Seed germination phenotyping in controlled conditions to measure genetic diversity and address climate change

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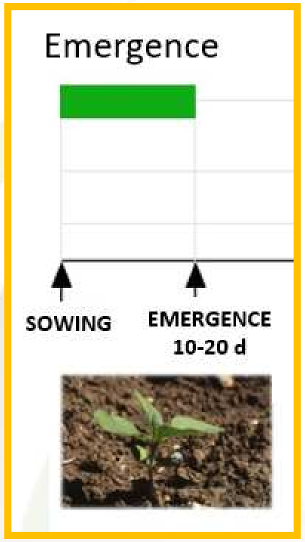
https://doi.org/10.15454/U2BWFJ

Outlines

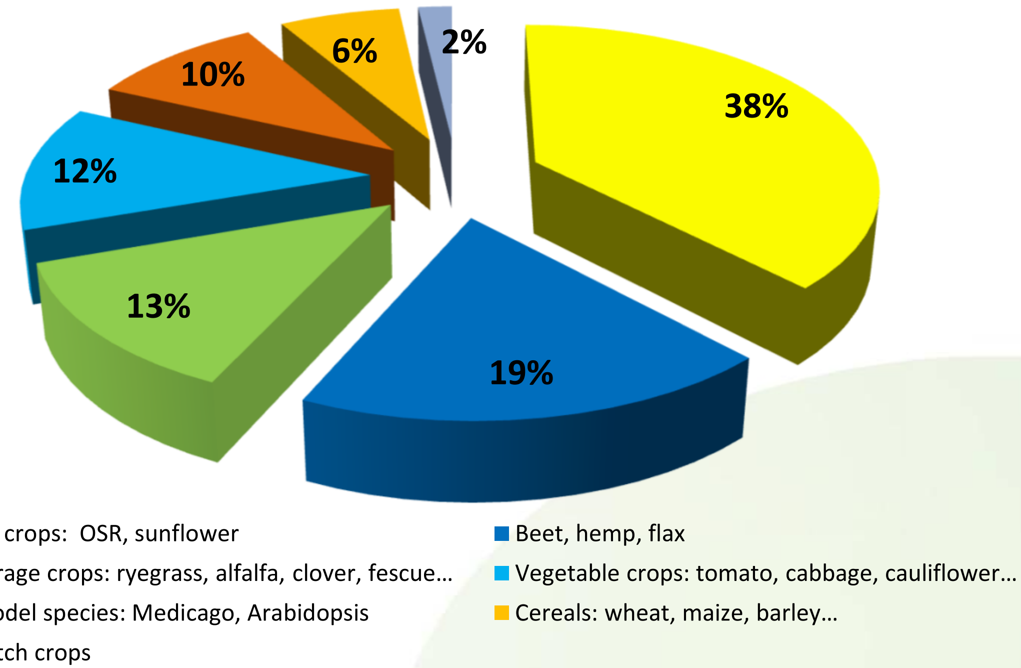
- Germination phenotyping: tools and data
- Three examples
- Methodological improvements to phenotype early stages of plant life



Introduction – Methodology – Results - Conclusion

High throughput germination phenotyping

Main crops phenotyped with automated germination tools since 2006

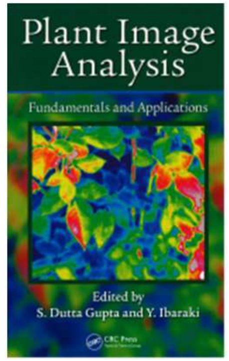


40 species validated for seed imaging at SNES with more than 10^6 single seeds analysed for:

- Seed physiology
- Seed testing: seed vigour or seed technology
- Breeding: genetic resources screening

Digital imaging traits: not only germination

- Automated germination time progress curves + 32 related seed traits
- Depend on species and temperature



Demilly et al., 2015 *in Plant Image analysis*, CRC Press

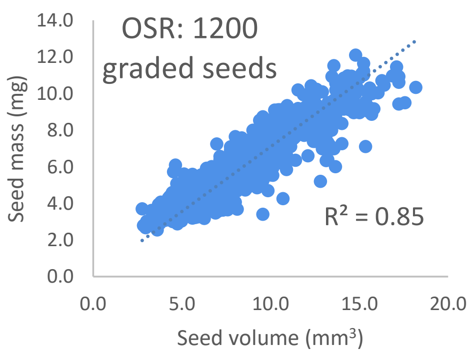
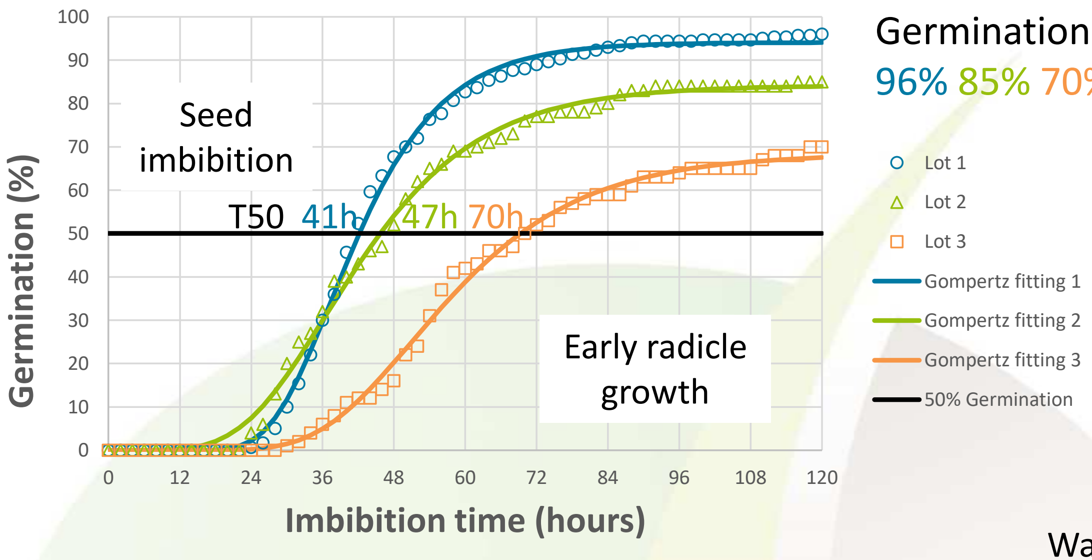


Image 0: dry seed size

Volume for spheric seed

Area for flat seed



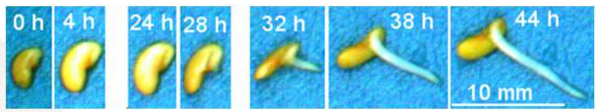
Germination ∑n

96% 85% 70%

Germination rate

T50 or MGT =∑ (n.t)/∑n

MGT 46h 47h 62h



Wagner et al., 2010 in *ISTA Symposium Proc.*

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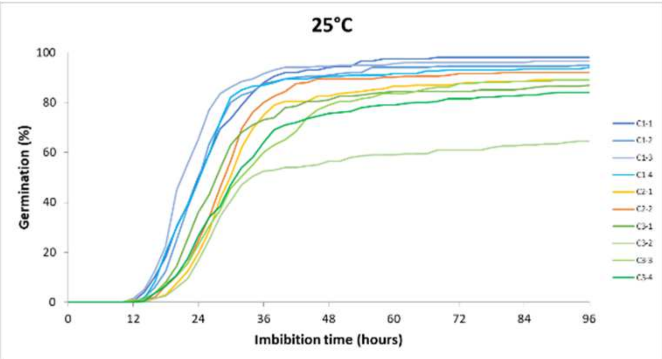
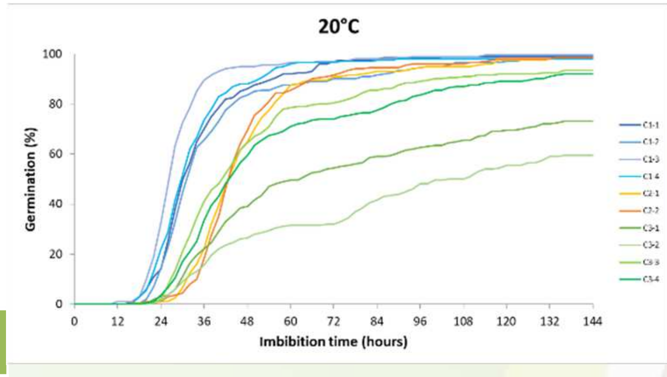
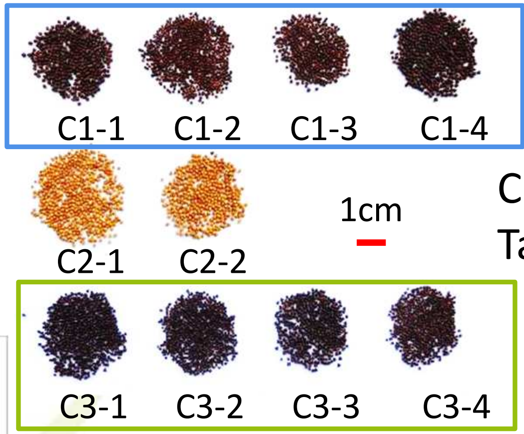
Genetic x Environment contribution to seed germination

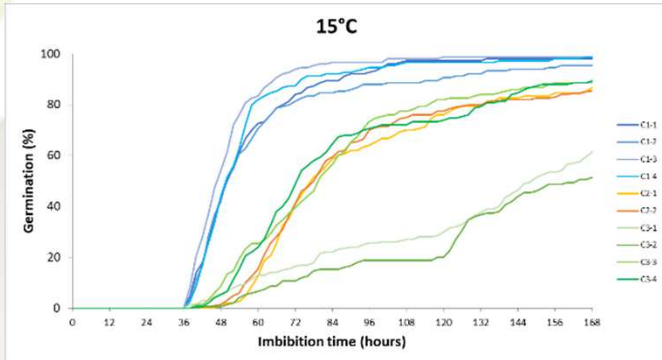
- Black mustard (*Brassica juncea*) from ISTA 19-3 special project







Courtesy of Takashi Shinohara

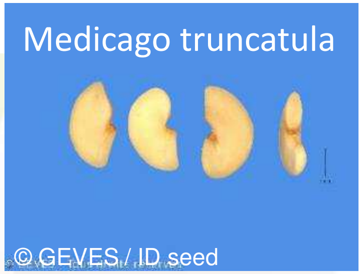


Temperature (°C)	25	20	15
MGT range (h)	13	38	61
Germination range (%)	31	39	47

Introduction – Methodology – Results - Conclusion

3 examples related to climate change

- 2 strategies to avoid abiotic stress: drought, warm temperature
  - Early sowing to escape dry weather during flowering
  - Screening adapted genetic resources
- Seed research on model species: courtesy of Julia Buitink (ANR-REGULEG)
  - measuring consequences of CC on progeny



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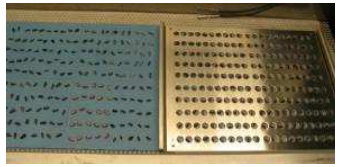
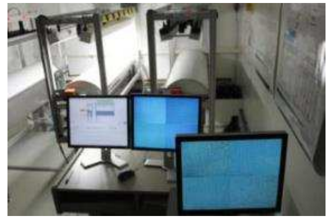
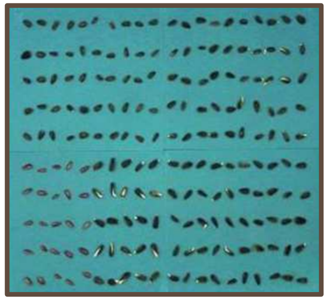
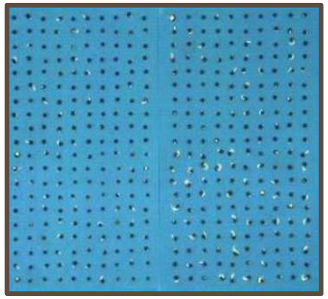
Lab screening methodology

- Automated germination time courses from 5 to 30°C
- Top of thick blotting paper sown with sub-replicates of 25 seeds per camera

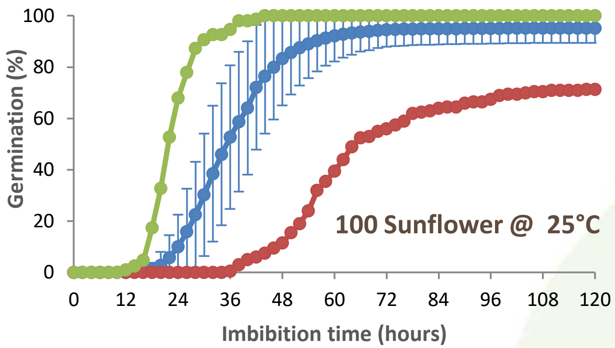


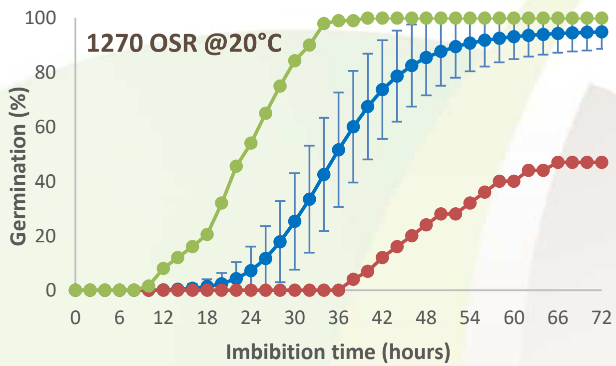
Sunflower
8 x 25

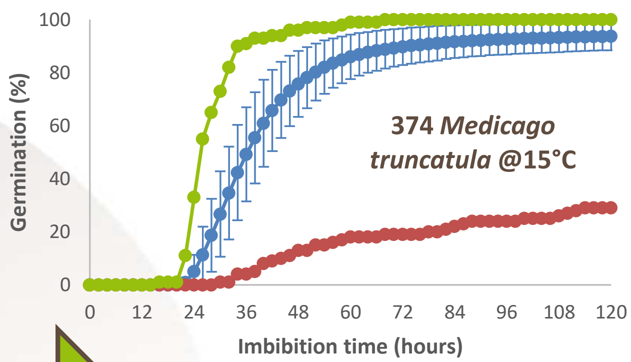


Oilseed rape
16 x 25

Medicago
24 x 25





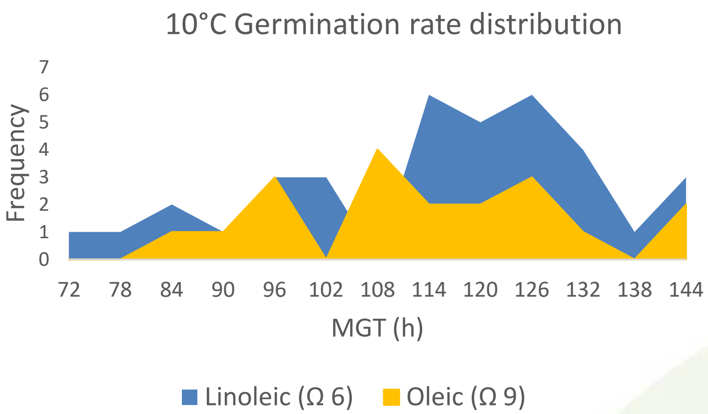


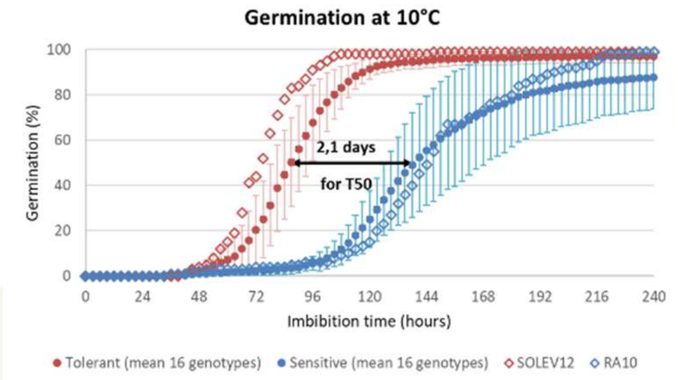
- mean of n samples @ T°C
- Min: slowest seeds from n samples
- Max: more rapid seeds from n samples

Introduction – Methodology – Results - Conclusion

Cold tolerance in sunflower

- No effect of oil composition on germination rate at 10°C





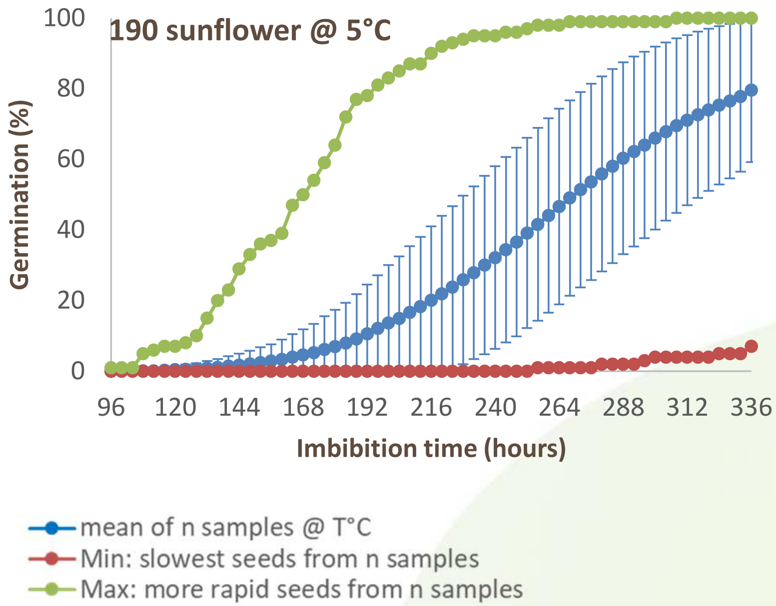
- Germination and growth differ for temperature response

Genetic type	Oil	**Base T°C germination**	**Base T°C seedling growth**
Hybrid	Linoleic	2.7 ± 0.24 bc	5.8 ± 0.48 bc
Female Line	Linoleic	2.3 ± 0.30 bc	5,6 ± 0.61 bc
	Linoleic	2.2 ± 0.51 b	6.3 ± 0.56 b
	Oleic	1.7 ± 0.08 bc	6.3 ± 0.65 b
	Oleic	1.6 ± 1.21 cd	5.5 ± 0.67 c
Male Line	Linoleic	2.6 ± 0.25 bc	5.7 ± 0.35 bc
	Linoleic	1.9 ± 0.36 d	5.8 ± 0.39 bc
	Linoleic	3.6 ± 0.22 a	8.3 ± 0.67 a
	Oleic	0.4 ± 0.04 e	6.2 ± 0.37 bc
General mean		**2.2 ± 0.9**	**6.2 ± 0.8**

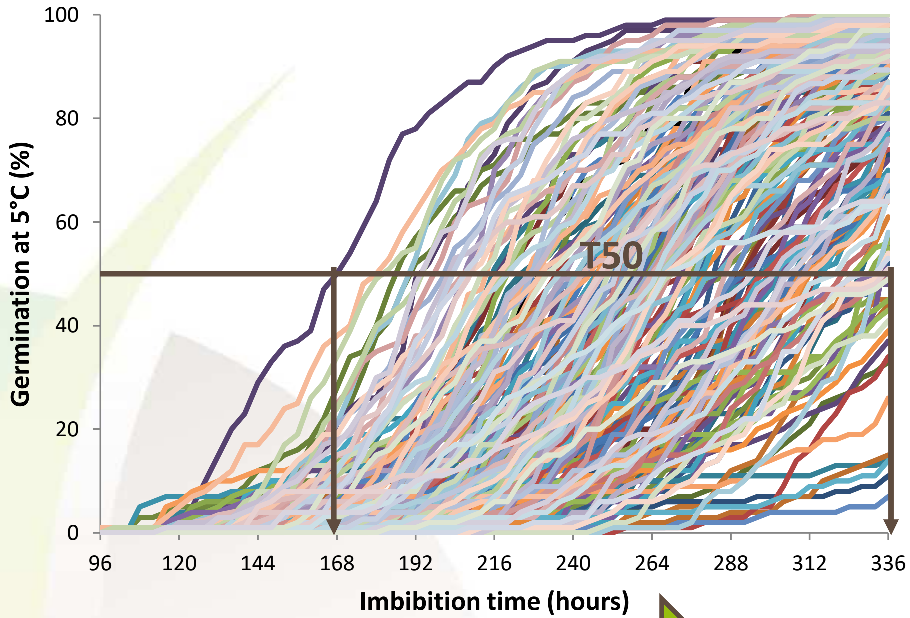
Wagner et al., 2019 in *ISTA Symposium Proc.*

Higher diversity at lower temperature

- 190 samples (4 x25) at 5°C during 14d



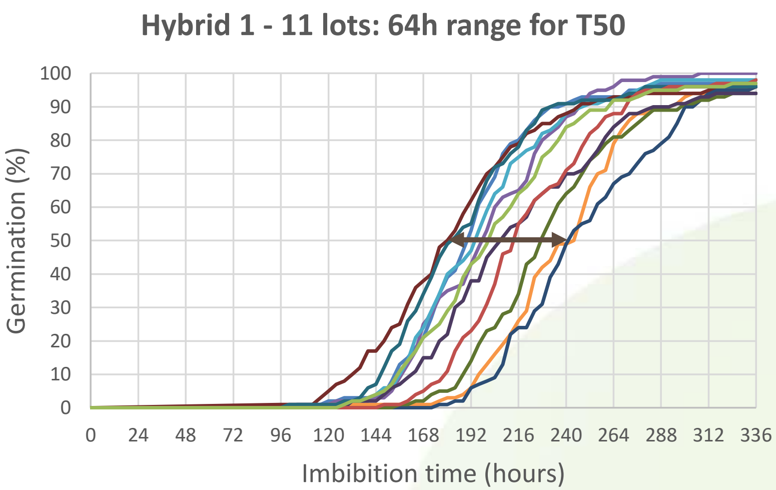
- From 7 to 100% germination and one week range for sunflower T50 at 5°C **± 0.5°C**

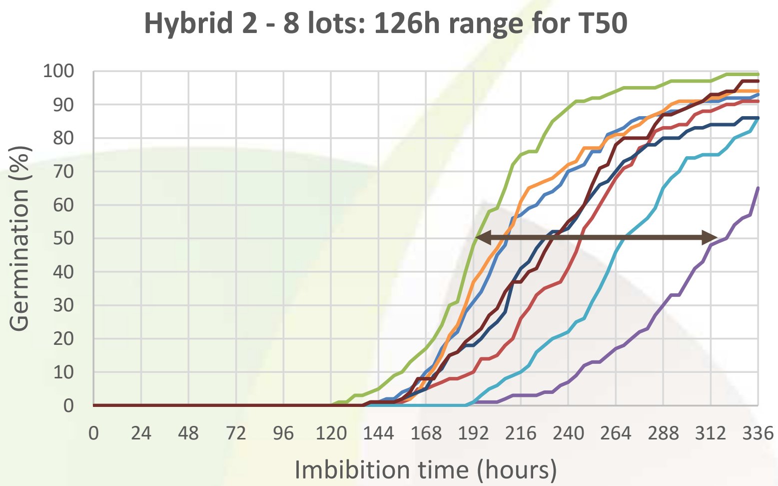


Adapted hybrid for early sowings but also suitable seed lot

- 2 hybrids produced in 2019 in 8 environments
- Germination response to 5°C depends on hybrid and seed lot

Plus la levée sera rapide, plus la durée d'exposition des graines et jeunes plantules vis-à-vis des oiseaux et autres ravageurs sera limitée dans le temps. (©Terres Inovia)



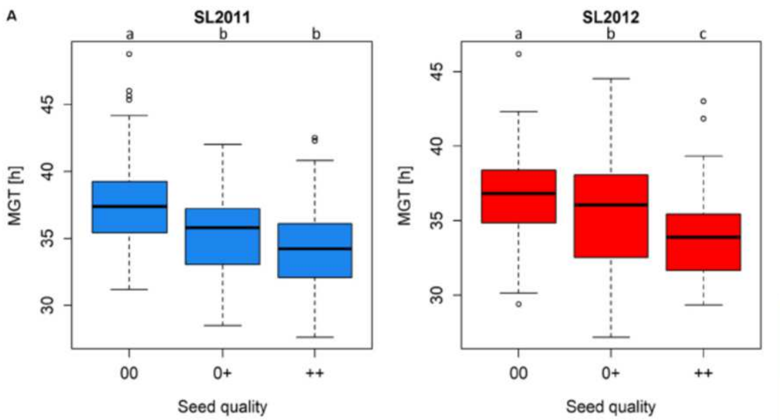


Introduction – Methodology – **Results** - Conclusion

Screening for winter oilseed rape vigour

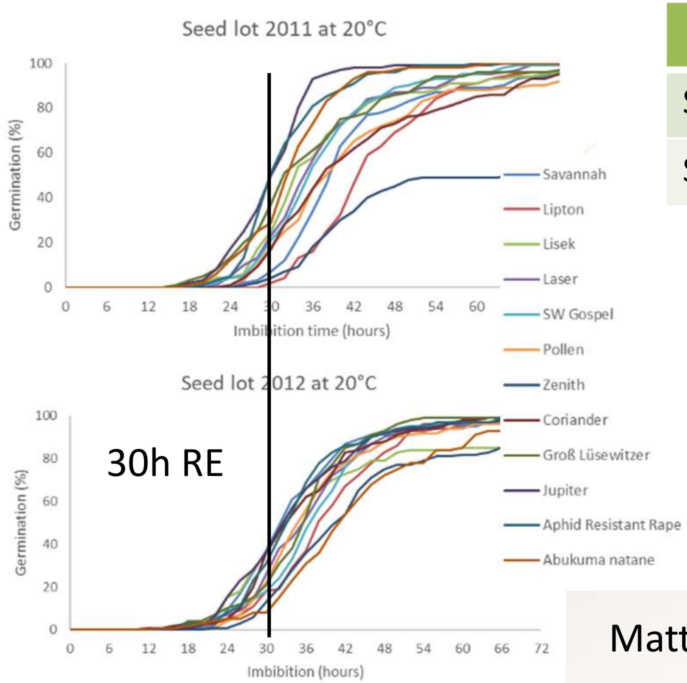
PLANT-KBBE
CONVIGOUR
2011-2014

- Impact of breeding on seed germination rate
- Diversity set of 248 cultivars produced twice

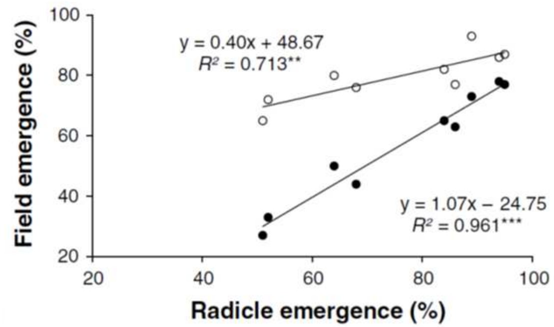


Hatzig *et al.*, Frontiers in Plant Science 2018

- 12 DS as extreme for field trials



	MGT (h)	GER (%)
SL2011	39.9 ± 1.5	93.5 ± 13.3
SL2012	36.5 ± 0.6	96.8 ± 3.6



Matthews *et al.*, Seed Sc.&Technol. 2012

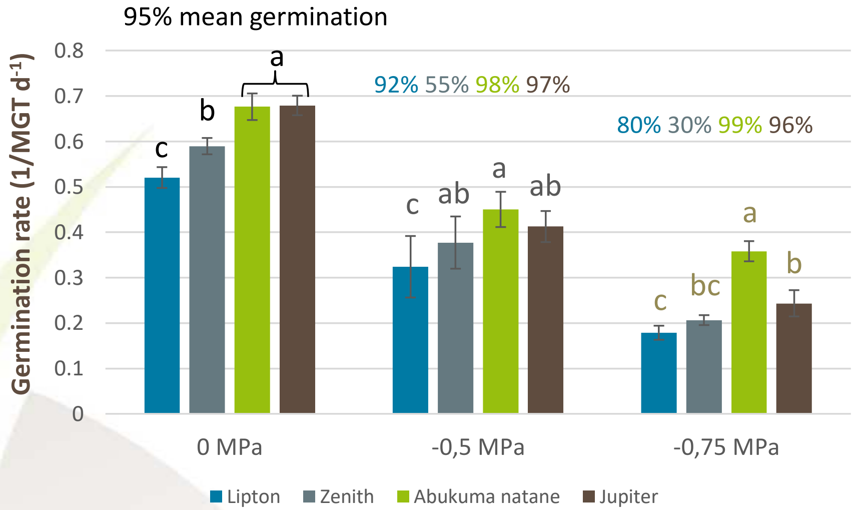
Introduction – Methodology – Results - Conclusion

Screening for WOSR water stress

- PEG solutions in plastic boxes at 20°C and lateral lightening for imaging

248 cultivars
2 DH populations } x 2 lots → 36 extreme genotypes
45 elite lines

Mean results SL2011	11°C	15°C	20°C	24°C	0 MPa	-0.5 MPa	-0.75 MPa
T50 (hours)	115.4 [64-419]	63.6 [42-167]	37.1 [27-72]	26.0 [19-44]	40.6 [31-69]	71.1 [49-135]	119.6 [66-171]
Germination (%)	79 [13-99]	93 [26-100]	93 [52-100]	97 [77-100]	95 [75-100]	88 [21-100]	79 [30-99]



Introduction – Methodology – Results - Conclusion

CONVIGOUR
2011-2014

Field emergence screening towards virtual modeling

- Space and time consuming trials
  - Several locations and sowing dates
- Early sowing conditions difficult to reproduce
- Multi–abiotic stress + biotic

Early sowing 2012

Asendorf	Einbeck	Hohenlieth	Le Rheu	Leutewitz	Malchow	Prémesques	Seligenstadt	Vierville	FE Mean
60.1	58.2	79.1	45.5	56.6	55.7	30.8	10.7	48.0	49.4

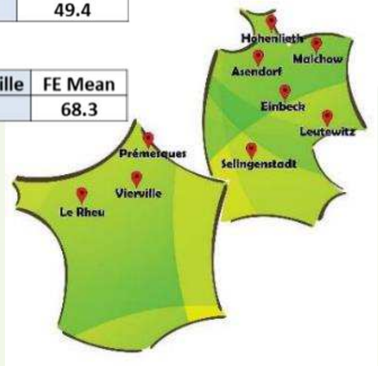
Early sowing 2013

Asendorf	Einbeck	Hohenlieth	Le Rheu	Leutewitz	Malchow	Prémesques	Seligenstadt	Vierville	FE Mean
82.1	57.1	82.5	62.9	62.3	47.6	65.6	87.0	-	68.3

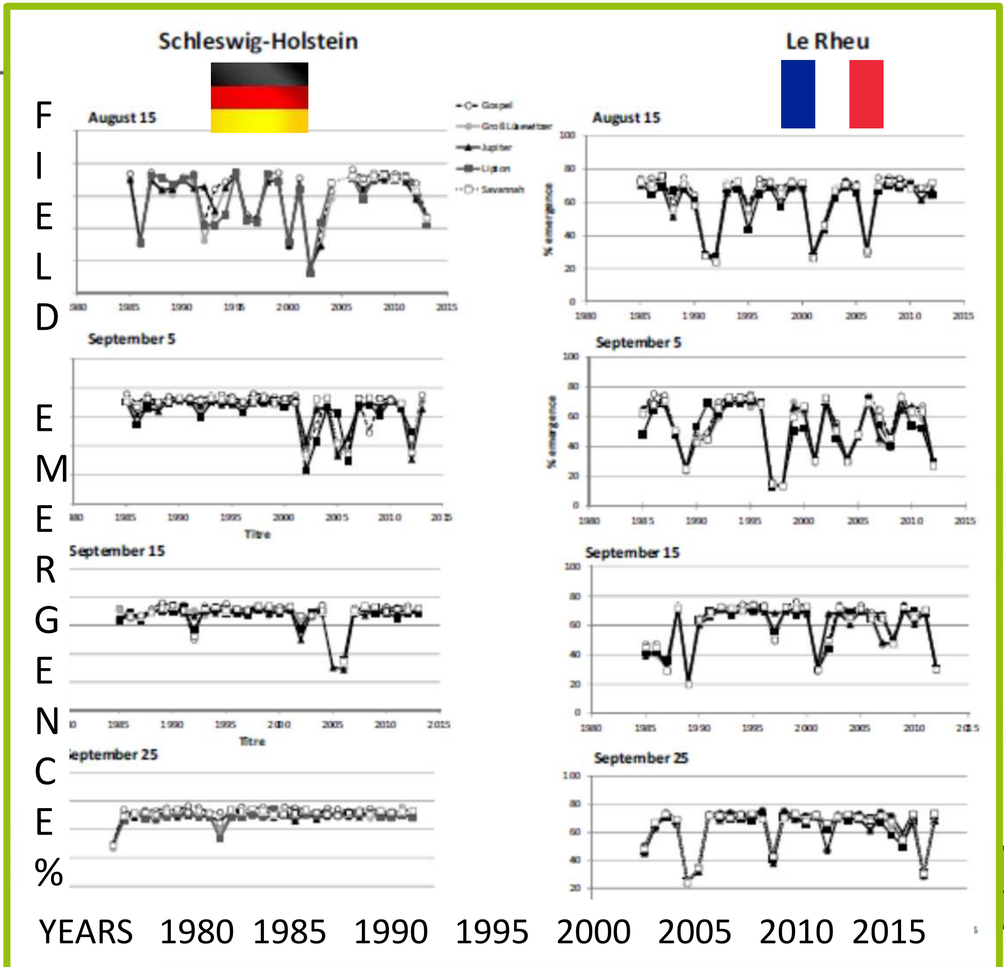
Emergence ≥75%

Emergence <50%

WOSR field trials



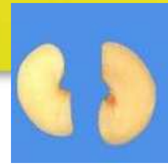
Field emergence screening towards virtual modeling



- Deep phenotyping for 5 cultivars: germination + growth
- Climatic and soil data inputs
- Model validation with 2014 field trials
- 1080 virtual sowings:
  4 sowing dates, 2 locations, and 27 years (1985-2012)

Dürr *et al*., 2016, Europ. J. Agronomy

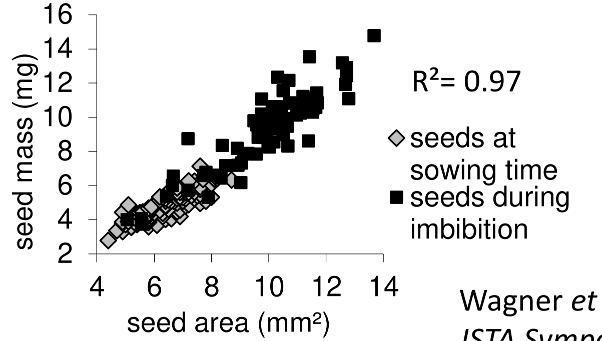
Conclusion



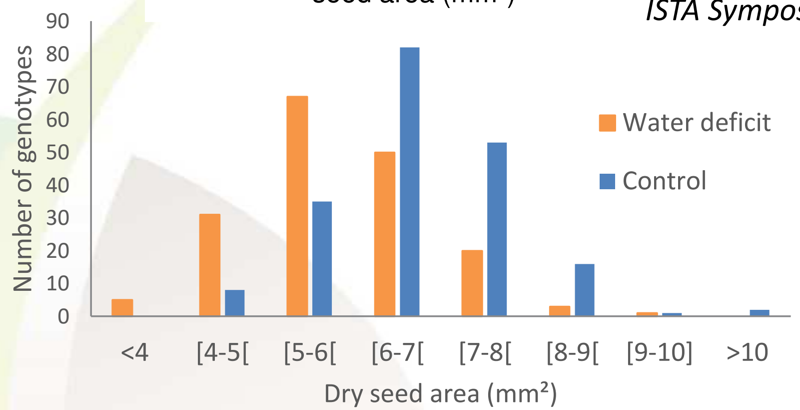
What happens to seed traits when mother plants are grown under water stress conditions?

ANR REGULEG (2016-2021) : Identifying regulators of legume seed adaptation to environmental changes , Coord. Julia Buitink

- 200 sequenced ecotypes x 3 plants cultivated in two environments
  - Well-watering
  - Water-stress after flowering start
- Less seed per plant and some accessions not available for phenotyping
  - 197 accessions for control
  - 177 for water-deficit
- Lighter and smaller seeds for water stressed plants



Wagner *et al.*, 2013 in *ISTA Symposium Proc.*



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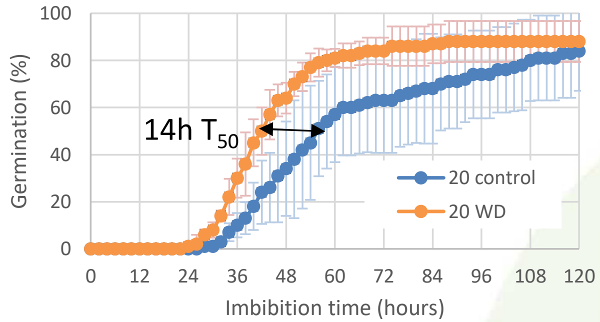
Faster germination for seeds produced with water stress

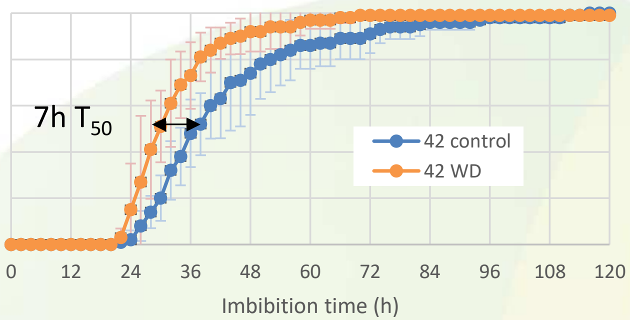
- Mean germination traits at 15°C: 177 accessions for each condition and [range]

Condition	MGT (h)	SD	T10 (h)	T30 (h)	T50 (h)	T70 (h)	Uniformity	Germination (%)
Control	42.4 [33-60]	14.1	28.1	33.6	38.8 [30-56]	46.2	22.7 [12-67]	94.7 [84-100]
Water stress	38.0 [30-65]	13.3	25.9	30.2	34.2 [26-70]	41.8	21.7 [8-66]	92.6 [30-100]

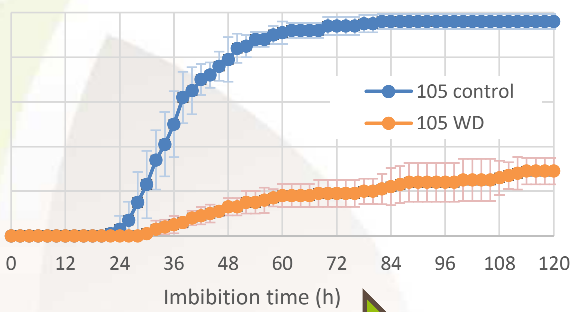
Genotype 20: 84% germination



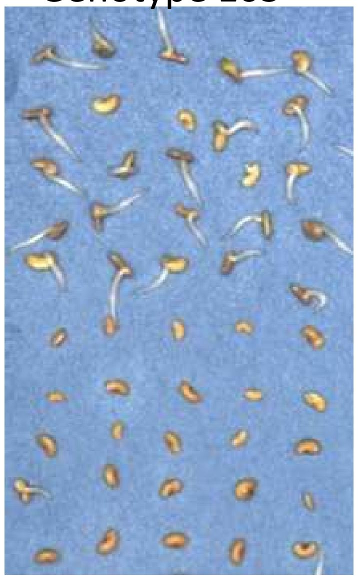
Genotype 42: 100% germination



Genotype 105: 30% germination



Genotype 105



Fresh and hard seeds for genotype 105 WD

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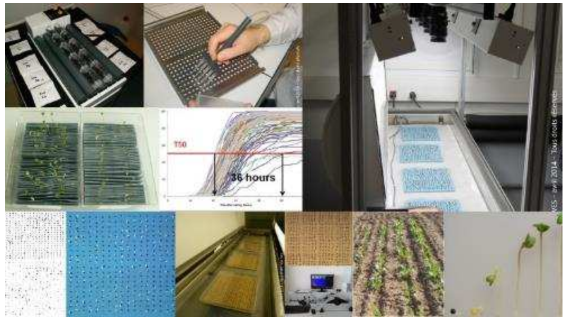
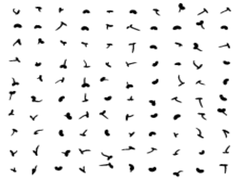
Germination: first stage of plant development, main seed functionnal trait

- Accuracy and reproducibility of germination curves
  - total germination versus germination rate
- Single seed traits thanks to imaging
  - germination but also size, imbibition, radicle early growth
- Emergence = germination + growth
  - Automation of seedling heterotrophic growth
  - Germination a water-limited process
    water stress studies to improve
  - Interaction with biotic factors





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Many thanks to…

- Amine Abbadi, Fabien Ancellin, Thierry André, Christophe Bailly, Benoit Bleys, Julia Buitink, Audrey Dupont, Carolyne Dürr, Philippe Garreau, Simon Goertz, Véronique Hansen, Marion Laporte, Lydie Ledroit, Pierre Lerebours, Luc Mallet, Nathalie Nesi, Stan Matthews, Alison Powell, Takashi Shinohara, Sandrine Stievenard

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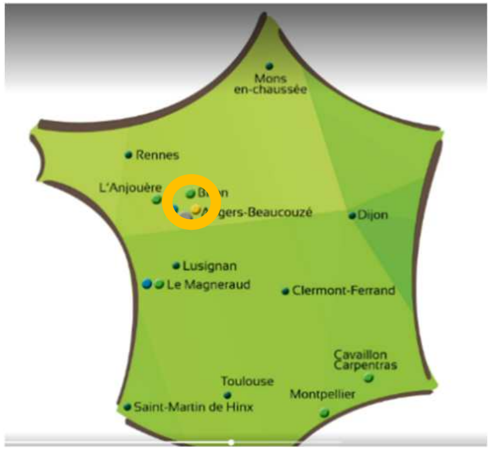

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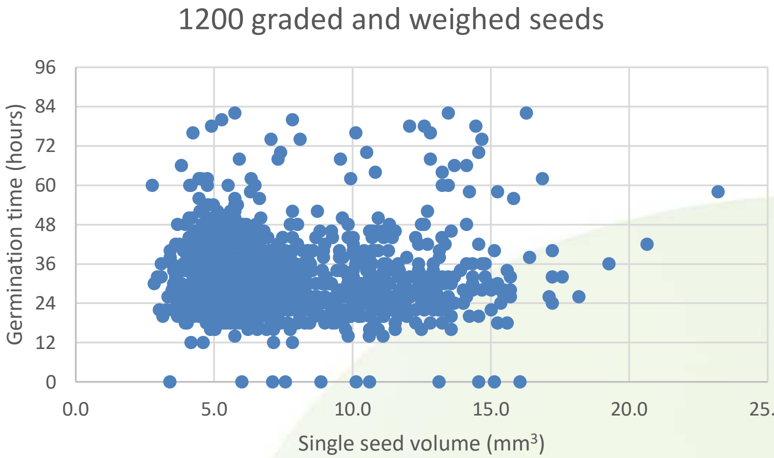
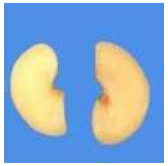
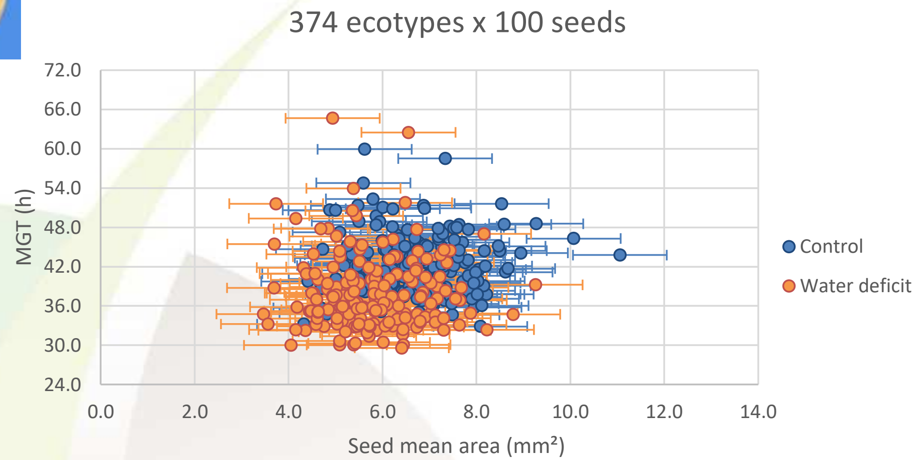
Germination rate and seed size: is there a link?

- No relationship found for the two species which seed size is a good proxy of seed mass

Code	TSW (g)
H1C1	5
H1C2	6,9
H1C3	9,1
H2C1	3,8
H2C2	6
H2C3	8,5
L1C1	3,6
L1C2	3,9
L1C3	4,8
L2C1	3,7
L2C2	4,1
L2C3	5,1







Bonus Results