

Engineering Canola Architecture

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Diversity in Flower and Inflorescence Architecture



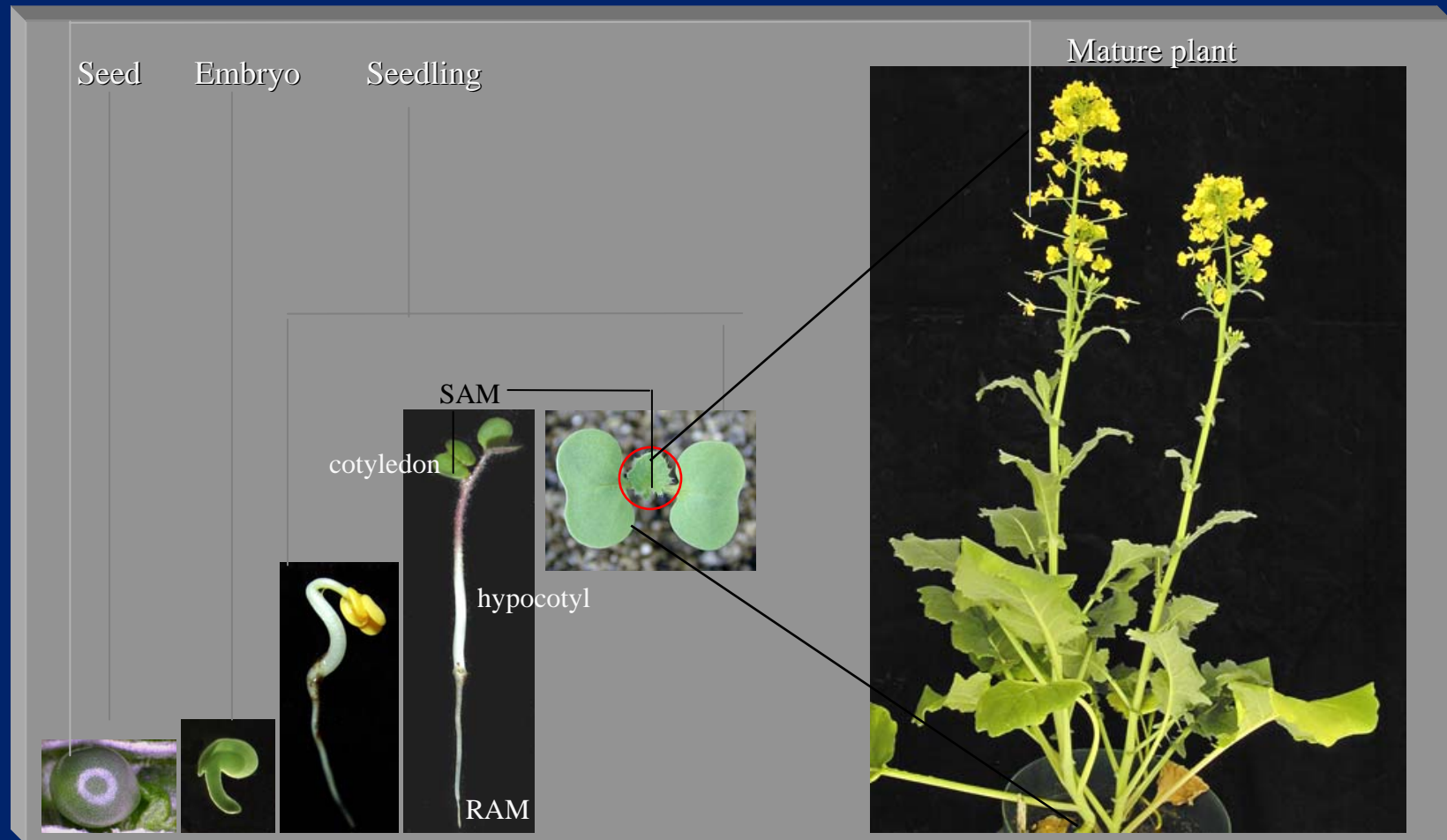
Canola inflorescence and pods



Hypothetical canola

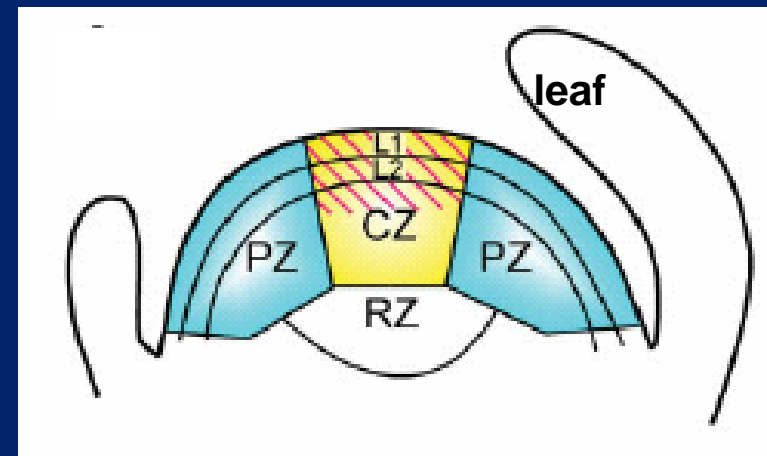


Brassica life cycle

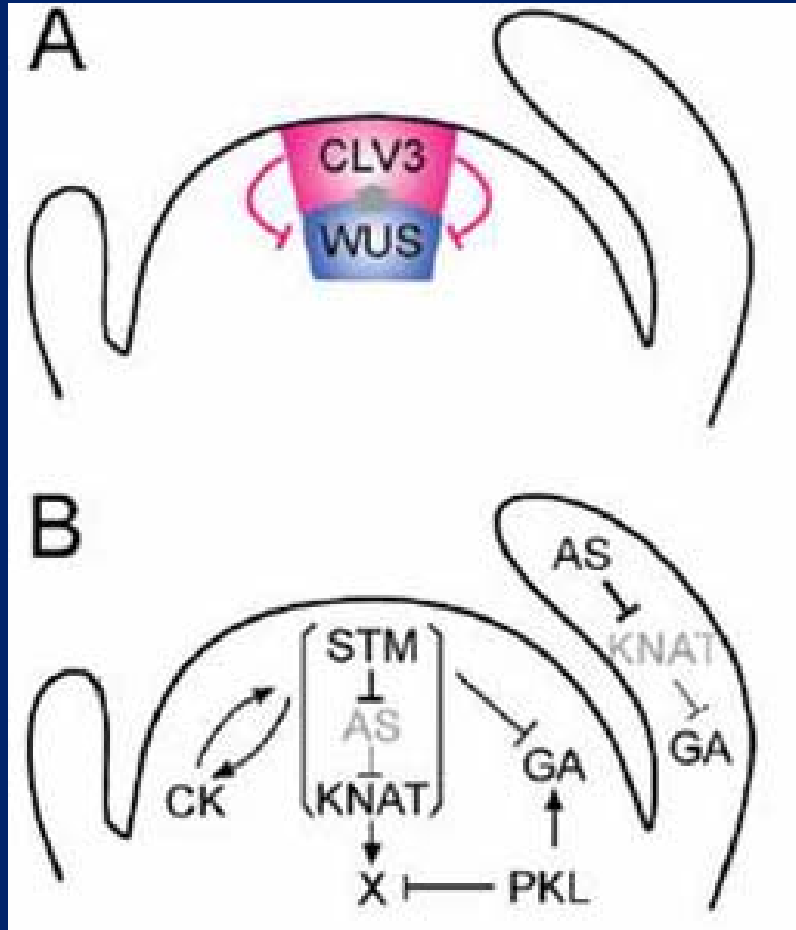


SAM - Shoot Apical Meristem

- Central role in plant growth and development
- The SAM:
 - produces lateral organs
 - forms stem of plant
- CZ : Central Zone
 - slowly dividing; replenishes cells to peripheral zones
- PZ : Peripheral Zone
 - rapid cell divisions; gives rise to lateral organs through founder cells
- RZ : Rib Zone
 - stem pith



Regulatory pathways in the shoot meristem



Stem cell formation by
CLV WUS loop

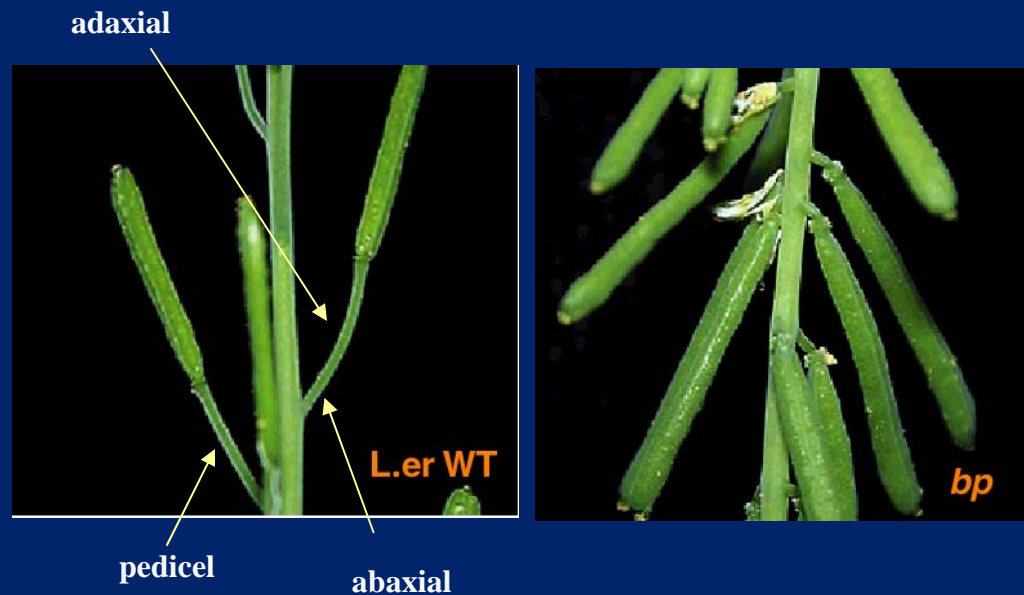
Factors regulating meristem
Maintenance and organ initiation

Homeobox genes

- Class 1 KNOX gene (STM & BP) encodes homeodomain protein
 - Transcription factor which will bind specific DNA sequences
- Expressed in meristems but not organ primordia
- Good target candidates to engineer architecture

Brevipedicellus (BP)/ KNAT1

- Reduced pedicels, downward siliques and flowers, reduced overall size
- Defective cell division, differentiation and elongation



Developmental Model for diversity in pedicel architecture



abaxial > adaxial



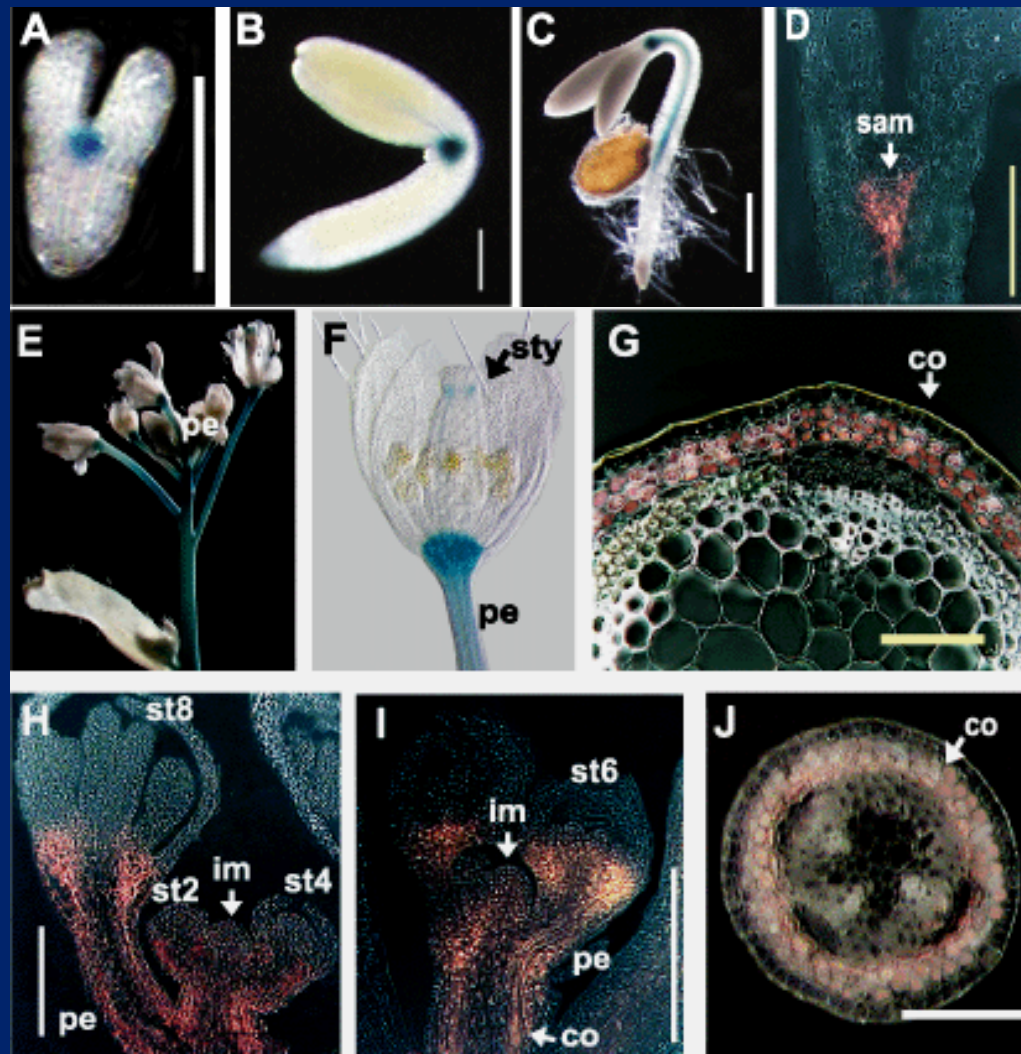
abaxial = adaxial



abaxial < adaxial

Cell differentiation and growth (asymmetric/symmetric) in abaxial and adaxial regions of pedicel defines the angle of attachment to peduncle

BP::GUS expression patterns



Brassica BP homologs share sequence and functional identity with Arabidopsis

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                *           20           *           40
AtBP : MEEYQHDNSTTPRVSFLYSPISSSNKNDNTSTNNNNNNNNSNYG : 47
BnBP : MEEYQHESSTTPRVSFLYSPISSSNKNDNTTNNNN-----TNYG : 41
BrBP : MEEYQHESSTTPRVSFLYSPISSSNKNDNTTNNNN-----TNYG : 41
BoBP : MEEYQHESSTTPRVSFLYSPISSSNKNDNTTNNNN-----TNYG : 41
MEEYQHesr3TPhRVsFLYsPISsSNkNDNT3 TNNNN 3NYG

                *           60           *           80           *
AtBP : PGYNNTNNNNHQHMLFPHMSSLLPQTTENCFRSDHDQPTNNNNNS : 94
BnBP : SGYNNTNNNNHQHMLFPHMSSLLPQTTENCFRSDHDQPTNN---AS : 84
BrBP : SGYNNTNNNNHQHMLFPHMSSLLPQTTENCFRSDHDQPTNN---AS : 84
BoBP : SGYNNTNNNNHQHMLFPHMSSLLPQTTENCFRSDHDQPTNN---AS : 84
sGYNNTNNNNHq QHMLFPHMSSLLPQTTENCFRSDHDQpTn aS

                100           *           120           *           140
AtBP : VKSEASSRINHYSMLRAIHNTQEANNNNNDNVSDVEAMKAKIIAH : 141
BnBP : VKSEASSRINHYSMLMKAIHNTQEANNNNND---EMKAKIIAH : 127
BrBP : VKSEASSRINHYSMLMKAIHNTQEANNNNNN---EMKAKIIAH : 129
BoBP : VKSEASSRINHYSMLM4AIHNTQEANNNNND---EMKAKIIAH : 127
VKSEASSRINHYsMLM4AIHNTQE NNNNN1 EsMKAKIIAH

                *           160           *           180
AtBP : PHYSTLLAYLDCQKIGAPPVVDRITAARQFEARQQRTSSVSAS : 188
BnBP : PHYSTLLAYLDCQKIGAPPVVDKITAARQFEARQQRTSSVTA : 174
BrBP : PHYSTLLAYLDCQKIGAPPVVDKITAARQFEARQQRTSSVTA : 176
BoBP : PHYSTLLAYLDCQKIGAPPVVDKITAARQFEARQQRTSSVTA : 174
PHYSTLLhAYLDCQKIGAPPeVVD4ITAARQeFEARQQRpTaSV3A1

                *           200           *           220           *
AtBP : SRDPELDQFMEAYCDMLVKYREELTRPIQEAMEFIRRIESQLSMLCQ : 235
BnBP : SRDPELDQFMEAYCDMLVKYREELTRPIEEAMEYIRRIESQISMLCQ : 221
BrBP : SRDPELDQFMEAYCDMLVKYREELTRPIEEAMEYIRRIESQISMLCQ : 223
BoBP : SRDPELDQFMEAYCDMLVKYREELTRPIEEAMEYIRRIESQISMLCQ : 221
SRDPELDQFMEAYCDMLVKYREELTRPI2EAME5IRRIESQ6SMLCQ

                240           *           260           *           280
AtBP : SPIHLNNPDGKSGIMSSDEEQqNNSGGEELPEIDPRAEDRELK : 281
BnBP : GPIHLNNPDGKSGIMSSDEEQqNNSGGEELPEIDPRAEDRELK : 268
BrBP : GPIHLNNPDGKSGIMSSDEEQqNNSGGEELPEIDPRAEDRELK : 270
BoBP : GPIHLNNPDGKSGIMSSDEEQqNNSGGEELPEIDPRAEDRELK : 268
gPIHLNNPDGKSG6eSSDEEQqnNNSGGEaELPEIDPRAEDRELK

                *           300           *           320
AtBP : NHLLKYSGYLSSLQELSKKKKKGKLPKEARQLLTWWELHYKWPY : 328
BnBP : NHLLKYSGYLSSLQELSKKKKKGKLPKEARQLLTWWELHYKWPY : 315
BrBP : NHLLKYSGYLSSLQELSKKKKKGKLPKEARQLLTWWELHYKWPY : 317
BoBP : NHLLKYSGYLSSLQELSKKKKKGKLPKEARQLLTWWELHYKWPY : 315
NHLLKYSGYLSSLQELSKKKKKGKLPKEARQLLTWWELHYKWPY
    
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Misexpression of *Brassica BP* leads to asymmetric and novel leaf phenotypes

WT

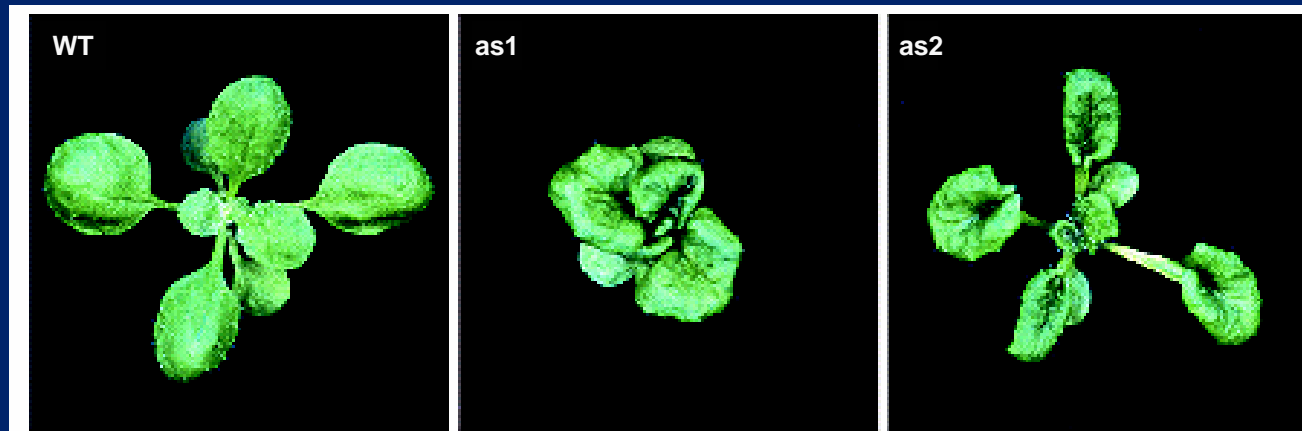


Upstream regulators of *BP*: *AS1* and *AS2*

Transcription factors containing MYB domain(*AS1*) and LOB domain(*AS2*)

Expressed in lateral organ primordia and down regulate *BP* expression and function

as1 and *as2* mutants - “lobed” leaves phenocopies *BP* mis expression phenotype



AS1 and AS2 are negative regulators of BP function

**Misexpression of AS1 or AS2: potential *bp* mutant
phenotype**

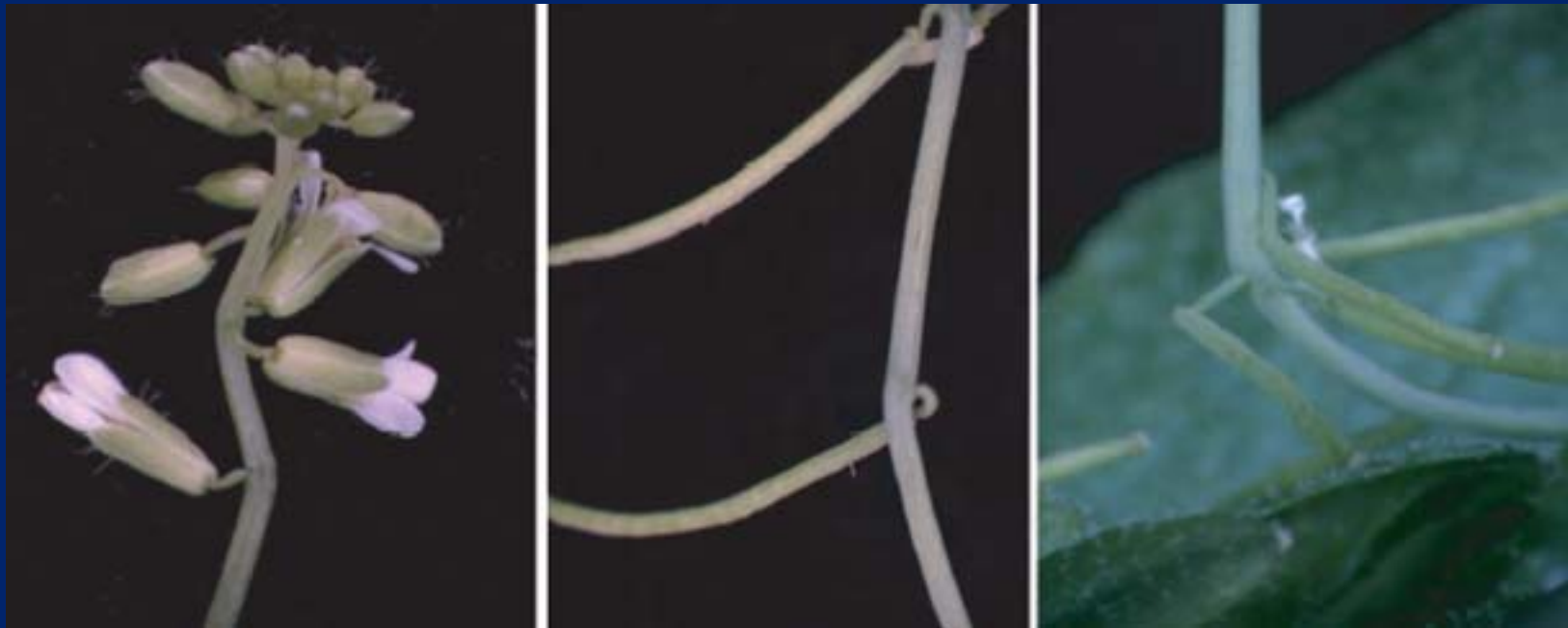
Constitutive : AS1 and AS2 under double 35s promoter

Targeted : AS1 and AS2 under BP promoter

Over expression of AS1 leads to BP phenotype



**Regulated misexpression of BP-AS2
show bp phenotype**



Potential approaches for bp Canola

1. Mutate Canola BP by
 - screening mutated population (non transgenic)
 - anti-sense approach
 - RNAi approach
2. Mis express BP negative regulators AS1 and AS2
 - constitutive promoter
 - regulated promoters

Canola inflorescence

Current



Hypothetical



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