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ERA-NET for Coordinating  
Action in Plant Sciences



# The SeedAdapt Project

- Dimorphic fruits, seeds, and seedlings as adaptation to abiotic stress in unpredictable environments
- *Aethionema arabicum* – 2 distinct types of diaspores



# The consortium

- 6 partners + 1 associated group

Royal Holloway, University of London	UK
University of Osnabrück	Germany
Gregor Mendel Institute, Vienna	Austria
Philipps-University Marburg	Germany
Wageningen University	Netherlands
Friedrich-Schiller-University Jena	Germany
University of Olomouc	Czech Republic

# The Aims of the Research

- Dimorphic diaspores represent distinct adaptations as dormancy bet-hedging strategy
- Evolved in annual plant species as adaptations to abiotic stresses
- Regulatory basis of fruit, seed, and seedling trait diversity
- Comparative investigation of the epigenomes, hormonomes and transcriptomes

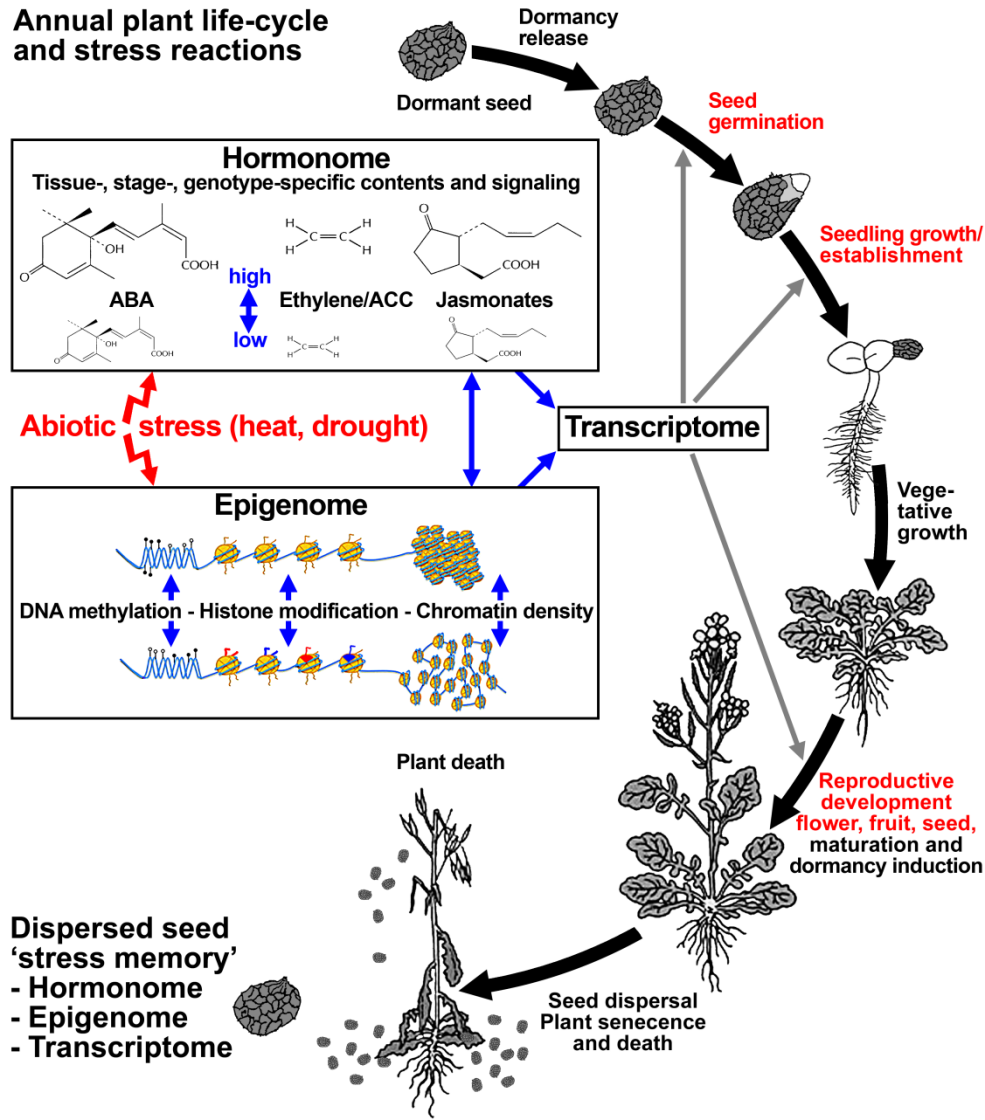
# Scientific background and programme





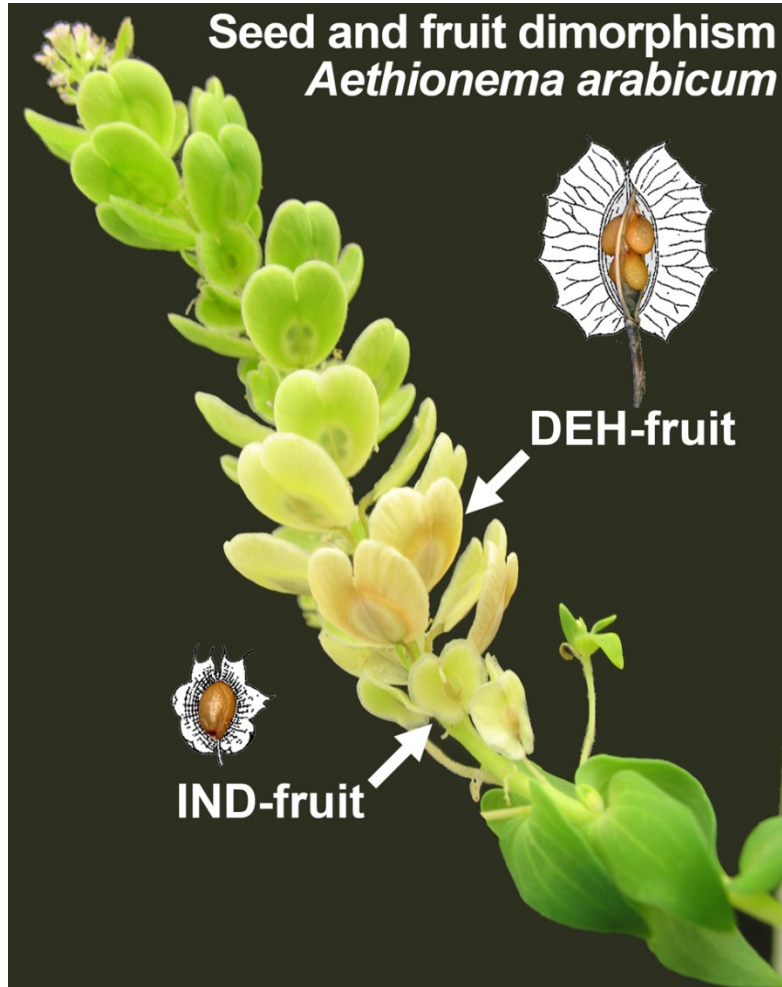
# Typical annual plant life-cycle

## Annual plant life-cycle and stress reactions



- Abiotic stress during life-cycle  
> seed 'stress memory'
- Monocarpic life-style:  
one type of fruit  
one type of seed

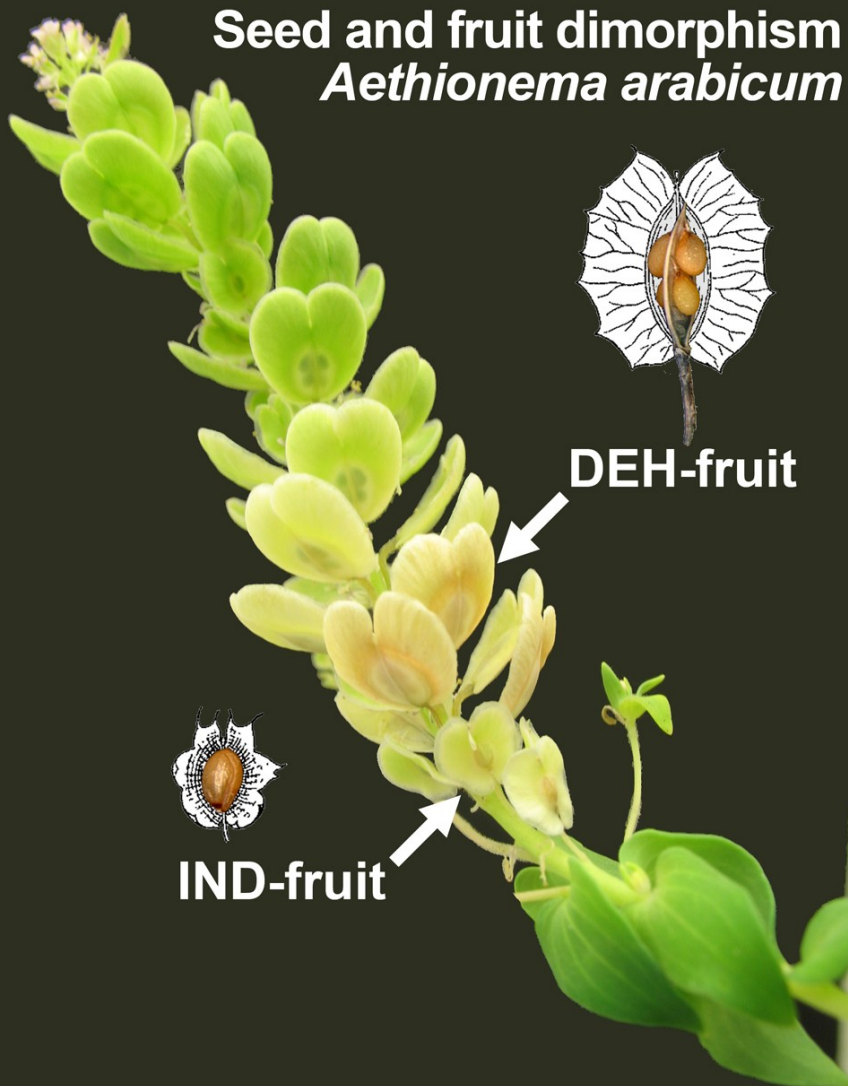
# Heterocarpy



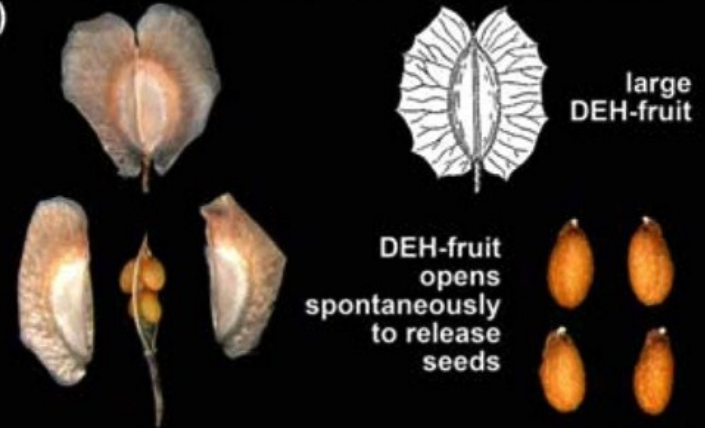
- Multiple types of fruits on the same plant

# Dimorphic diaspore syndromes in *Aethionema arabicum*

## Seed and fruit dimorphism *Aethionema arabicum*



### Dehiscence: DEH-fruit with several $M^+$ seeds (B)



### Indehiscence: IND-fruit with one $M^-$ seed (C)



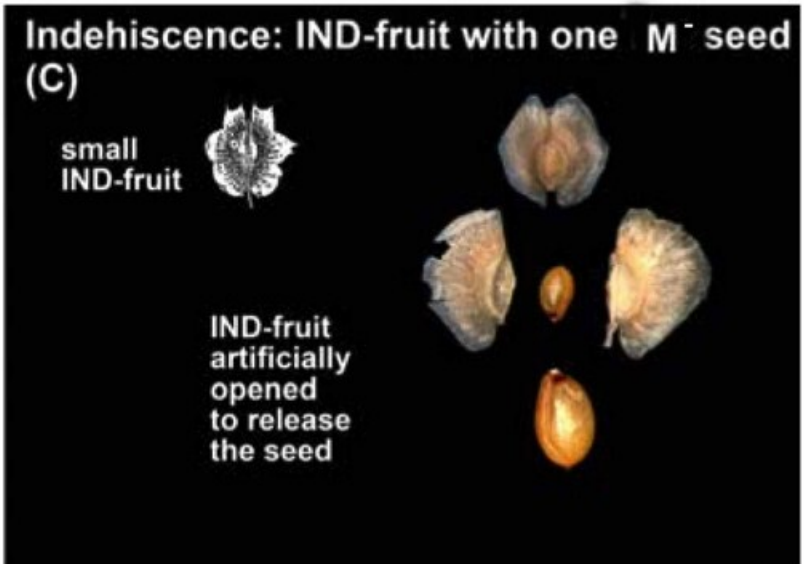
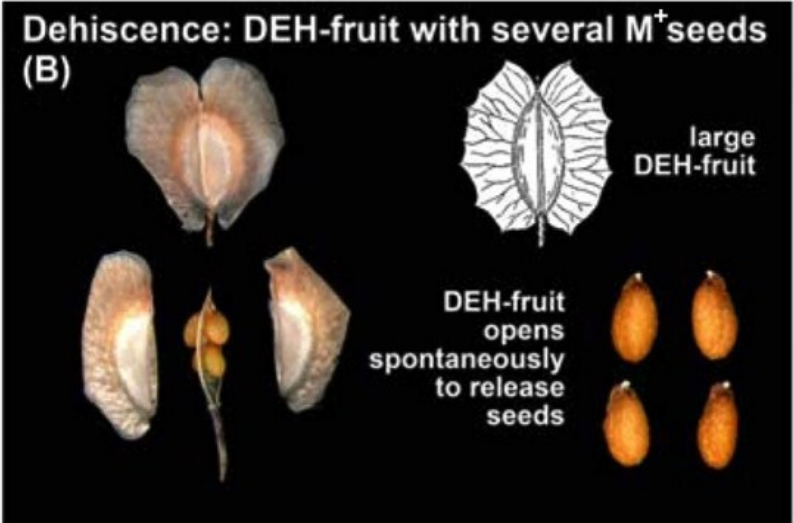


# Dimorphic diaspore syndromes in *Aethionema arabicum*

dry



imbibed



# *Aethionema arabicum*

## heteromorphous life-cycle

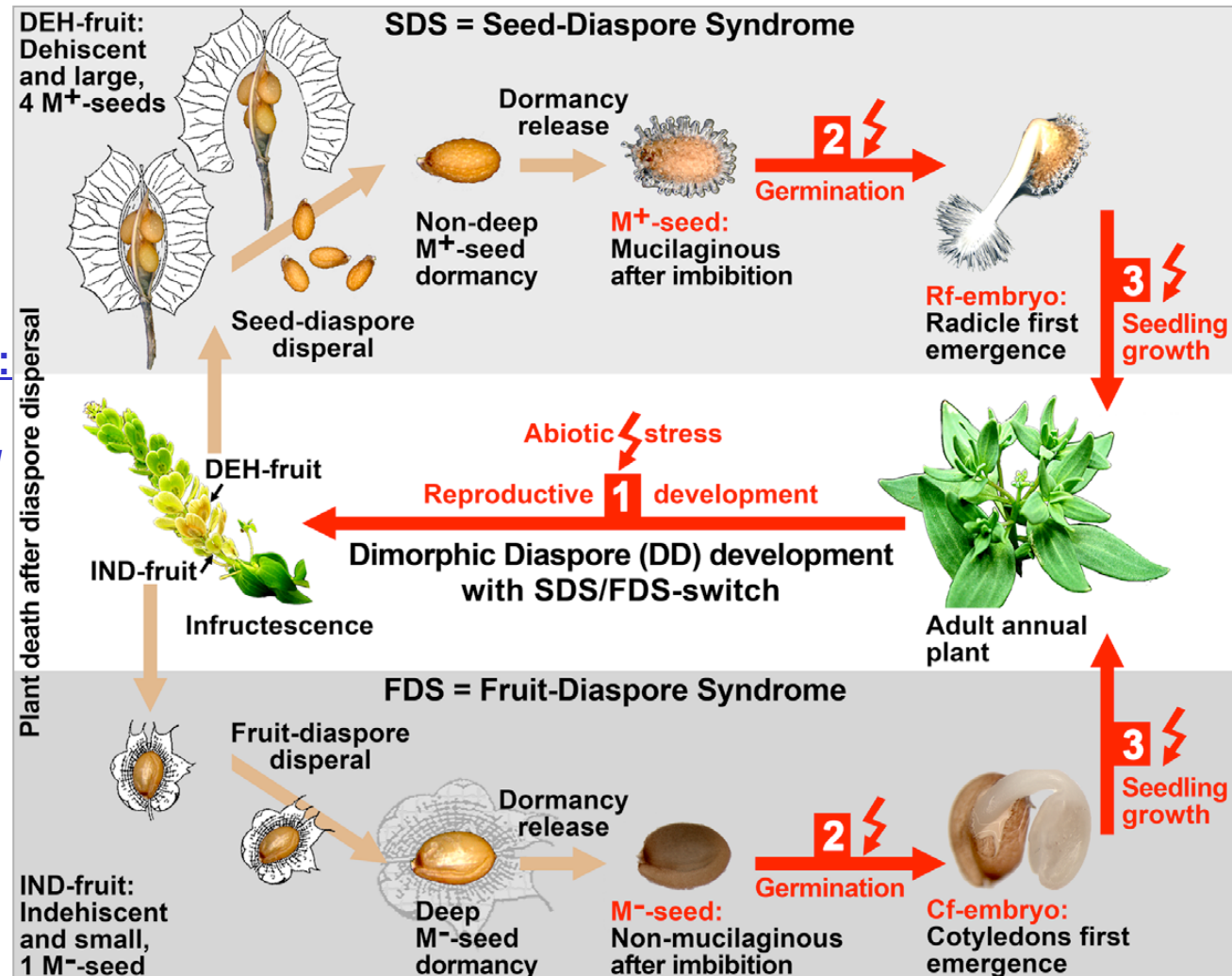
*high dispersal / low dormancy*  
escape in space



**Fruit/seed heteromorphism:**  
*Bet-hedging strategy in environmentally fluctuating (unpredictable) habitats; Reduces risk of failure under temporal environmental uncertainty*



*low dispersal / high dormancy*  
escape in time

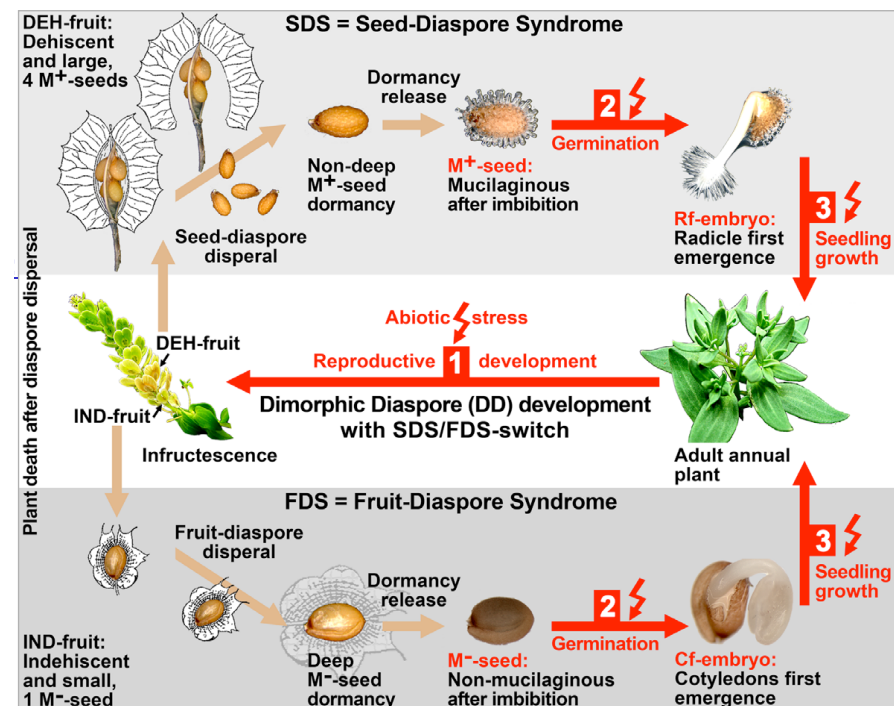


# SeedAdapt – aim and work packages

The aim of the SeedAdapt project is to elucidate the molecular mechanisms of heterocarpic fruit/seed-related life history traits that evolved in annual plant species as adaptations to abiotic stresses.

## Core experiment:

Investigate the influence of abiotic stress (heat) on epigenomes, hormones and transcriptomes during seed germination, seedling establishment and reproductive development of *A. arabicum*.



> Six work packages contribute to the core experiment

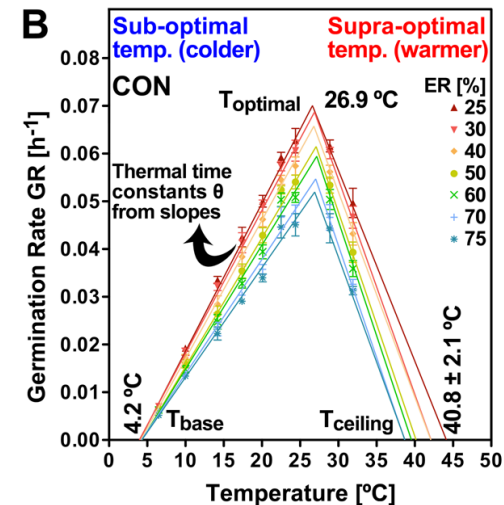
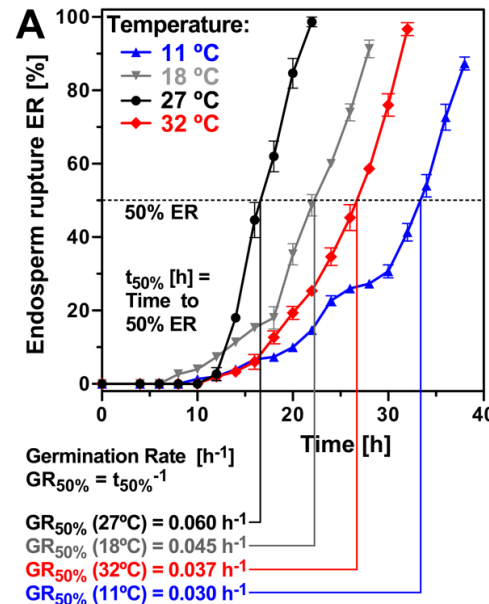


# Work package 1

(Gerhard Leubner, London)

Abiotic stress physiology, hormone analysis, seed biomechanics and growth imaging of *Aethionema* dimorphic diaspore syndromes

- Comparative germination analysis in relation to heat and water availability
- Seed hormone analysis
- Biomechanical and imaging analysis of germination in relation to abiotic stress

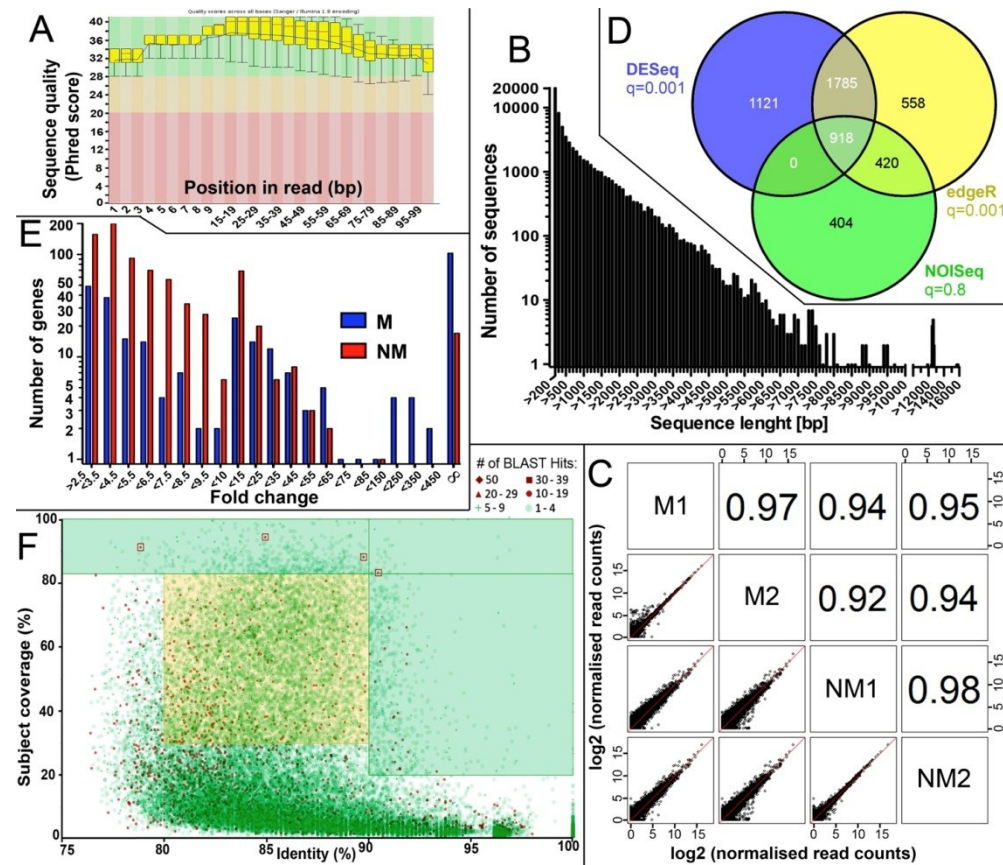


# Work package 2

(Kai Graeber, London)

## Global and targeted comparative analyses of transcriptomes and small RNAs in dimorphic seeds and seedlings upon abiotic stress

- Genome-wide comparative seed- and seedling-type RNA-Seq analyses (mRNA, sRNA)
- Targeted expression analyses (qRT-PCR) to validate DEG expression patterns from global analyses and to extend analysis (accessions, time-points, treatments)



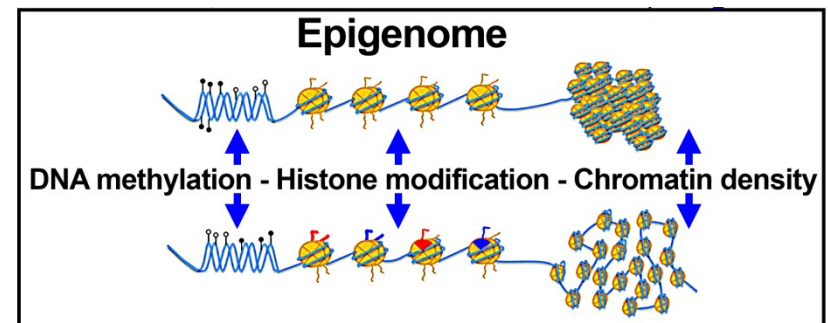


# Work package 3

(Ortrun Mittelsten-Scheid, Vienna)

## Comparative analyses of the epigenetic mechanisms regulating dimorphic seed and seedling responses upon abiotic stress

- Establishing epigenetic facts and protocols
- Characterizing the chromatin state at specific target genes known to determine fruit and germination/dormancy traits
- Determining chromatin states (DNA methylation, histone modification) genome-wide during developmental transitions and upon abiotic stress by BS-Seq and ChIP-Seq

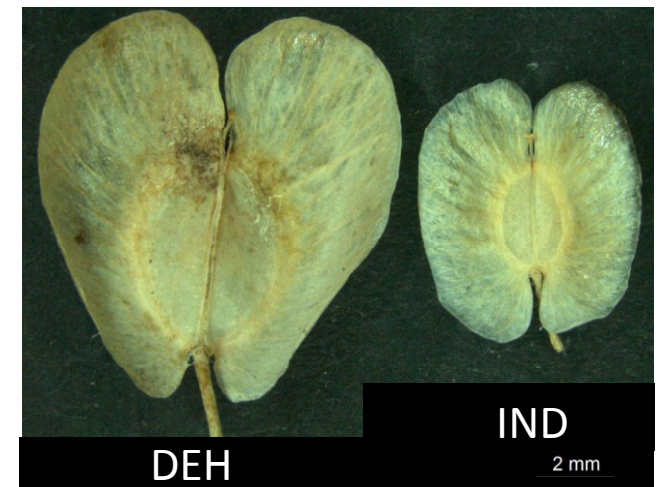
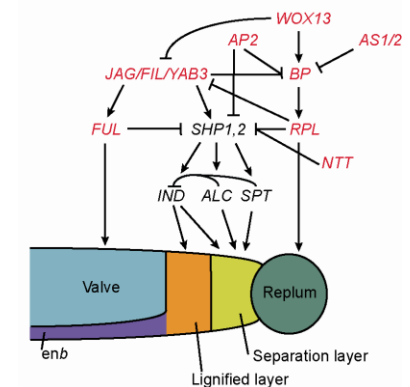


# Work package 4

(Klaus Mummenhoff, Osnabrück)

## Ecophysiology, ecobiochemistry, evolution and molecular genetics of *Aethionema* dimorphic diaspore development and dispersal

- Analysis of abiotic stress (heat) and biotic stress (herbivory) during reproduction on the DEH/IND fruit ratios
- Qualitative and quantitative analysis of glucosinolate patterns in fruit valves and seeds of DEH and IND fruits
- Comparative evolutionary and developmental (EvoDevo) genetics of the formation of IND and DEH fruits investigating known fruit development genes



# Work package 5

(Eric Schranz, Wageningen)

Comparative forward and reverse genetics of dimorphic diaspore syndromes and generation of *Aethionema* genetic resources

- QTL analysis of fruit/seed syndromes
- Forward genetics EMS population generation
- EMS mutant screen different conditions
- Establishing genetic transformation

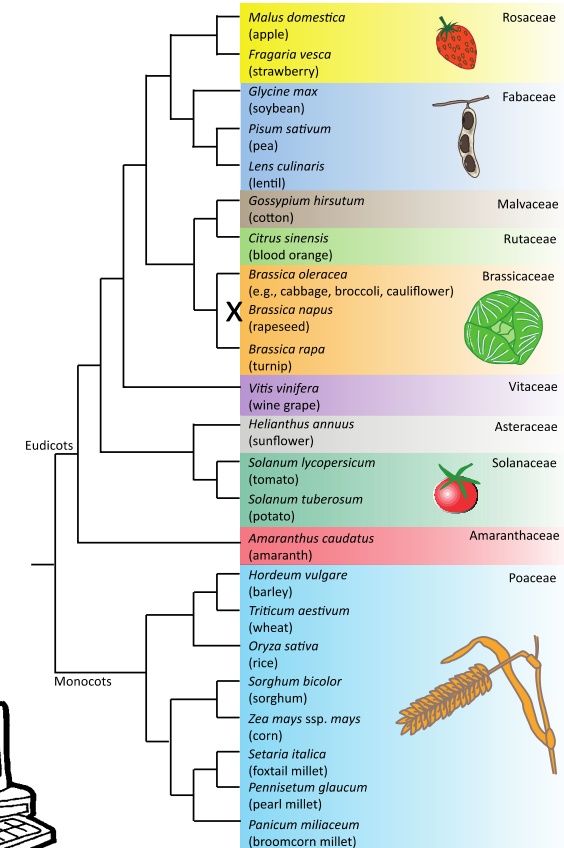


# Work package 6

(Stefan Rensing, Marburg)

## Integration of SeedAdapt project data and transfer of results to other species

- Comparative expression and population genetic analysis of key DEGs between *Aethionema* populations/species
- Identifying TFBSs and TFs involved in seed/fruit syndromes by cross-species phylogenomics of DEGs
- SeedAdapt data management and web-tool establishment
- Integration of SeedAdapt project data and dissemination of knowledge





# Use of joint facilities

- Core project will allow group to access to state-of-the art facilities in the collaborating parties institutions.
- For example consortium will have access to:
  - Greenhouse facilities at Wageningen
  - Sequencing facilities at Vienna
  - *Aethionema arabicum* genome sequence
  - Marburg bioinformatics technology



# Other benefits of collaborative project

- Highly inter-disciplinary project brings together wide-ranging expertise. For example:
  - RHUL expertise in seed biology and biomechanics and molecular techniques
  - Evolutionary biology expertise at Jena
  - Fruit biology and *Aethionema* accessions at Osnabrück
  - Plant epigenetics at Vienna

# Expectations of ERA-CAPS / Comments on call

- Aims of ERA-CAPS fits well with our research
  - Cross-disciplinary collaborative calls on fundamental science topic
- Processes and support have been good
- Consortium looking forward to an exciting project



Gerdhard Leubner  
Kai Graeber  
Joanna Cox  
Lorna Ravenhill  
Klaus Mummenhoff  
Katja Sperber  
Sara Mayland-Quellhorst  
Ortrun Mittelsten-Scheid

Stefan Rensing  
Christopher Grosche  
Eric Schranz  
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Günter Theißen  
Teresa Lenser  
Miroslav Strnad



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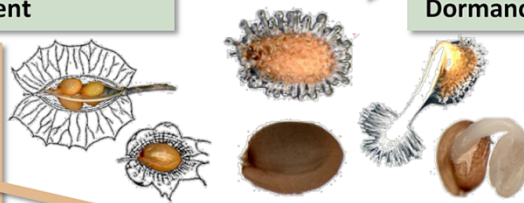
# SeedAdapt core experiment

Core experiment for dimorphic diaspores with distinct **syndrome** and **stress** memories

Generation of 'dimorphic memory'  
Seed and fruit development

- DEH/IND-fruit biology (glucosinolates) WP4
- Adaptation to different climates WP4+5
- Abiotic stress during reproduction WP1.1

- Differentially Regul. Genes (DEGs) M+/M- transcriptome WP2
- Natural variation (RIL, QTL) WP5
- Population, forward and reverse (transformation) genetics WP5



Utilization of 'dimorphic memory'  
Dormancy/Germination/Seedling growth

- M+/M- seed biology (all early stages) WP1+5
- Biophysical adaptation of early stages WP1.3+5
- Abiotic stress during early stages WP1.1+2+3

- DEGs (**syndrome** x **stress**) from global memory analyses WP2+3
- Generation genet. resources WP5
- Phylogenomics, evolution, transfer to crops, integration WP6

Global memory analyses:

- Hormonome WP1.2
- Transcriptome WP2.1
- small RNAs WP2.2
- Epigenome WP3

Six work packages contribute to the core experiment

Accessions differ in climatic adaptation	Reproduction temperature stress (1)	'Syndrome x stress memory' M+/M- imbibed seed, temperature stress (2)	'Establishment, resetting?' RF/Cf-seedling growth, temp. stress (3)
<i>Ae. arabicum</i> <i>RIL-TUR</i> origin: Turkey climate: cold-wet	1Temp.optimal	M <sup>+</sup> -seed 2Topti M <sup>+</sup> -seed 2Theat M <sup>-</sup> -seed 2Topti M <sup>-</sup> -seed 2Theat	Rf-seedlings 3Topti Rf-seedlings 3Theat Cf-seedlings 3Topti Cf-seedlings 3Theat
	1Temp.heat-stress	M <sup>+</sup> -seed 2Topti M <sup>+</sup> -seed 2Theat M <sup>-</sup> -seed 2Topti M <sup>-</sup> -seed 2Theat	Rf-seedlings 3Topti Rf-seedlings 3Theat Cf-seedlings 3Topti Cf-seedlings 3Theat
<i>Ae. arabicum</i> <i>RIL-CYP</i> origin: Cyprus climate: warm-dry	1Temp.optimal	M <sup>+</sup> -seed 2Topti M <sup>+</sup> -seed 2Theat M <sup>-</sup> -seed 2Topti M <sup>-</sup> -seed 2Theat	Rf-seedlings 3Topti Rf-seedlings 3Theat Cf-seedlings 3Topti Cf-seedlings 3Theat
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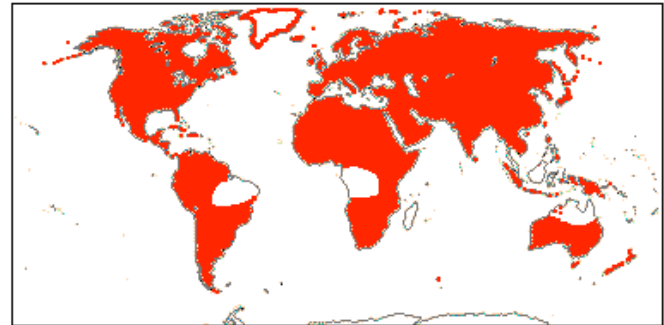
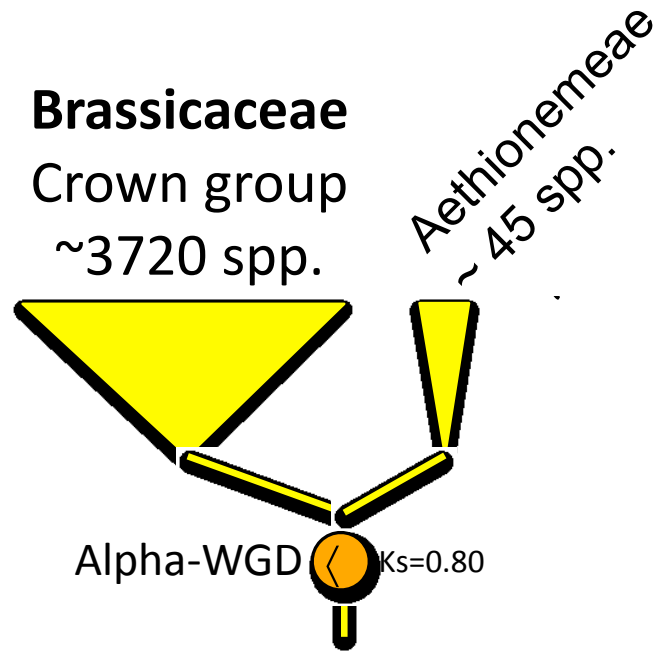
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mRNA-Seq, sRNA-Seq, BS-Seq, ChIP-Seq  
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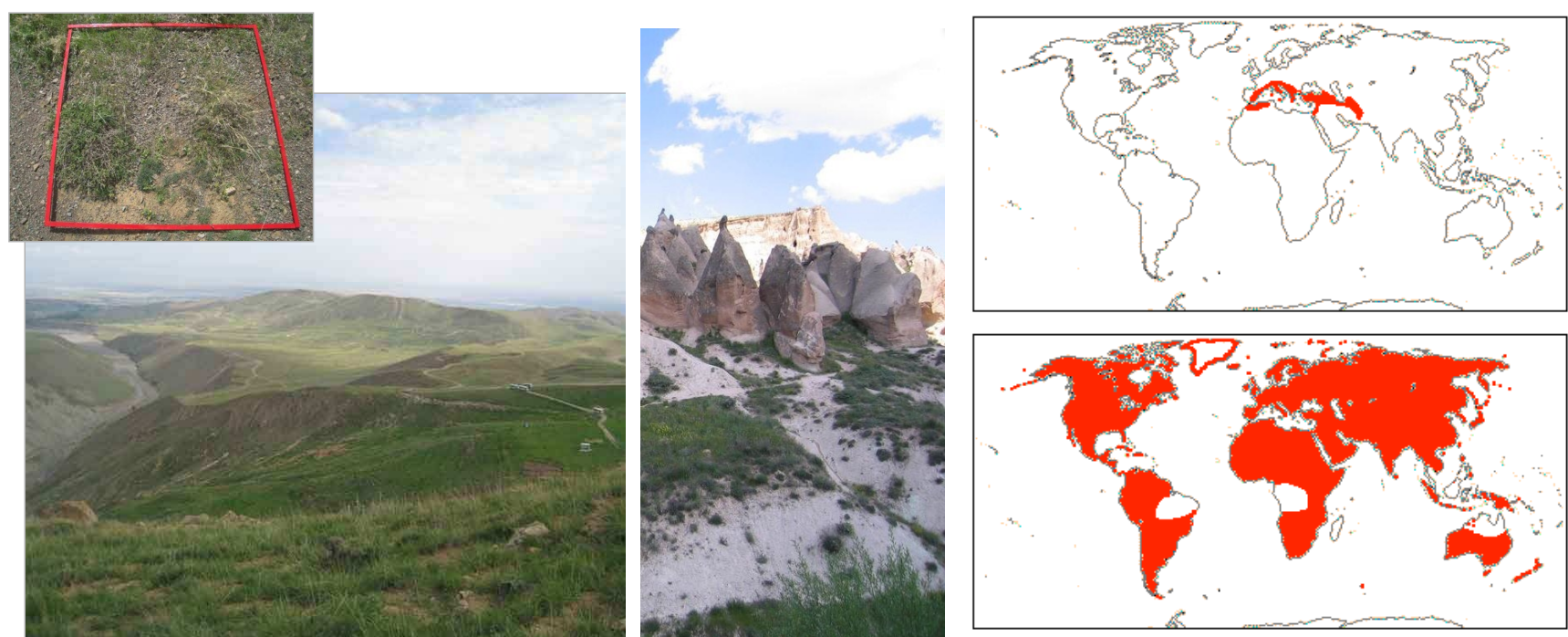


# Genus Aethionema



- Brassicaceae crown radiated ~ 32 mya
- Aethionema base diverged ~ 38 mya
- Alpha event occurred ~ 40 mya

# Genus Aethionema



- Brassicaceae crown radiated ~ 32 mya
- Aethionema base diverged ~ 38 mya
- Alpha event occurred ~ 40 mya