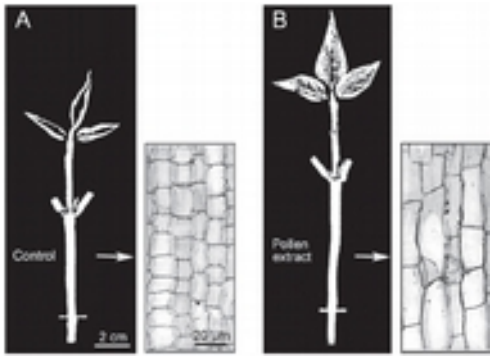
Actions of BRs in regulating Plant Development and Traits of Agronomic Importance in Model Plant Species and Crops.

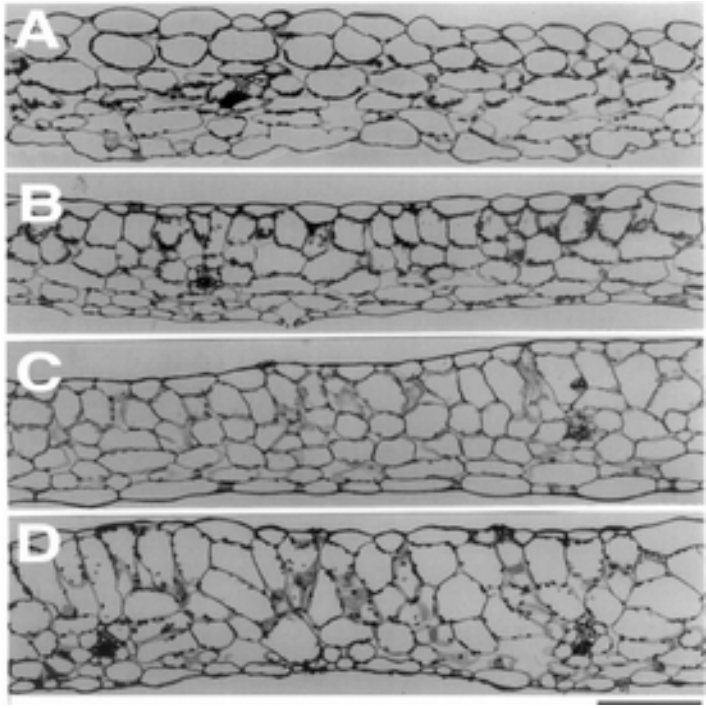
Positive effects of increased BR content are boxed in red. Mixed effects are boxed in orange. Does-dependent effects are observed on root development (positive effects at low concentration and negative effects at high concentration).

BR PROMOTE BOTH CELL EXPANSION AND CELL DIVISION IN SHOOTS:

Bean second internodes
Rice leaf lamina inclination

In genetic studies, leaves of BR deficient mutants showed not only smaller cell size but also fewer cells in the leaf blade.

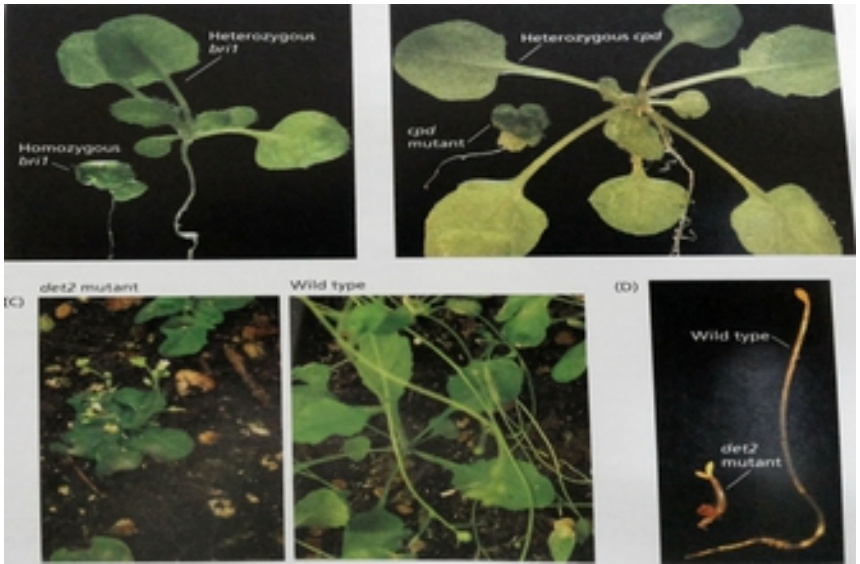




(A) Wild type; (B) dwf1-5 mutant; (C) det2-13 mutant; (D) det2-18 mutant. Bar = 100 µm.

Nakaya M et al. Plant Cell Physiol 2002;43:239-244

Phenotypes of Arabidopsis BR mutants

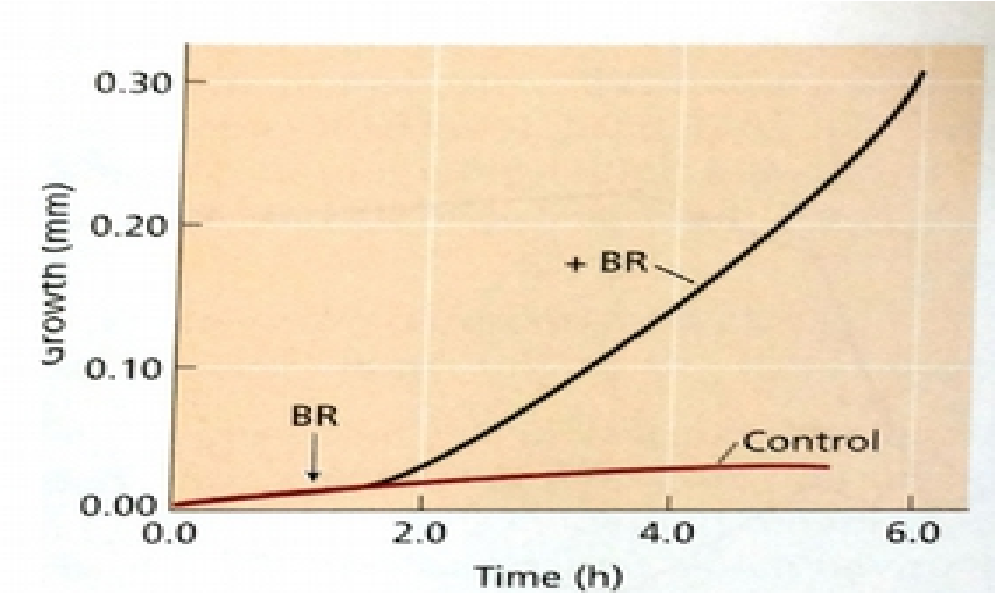


Arabidopsis WT and over expressing BT biosynthesis gene (*DWF4*)

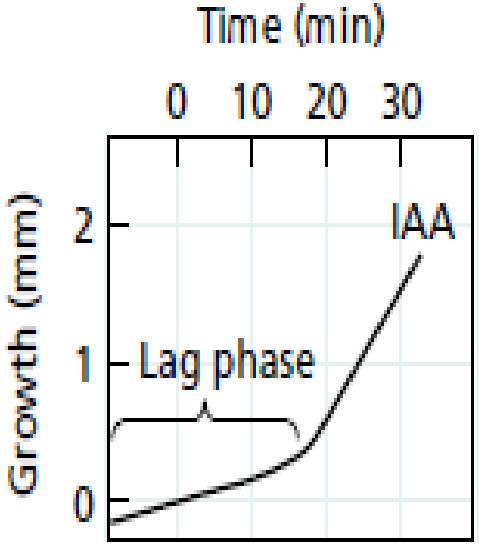
Reduced male fertility, and deetiolation in darkness (typical phenotypes of plants defective in BR biosynthesis)

These studies indicate that BR promote both cell elongation and cell proliferation

The kinetics of cell expansion in response to BR is differ from those of auxin induced



BL begins to enhance elongation rate after a 45 min lag period and reaches maximum only after several hours of treatment. In contrast, auxin stimulate elongation after 15 min and reaches maximum just after 45 min.

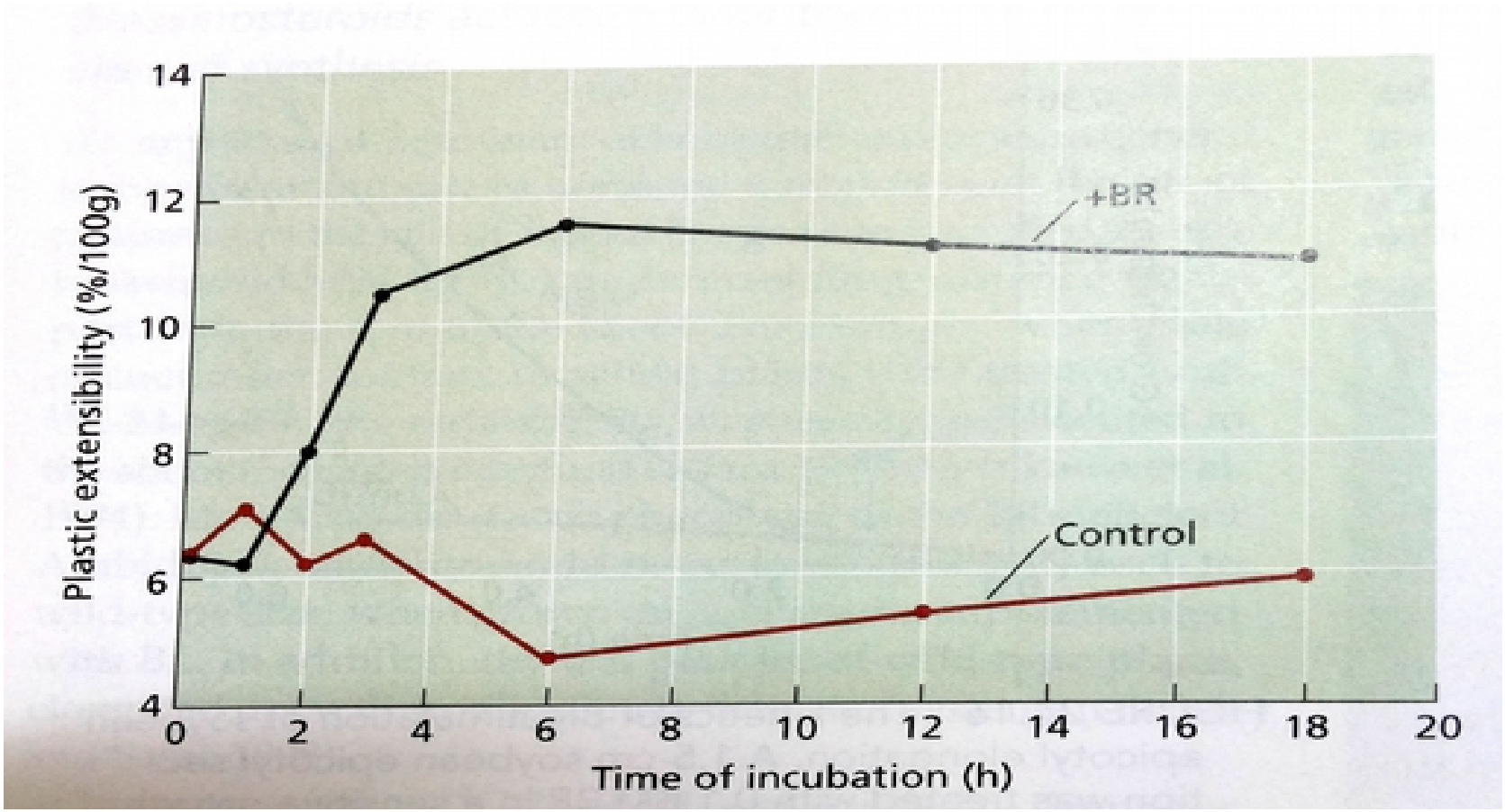


Auxin growth kinetics

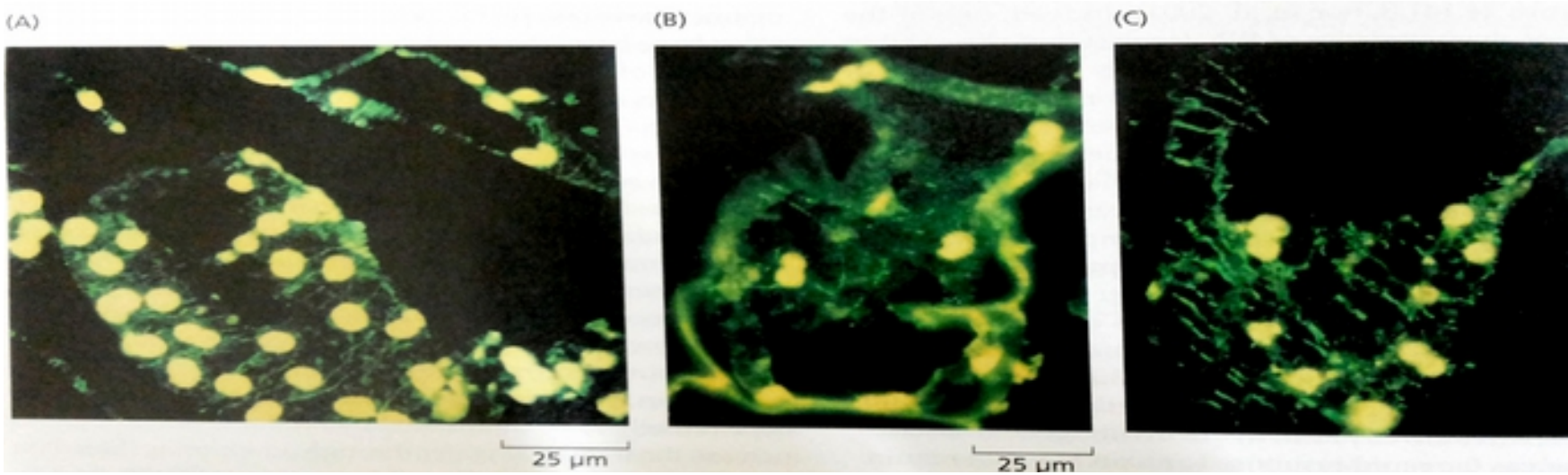
BR involves slower pathways involving gene transcription

BR are thought to increase the uptake of water through aquporins, enhance cell wall loosening and induce expression of xyloglucan endotransglycosylase/hydrolases (XTH) and expansins.

BR increases cell plastic extensibility.



Mutant cells contain very few microtubules and those present lack organization.

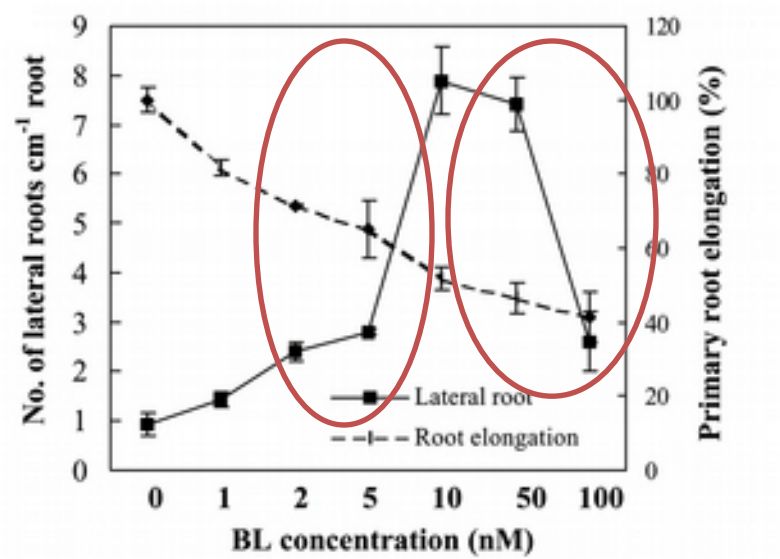


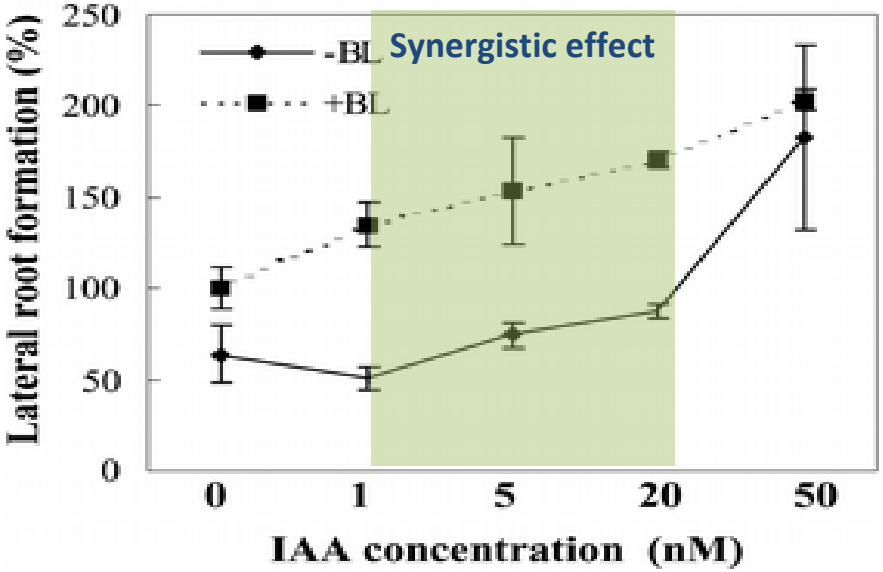
Microtubules in green
A.Wild type paranchyma
B. BR deficient mutantwith few nonaligned microtubules
C. BR deficient mutant treated with BR.

BR and cytokinin appear to regulate the cell cycle via similar mechanism.
24-epiBL can substitute for zeatin in growth of Arabidopsis callus and cell suspension culture.

BRs BOTH PROMOTE AND INHIBIT ROOT GROWTH:

- Response may have positive or negative effect depends on concentration (Low –Positive and Higher- Negative).
- Threshold concentration of inhibition depends on activity of BR analog used.





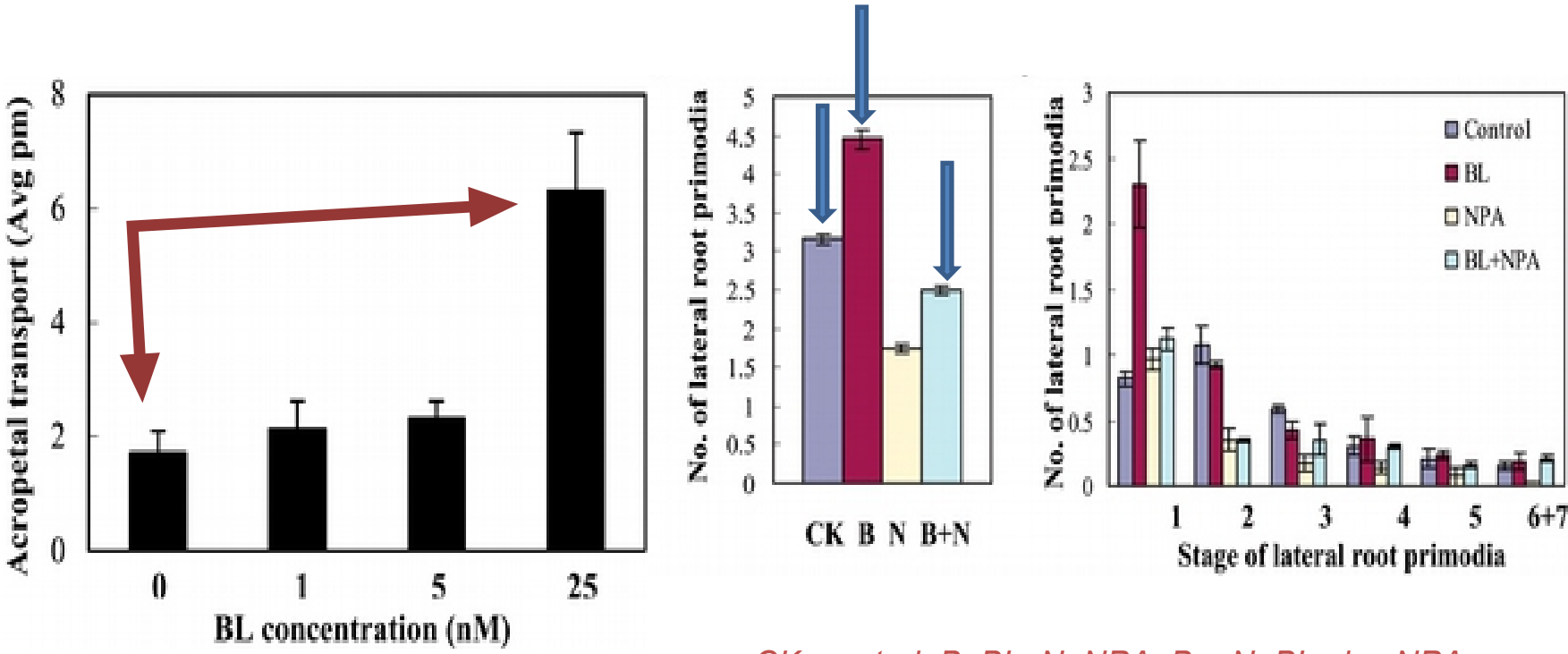
Arabidopsis seedling were grown vertically for 8 d on 1/2 MS plates containing 0, 1, 2, 5, 10, 50, and 100 nm BL, respectively.

Seedlings were grown vertically on agar plates containing 0,1,5,20 or 50 nM IAA with or without 1nM BL for 8 days. The number of lateral roots and visible lateral root primordia per centimetre of primary root were counted. And the percentage were calculated over number of lateral roots per centimetre in 1 nM BL, zero auxin treatment.

Bao F et al. Plant Physiol. 2004;134:1624-1631

- BR effect on root growth is independent from both auxin and GA..
- Auxin transport inhibitor does not prevent BR induced growth. And Br mutant not recovered by application of GA
- Higher concentration show inhibitory effect may due to higher ethylene.

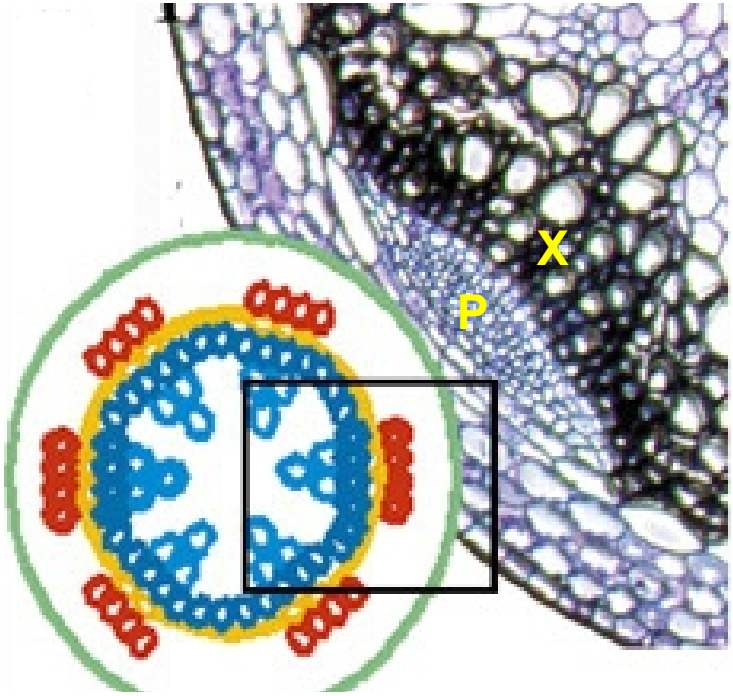
Current model suggests that BRs promote lateral root development partially by influencing polar auxin transport.



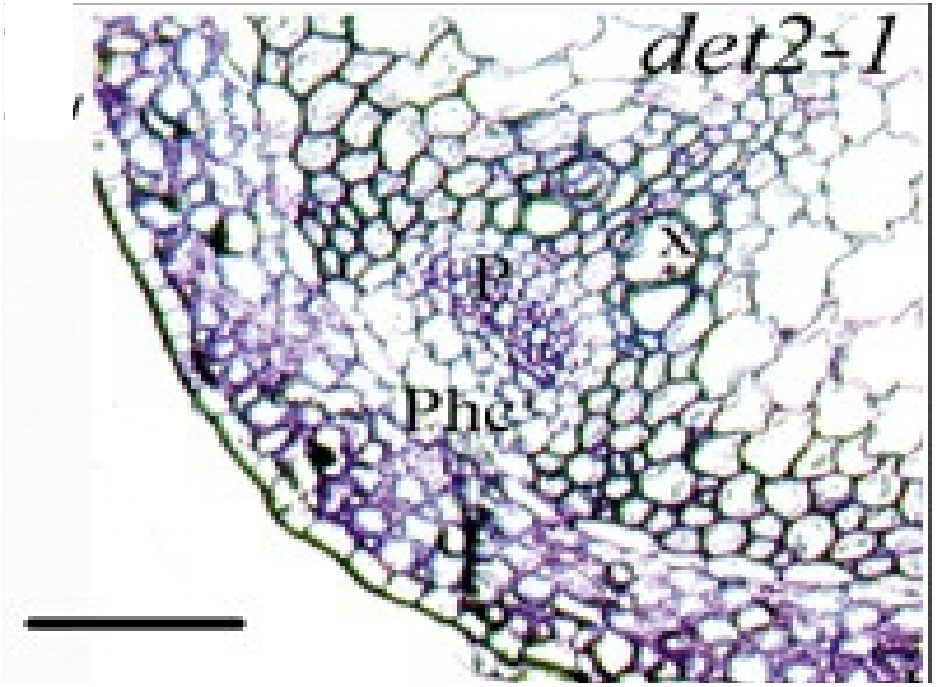
CK, control; B, BL; N, NPA; B + N, BL plus NPA.

BRs promote xylem differentiation during vascular development:

- BRs promoting differentiation of xylem and suppressing of phloem.



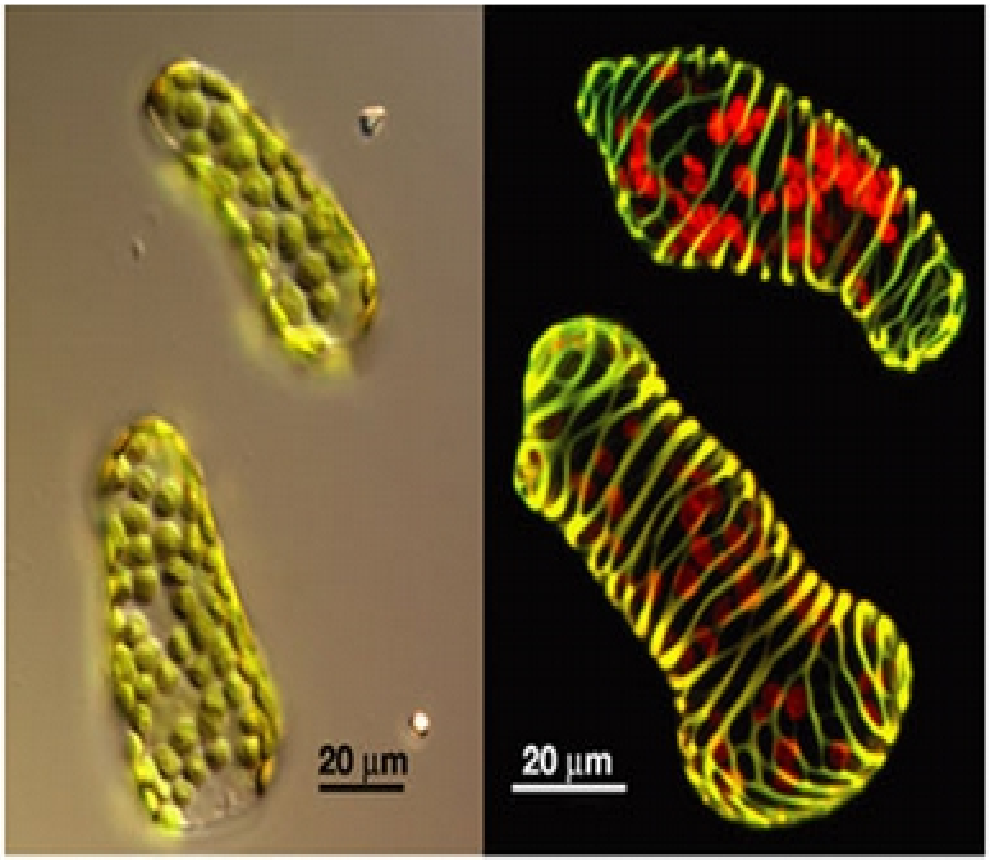
Wild type Arabidopsis stem cross section



Arabidopsis det-2 mutant stem cross section

The activity of the procambial cells (yellow) gives rise to phloem (red) tissue in the outer part and the xylem (blue) in the inner part of the vascular bundle. Pictures shown in this figure show the anatomy of a single vascular bundle (black box).
The vascular bundle of the BR deficient *det2* mutant has lower xylem to phloem ratio.

Caño-Delgado A et al. Development 2004;131:5341-5351



When single cell of zinnia (Zinnia elegans) cultured in liquid medium in dark, they differentiate into tracheary elements.

Measurements of BRs during xylem differentiation in this system have shown that BRs are actively synthesized and esssential for the differentiation.

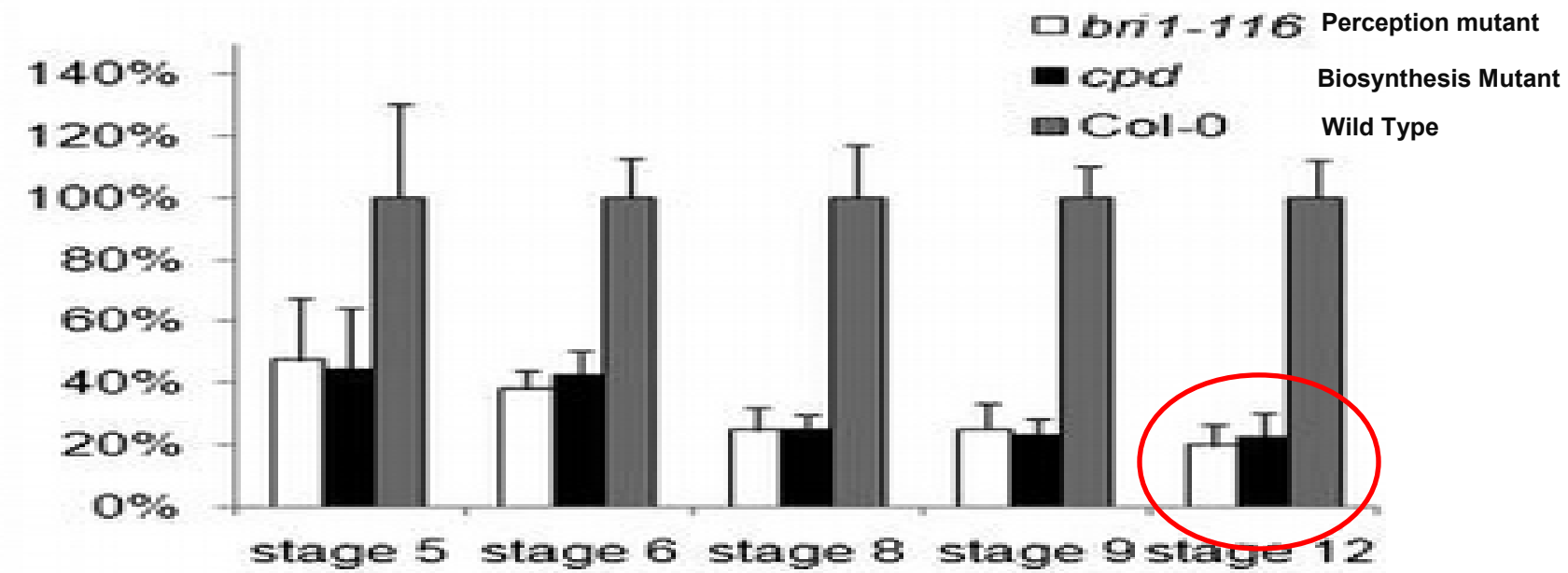
In vivo culture of zinnia leaf mesophyll cells before (left) and after differentiation into tracheary elements.

Nature Reviews Molecular Cell Biology **5**, 379-391 (May 2004)

BRs are required for the growth of pollen tubes

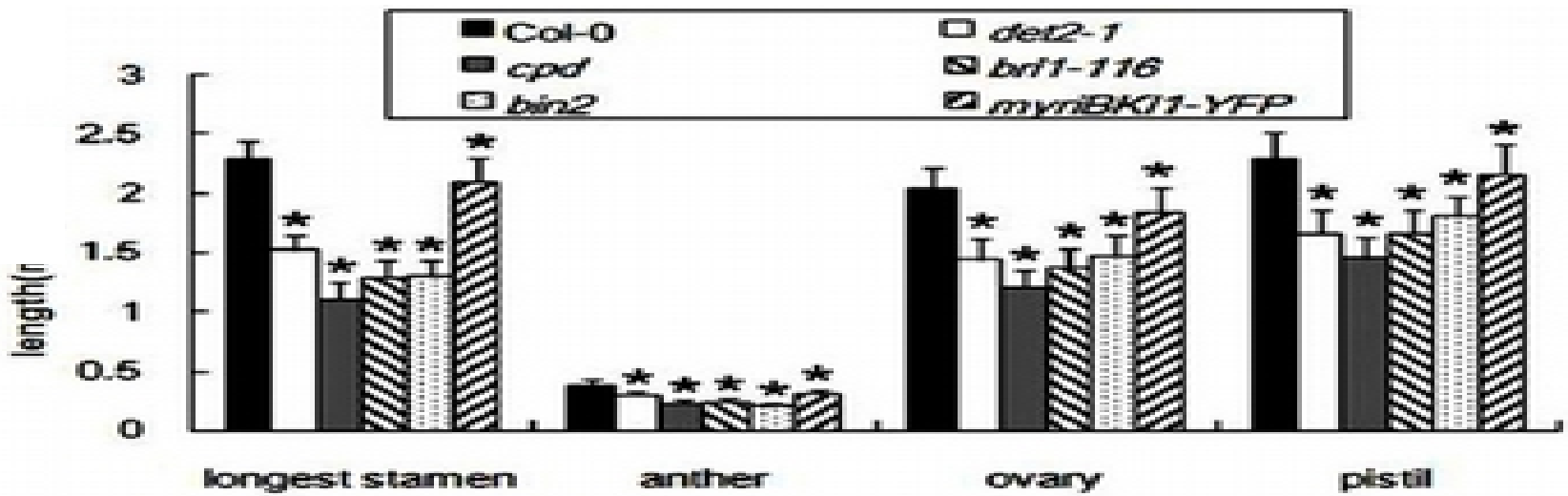
- As pollen are rich source of BRs, so not surprising that BRs are important for male fertility.
- Promote pollen tube growth.
- Pollen viability is slightly reduced.

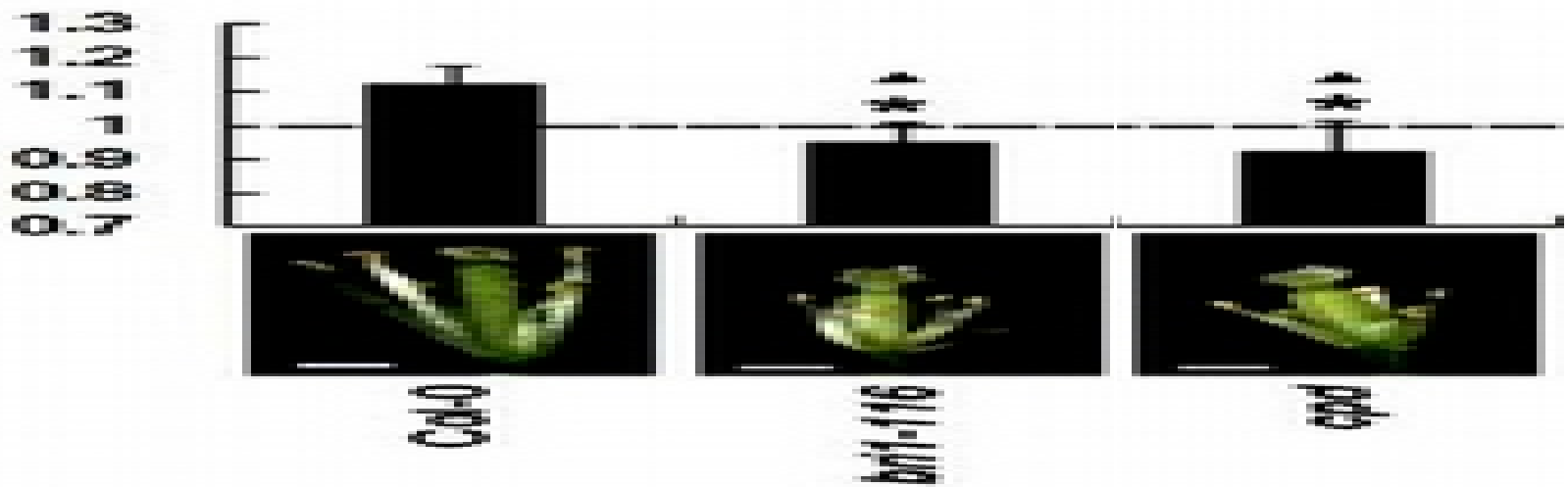
BRs control male fertility:



Ye Q et al. PNAS 2010;107:6100-6105

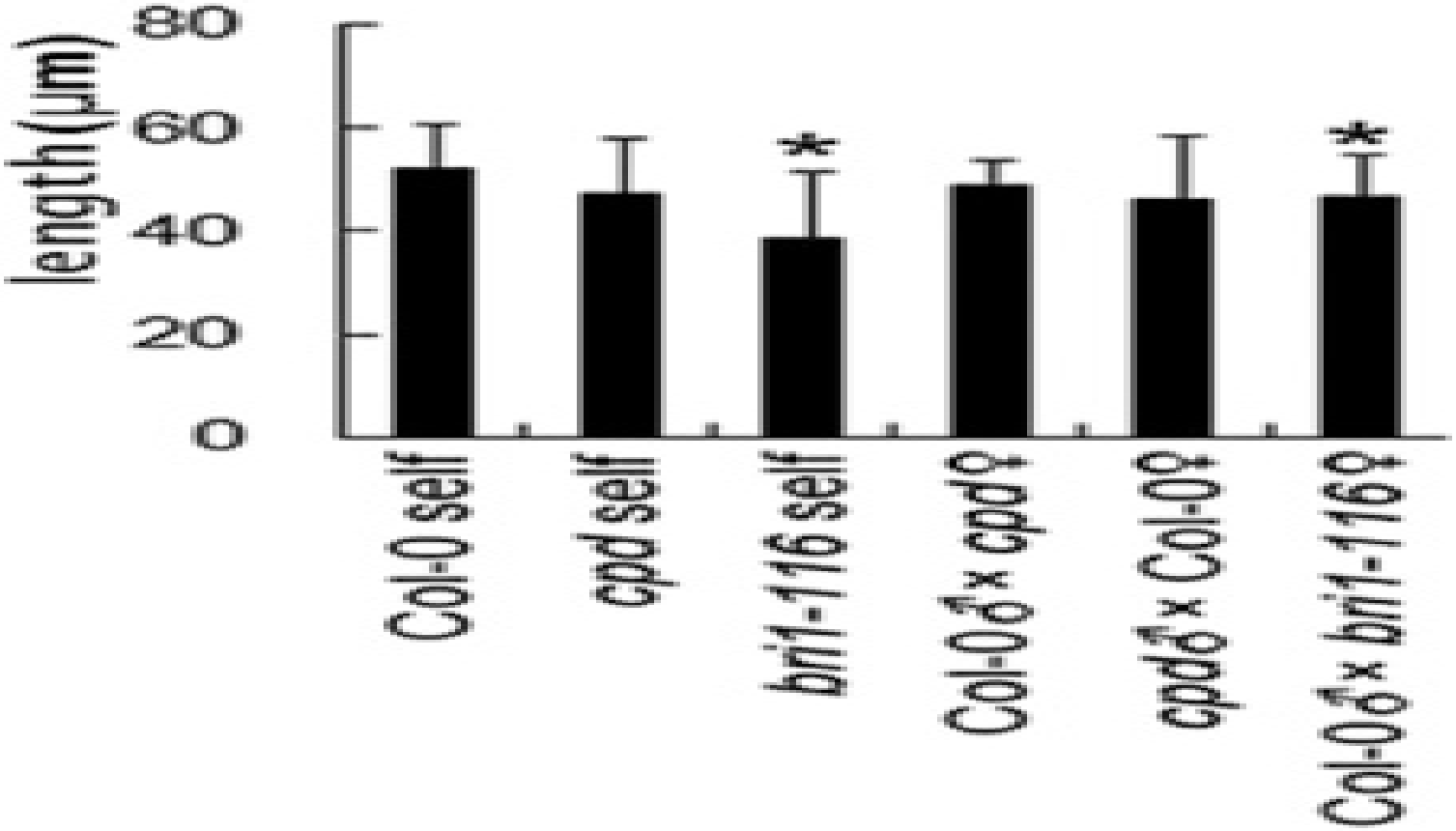
The length of male and female organs is significantly reduced in the brassinosteroids (BR)-related mutants





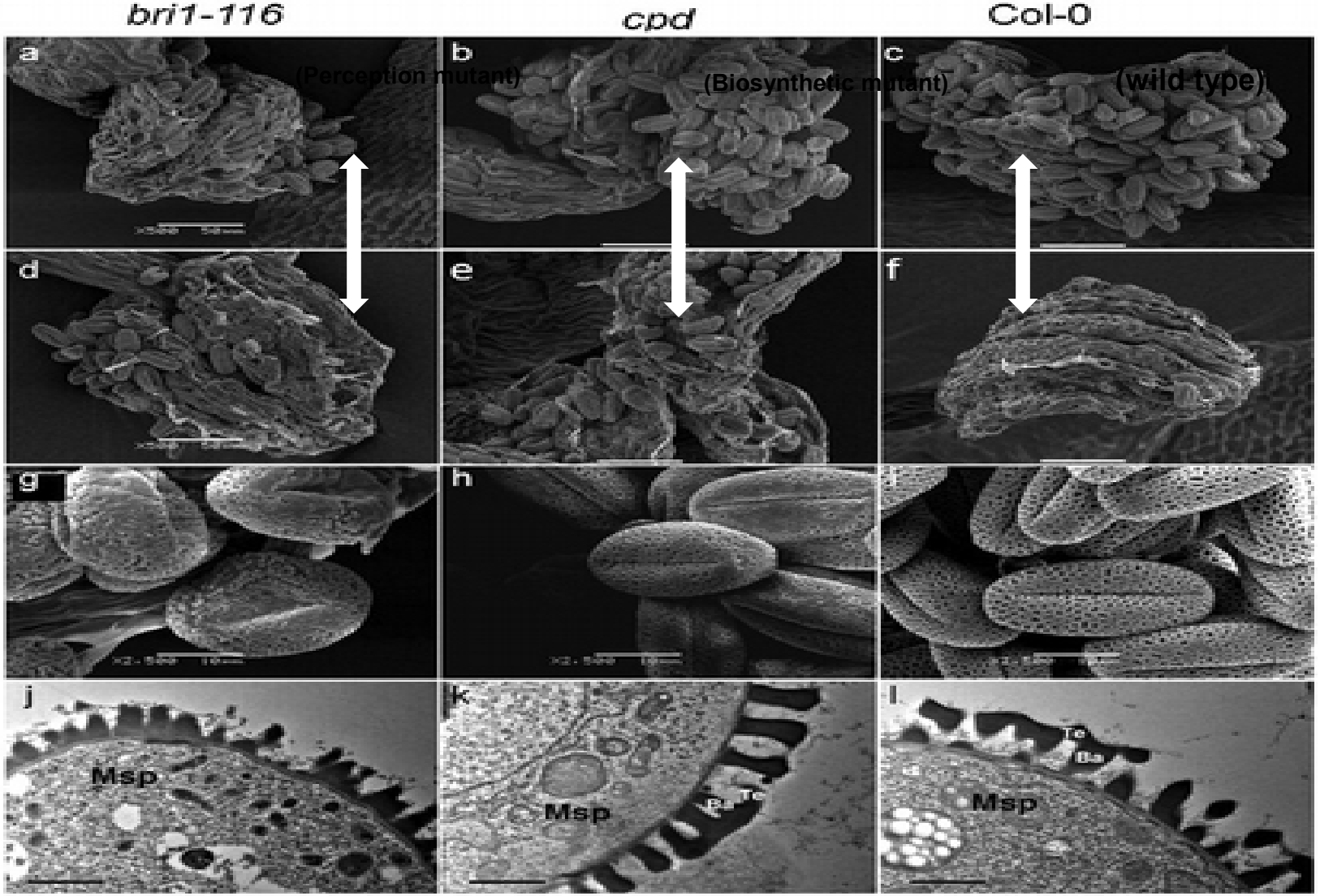
Ratio of the longest filament to the ovary in the same flower of the BR related mutants and the wild type.

Ye Q et al. PNAS 2010;107:6100-6105



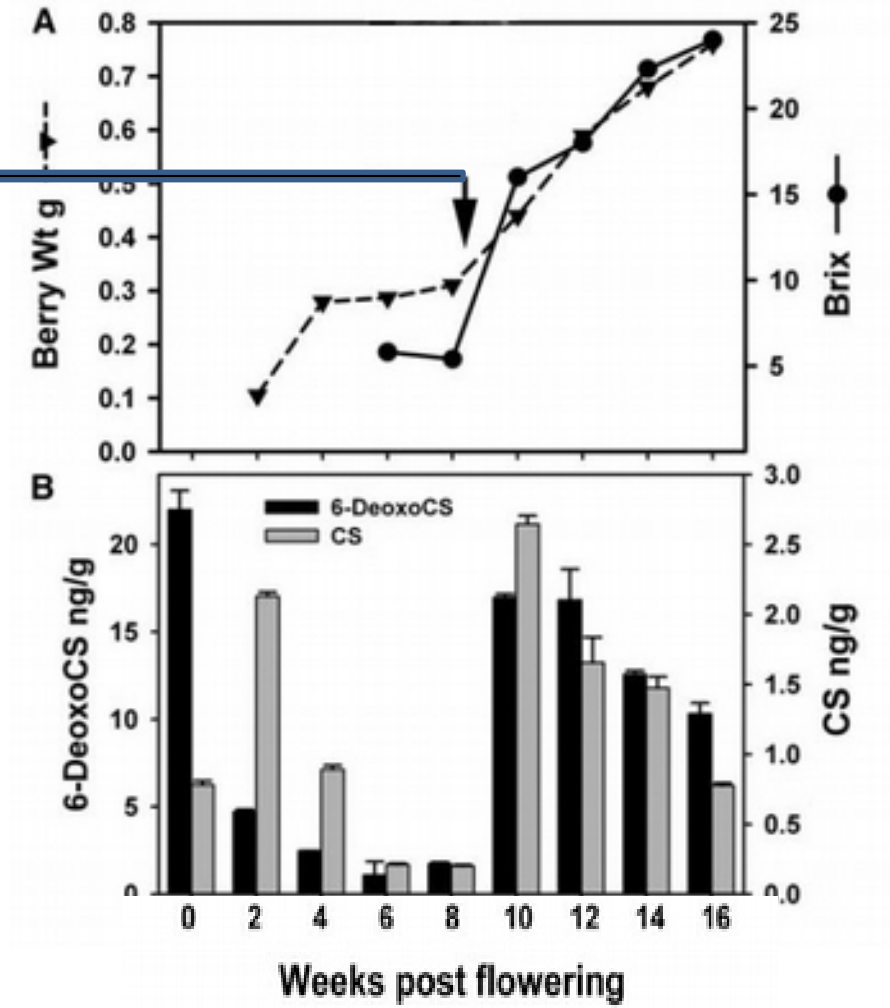
There was no significant difference between pollen tube growth between cpd and wild type.

Ye Q et al. PNAS 2010;107:6100-6105



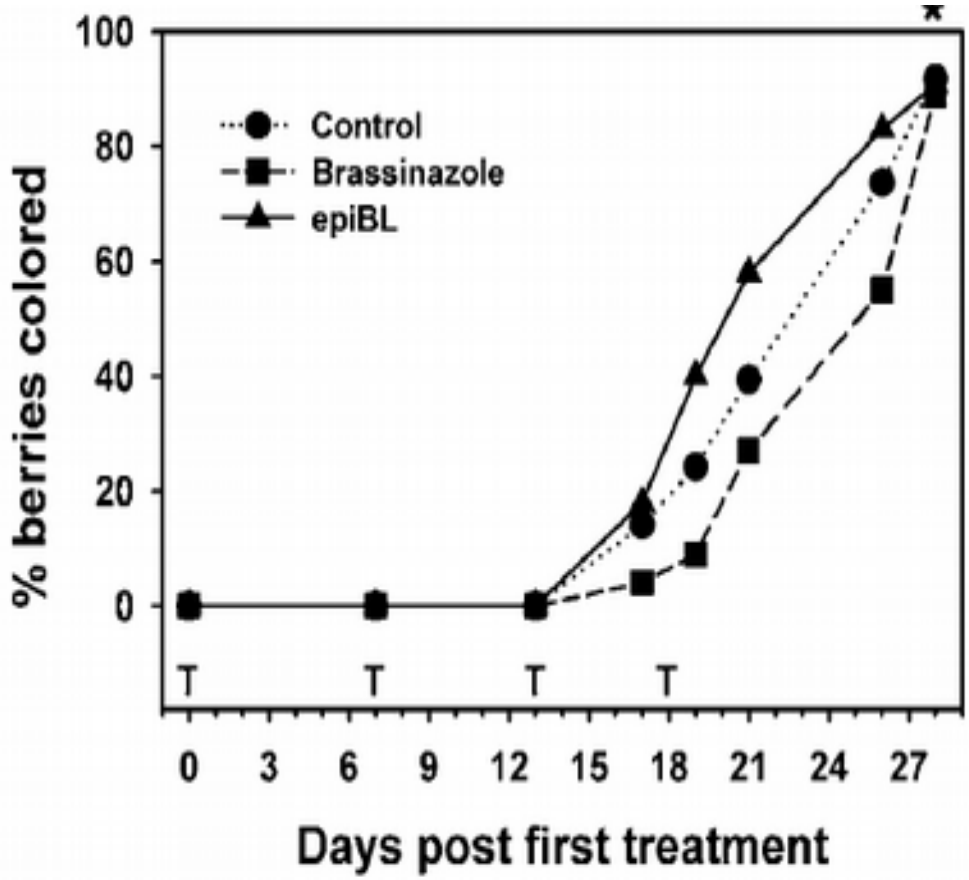
- ✓ Fewer pollen grain
- ✓ Defects in pollen release
- ✓ Abnormal tapetum development
- ✓ Abnormal exine developmnet.

Ye Q et al. PNAS 2010;107:6100-6105



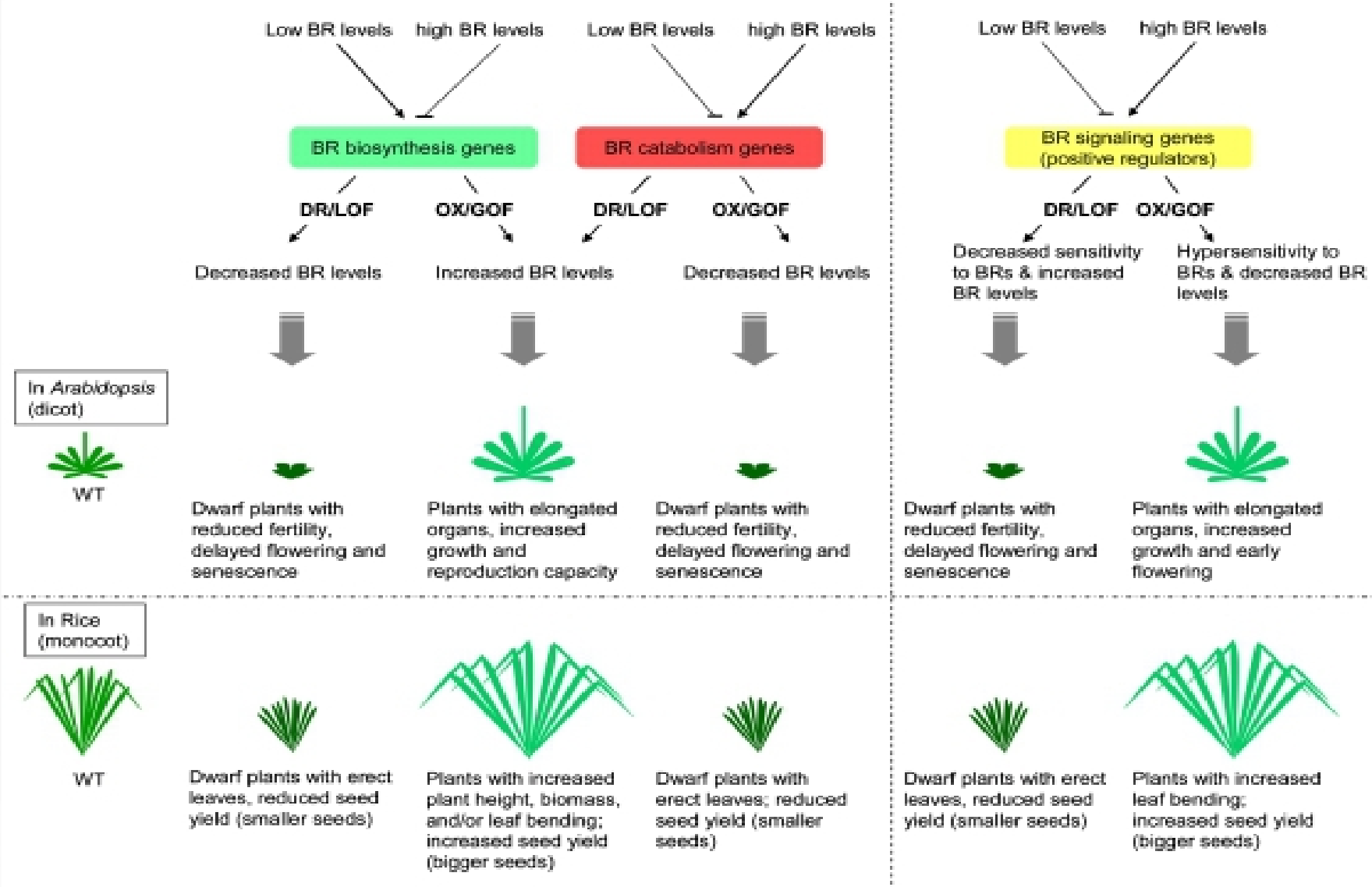
Symons et al. (2006) showed that increases in endogenous BR levels, but not indole-3-acetic acid or GA levels, are associated with ripening in grapes.

Application of 24-epibrassinolide to grape berries promoted ripening, while brassinazole, an inhibitor of BR biosynthesis, delayed fruit ripening.



epiBL & BRZ – applied to berry at each treatment time (T)

Boosting Crop Yield with Plant Steroids:



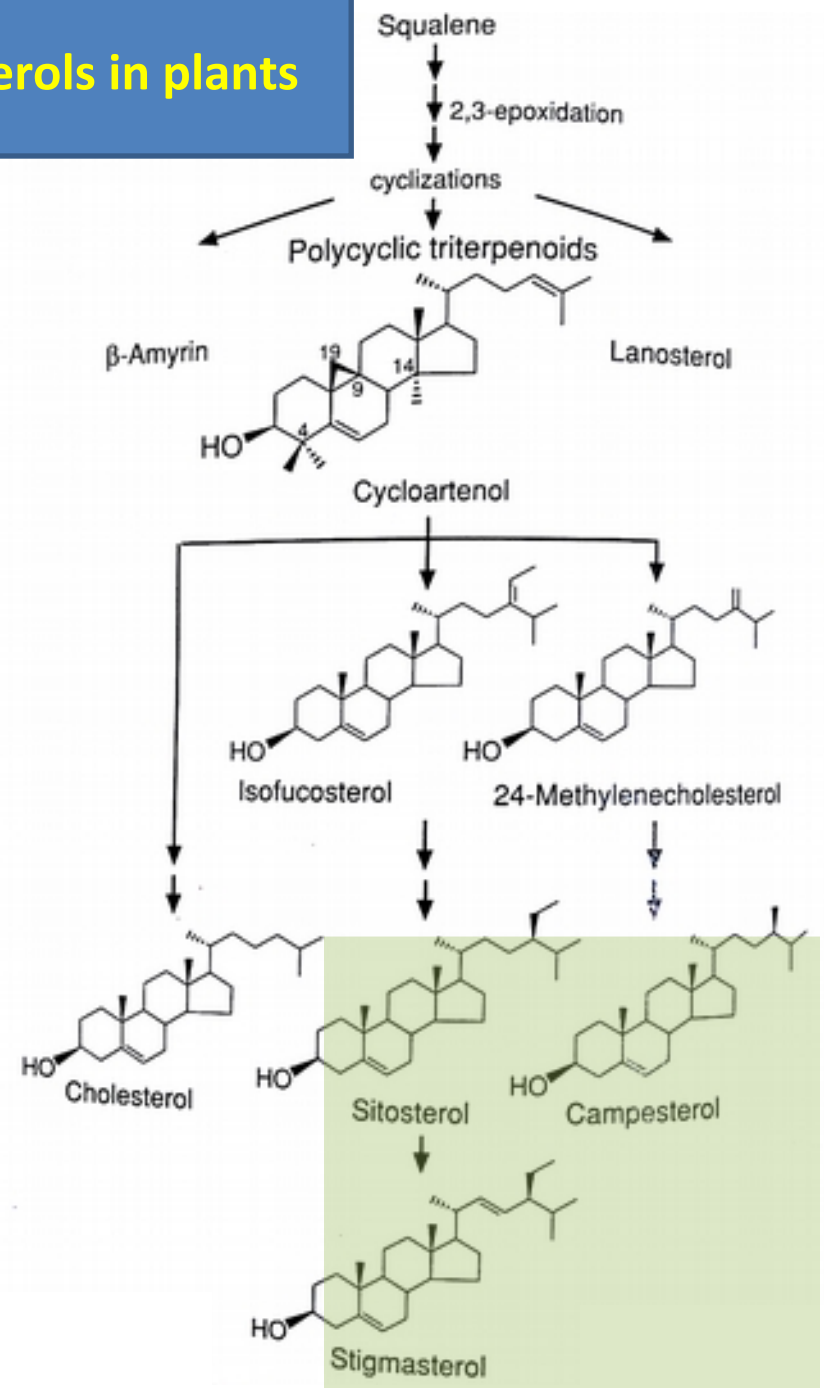
Cécile Vriet, Eugenia Russinova, and Christophe Reuzeau . (2012), The Plant Cell, Vol. 24:842-857

Practical use of BRs :

Yield increase in wheat, rice, barley etc.
Leaf weight in lettuce
Potato tuber growth.
Plant propagation of woody cuttings.

BRs application show most dramatic effect on yield under stress as compared to optimal condition.

Sterols in plants



All plant sterols derived from squalene, which in turn derived from Meavalonic acid pathway.

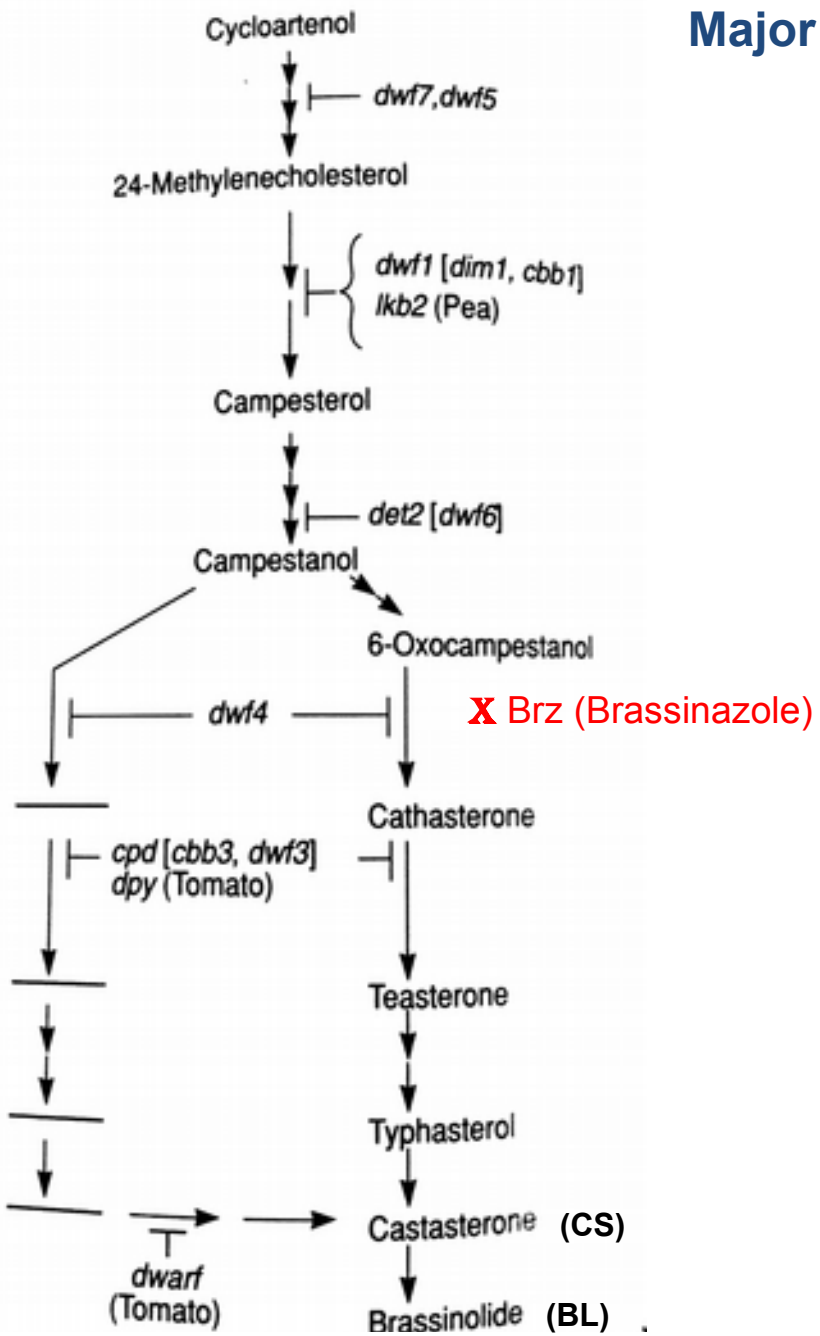
Some 300 different sterols are found in plants, among which sitosterol, stigmasterol and campesterol are the most common.

Cholesterol also found in plants, but is a minor component of plant sterol.

Sterol are important constituents of cellular membranes, modulate membrane stability, and permeability.

Plasma membrane has the highest sterol content and thus for growth and cell division sterol synthesis is required.

For human and insect?

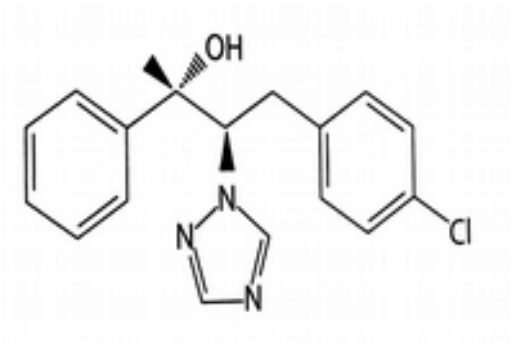


Major steps in BR biosynthesis:

Campesterol is the starting point for BL biosynthesis.

In some species eg. mung bean, tobacco and tomato, BL has not been found and CS seems to be final product. While some others eg. Arabidopsis, rice BL is the final product.

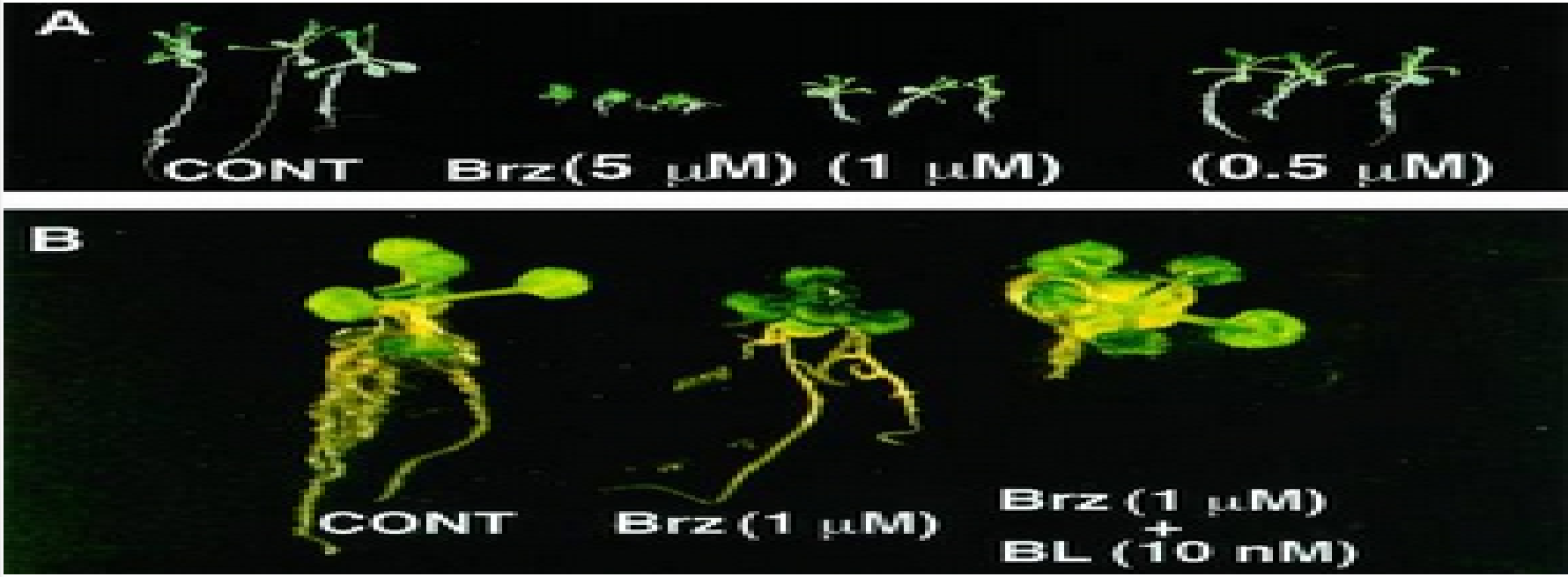
Mutant screening (Dwarfing, deetiolation in dark). (?-sensitivity to other hormones, endogenous content.)



BR SYNTHESIS INHIBITOR

Brassinazole, a trizole componds that inhibit brassinosteroid biosynthesis. It specifically inhibit the activity of DWF4 enzyme.

[4-(4-chloro-phenyl)-2-phenyl-3-(1,2,4-triazol)butan-2-ol]



Effect of brassinazole (Brz) on Arabidopsis seedlings grown in the light.
A, Brassinazole (5, 1, and 0.5 μM)-treated Arabidopsis (14-d-old) show dwarfism in a concentration-dependent manner. B, Brassinazole (1 μM)-treated Arabidopsis (14-d-old) show a BR-deficient mutant-like phenotype, which is rescued by the application of brassinolide (BL) (10 nM). CONT, Control.

Asmai et al, 2000, Plant Physiology, 123 (1):93-100

ASPEE College of Horticulture and Forestry

Thank You