BOKU UNIVERSITY

Legume Generation

Defensio

12. May 2025

Phenotyping of European soybean varieties with different time to maturity for drought tolerance

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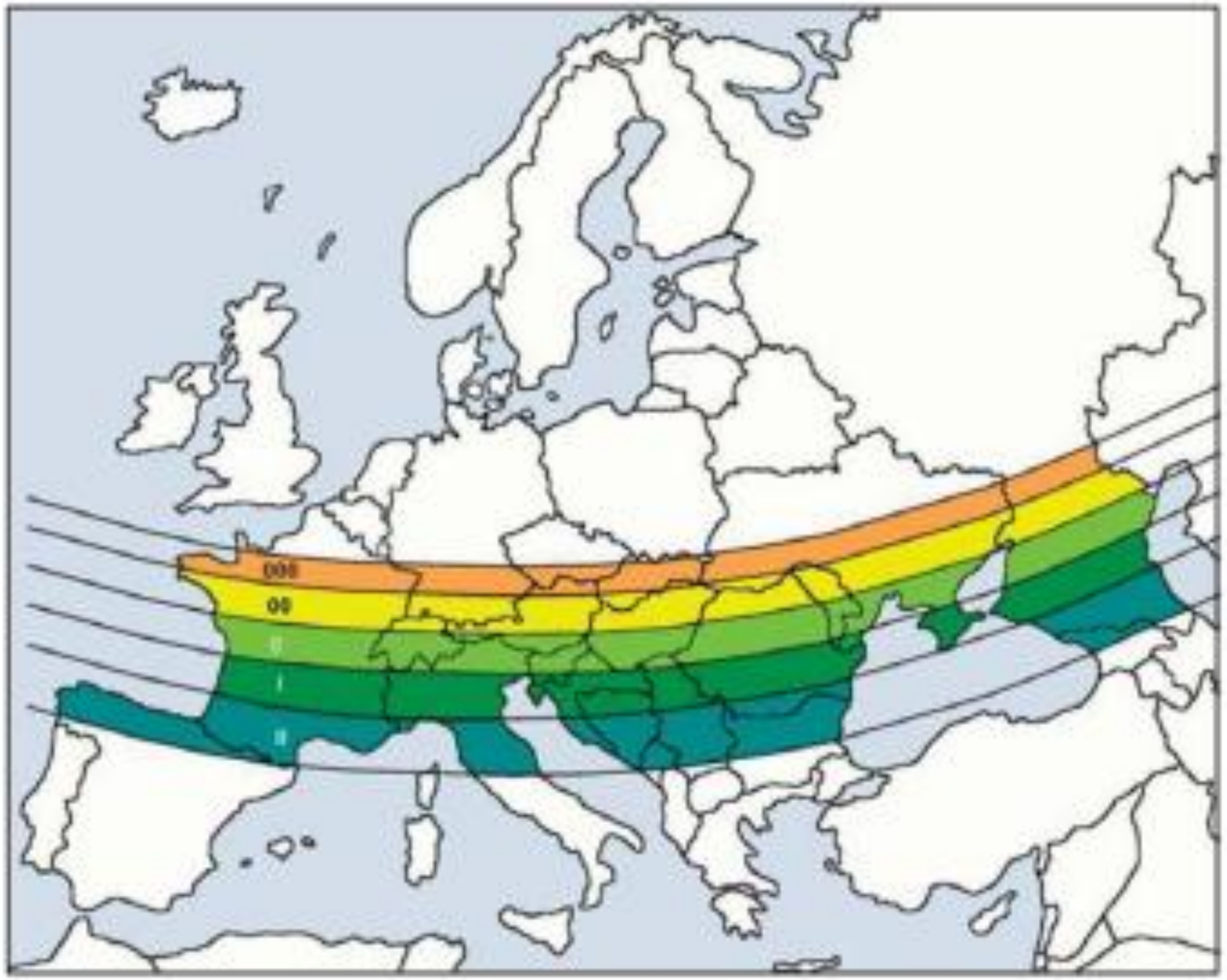
Overview

- Introduction
- Scientific questions
- Experimental design + Weather
- Phenotyping methods
- Results
- Discussion & Conclusion

Introduction – Soybean maturity groups



Maturity group classification

000 = very early matured
00 = mid-early matured
0 = normal to late matured
I = late matured
II = very late matured

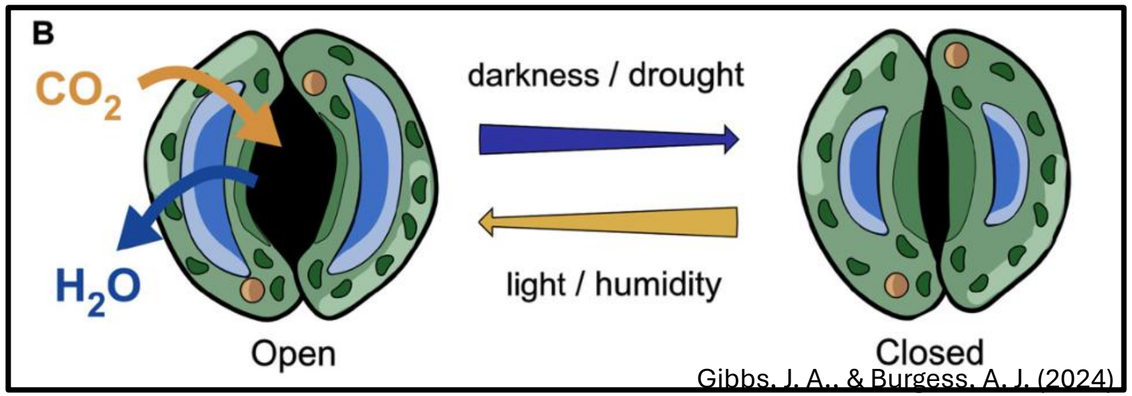
Miladinović, J., & Đorđević, V. (2011). Soybean morphology and stages of development. *Soybean*, 45-71.

Introduction – Drought stress

Soybean needs warm conditions: T-optimum at 25 – 30°C,
BUT: sufficient water supply is necessary, e.g. regular rain events.

At drought stress events …

… stomata are closed.
… transpiration is strongly limited.
… water content in leaves decreases.
… leaves warm up due to limited evaporation



Gibbs, J. A., & Burgess, A. J. (2024)

Complex task for plant breeders: phenotyping of drought stress!

Introduction – Phenotyping

Aim: Find measurement methods to identify optical traits as fast and reliable as possible

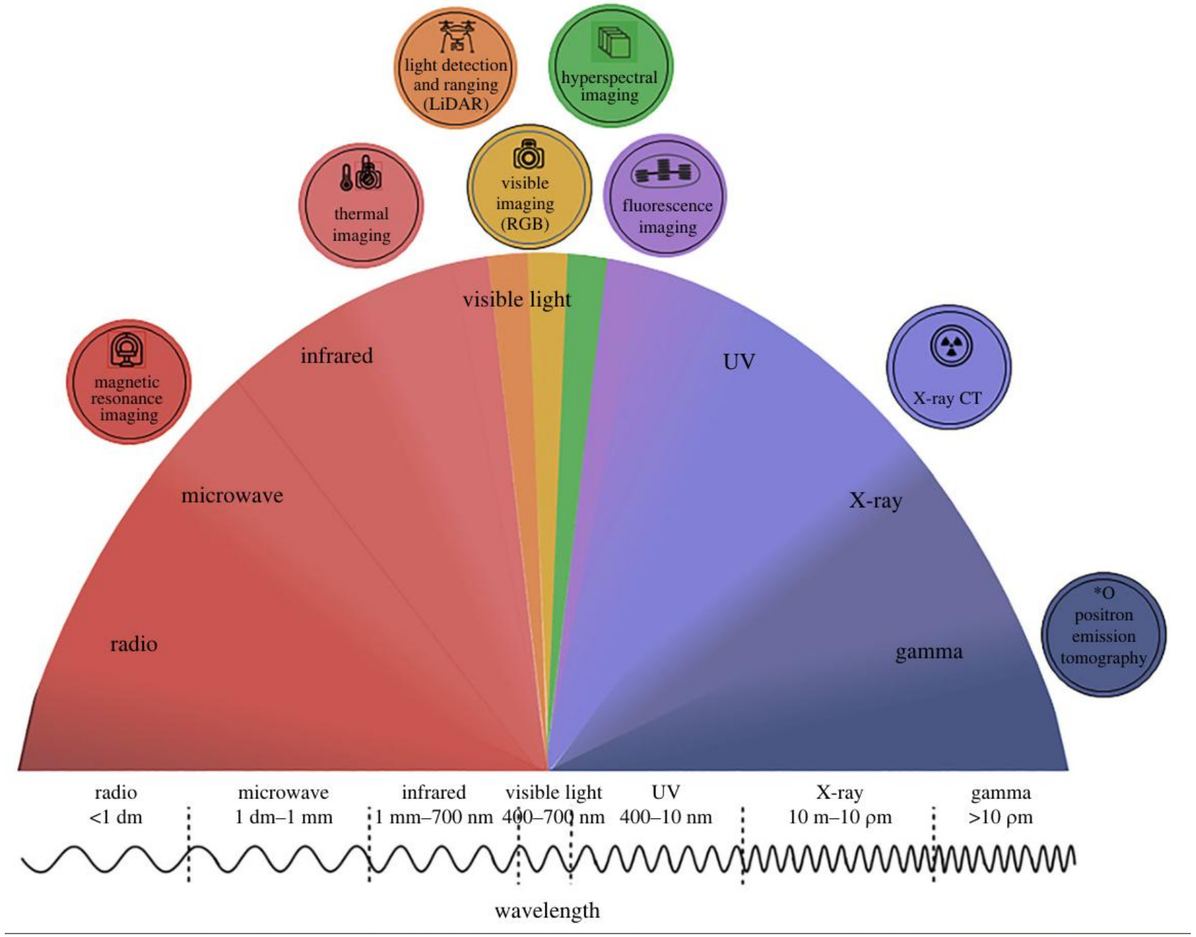
Thermography

Thermal measurement of canopy temperature

Hyperspectroscopy

Spectral reflection of leaf surface

➔Calculation of hyperspectral indices



Al-Tamimi, N., Langan, P., Bernád, V., Walsh, J., Mangina, E., & Negrão, S. (2022). https://doi.org/10.1098/rsob.210353

Scientific questions

1) Are there **significant differences between genotypes** for **grain yield** within MG 000, MG 00 and MG 0/I?

2) What is the influence of **time to maturity to grain yield** between MG?

3) Are there significant differences between genotypes of MG 00 for **stomatal density**?

4) Which of 16 **hyperspectral reflectance indices** can show highest **correlation** with **grain yield** in soybean MG 000, MG 00 and MG 0/I?

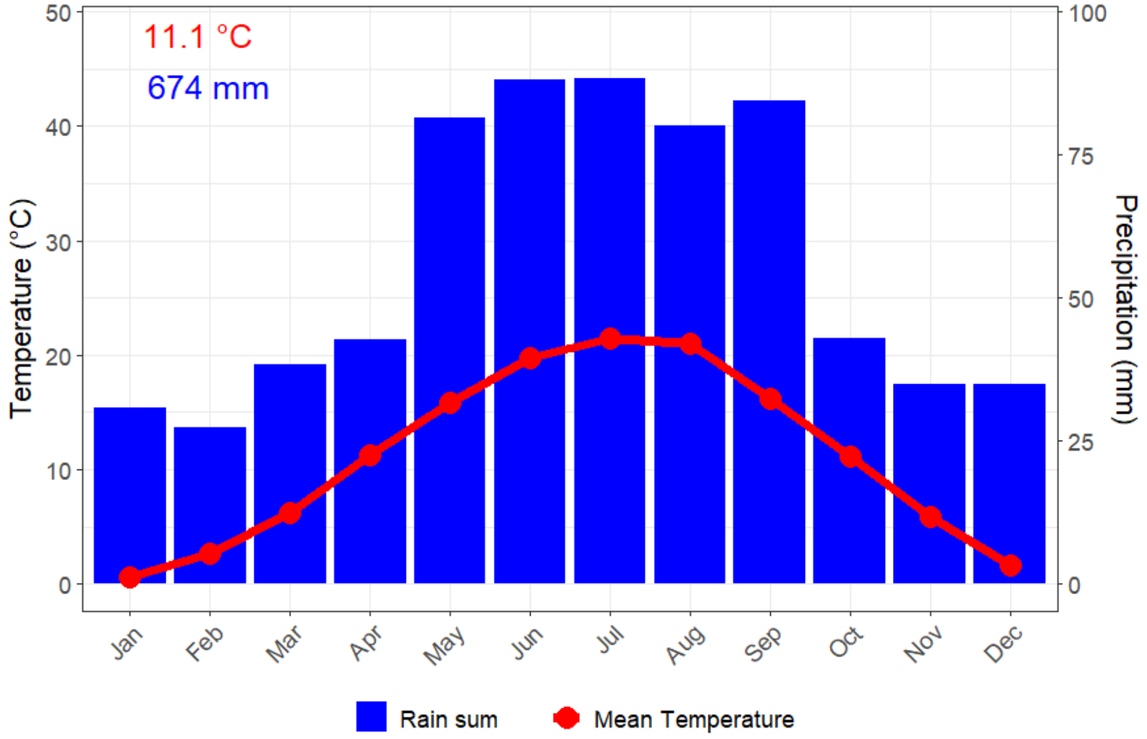
5) How strong is the **correlation** between **grain yield** and **siCWSI** in soybean varieties?

Location & Weather

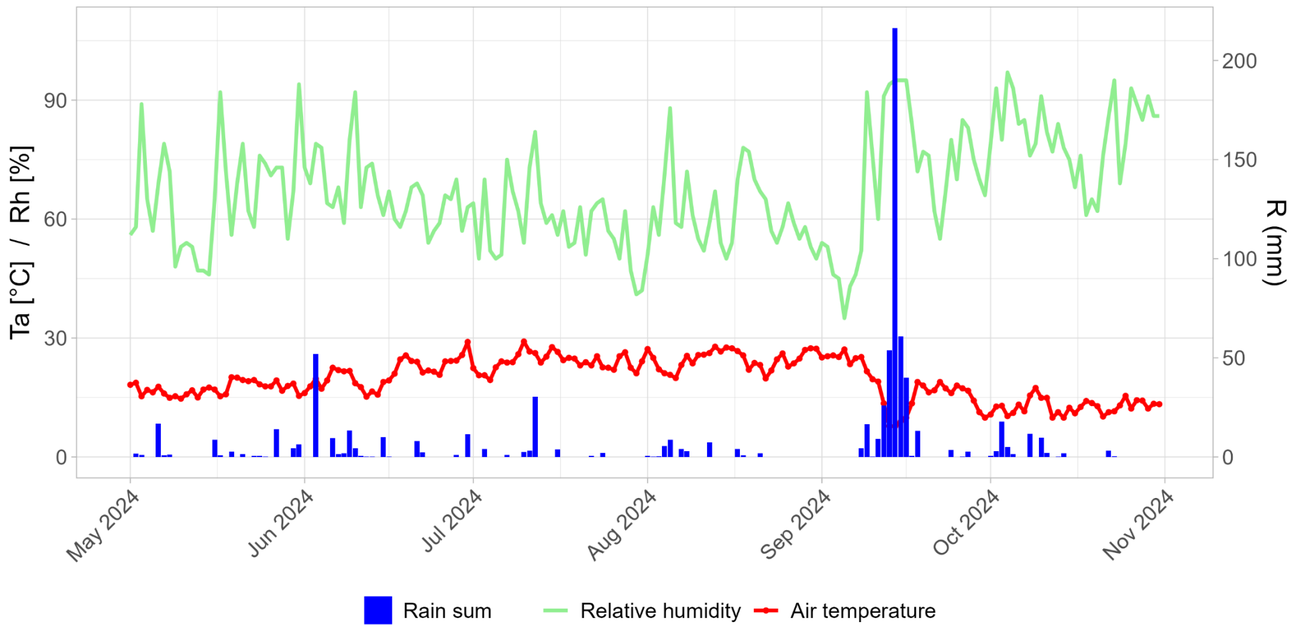
Month	May	Jun	Jul	Aug	Sep	Oct	**Total**
Mean air temperatures, T_a (°C)							*Mean*
Climate norm	15.8	19.8	21.4	21.0	16.2	11.1	**17.6**
2024	17.2	21.1	24.0	24.6	17.8	12.7	**19.6**
Precipitation sums, R (mm)							*Sum*
Climate norm	81.4	88.0	88.3	80.0	84.3	43.1	**465.1**
2024	60.9	116.2	47.3	35.9	447.2	56.6	**764.1**
Relative humidity, rH (%)							*Mean*
Climate norm	65.0	64.3	63.7	66.5	73.0	78.9	**68.6**
2024	65.5	66.8	58.6	61.5	69.8	81.3	**67.2**

Tulln, Austria (178m)
48°19'N / 16°04'E

Climate period: 1995 - 2024



Tulln, growing season 2024



Experimental design

- Field experiment in **Tulln** (Austria) in summer season **2024**
- 2,5 m long single-rows, 50 cm row spacing; lattice design
- Sowing date: 10. May 2024
- Harvesting date: September/October 2024
- EU-certified soybean varieties, partly breeding lines
- 7 European breeders
- 4 Trials with different maturity groups (MG)

Trial No.	Trial name	Abbr.	Maturity group	Plots	Rep	Plot No.	α-lattice design
1	Yield 1	Y1	000 and earlier	60	2	101 - 160	10x6
2	Yield 2	Y2	00	45	2	201 - 245	15x3
3	Yield 3	Y3	0 and I	30	2	301 - 330	10x3
4	Food	Food	000 – I	70	2	401 - 470	10x7

row	rep 1			Yield 1			rep 2					
30	119	152	112	159	125	133	131	152	128	105	108	142
29	135	126	118	127	156	141	117	136	133	106	154	139
28	120	131	124	139	150	101	127	140	144	143	125	160
27	146	137	117	109	114	102	156	124	115	145	113	146
26	136	108	147	116	145	130	153	122	135	107	102	104
25	106	113	155	132	104	160	149	112	103	130	120	114
24	149	134	128	107	111	144	157	155	151	141	147	109
23	153	151	143	142	129	148	116	110	159	138	150	137
22	110	121	122	154	115	157	121	132	126	158	134	148
21	105	140	158	103	138	123	129	101	123	118	119	111
col	1	2	3	4	5	6	7	8	9	10	11	12

row	rep 1			Yield 2			rep 2		
30	232	233	223	223	227	238	201	243	228
29	236	239	238	212	208	214	202	234	244
28	203	245	225	233	216	231	210	239	232
27	215	228	212	229	207	230	225	213	205
26	210	237	222	224	219	235	218	209	215
25	231	227	242	220	216	221	220	211	245
24	219	230	244	207	224	214	204	237	226
23	217	243	218	202	229	204	206	236	222
22	209	240	213	201	235	208	242	241	221
21	205	241	211	226	234	206	240	217	203
col	13	14	15	16	17	18	19	20	21

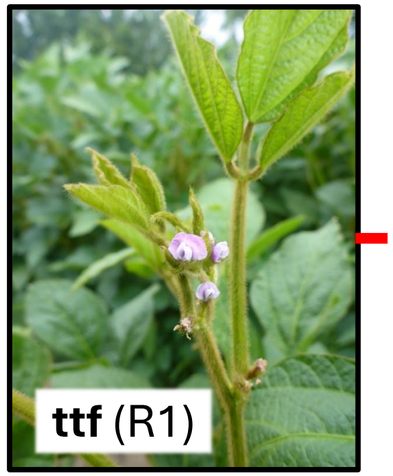
row	rep 1			Yield 3			rep 2		
30	308	310	311	304	314	309			
29	312	306	324	302	317	318			
28	319	316	328	323	305	312			
27	301	303	317	303	315	324			
26	323	325	326	310	330	307			
25	313	320	321	329	322	301			
24	330	314	302	327	328	311			
23	322	315	305	321	319	308			
22	304	327	307	325	313	316			
21	318	329	309	326	320	306			
col	22	23	24	25	26	27			

row	rep 1				Food			rep 2						
30	461	413	437	469	420	447	406	448	402	412	467	419	414	445
29	441	458	453	452	433	419	466	438	454	463	422	421	455	449
28	465	456	439	448	446	449	432	469	423	460	403	409	452	439
27	450	412	457	431	460	462	438	416	432	468	410	401	407	450
26	422	459	428	444	423	435	427	408	433	406	436	415	444	417
25	443	405	445	468	408	403	426	429	427	466	470	447	442	440
24	417	440	451	434	409	455	401	418	411	430	451	428	420	456
23	418	402	424	454	442	410	415	462	459	443	434	458	424	437
22	436	467	470	416	411	463	425	461	441	431	464	426	465	425
21	421	429	464	407	414	404	430	405	446	457	404	435	413	453
col	28	29	30	31	32	33	34	35	36	37	38	39	40	41

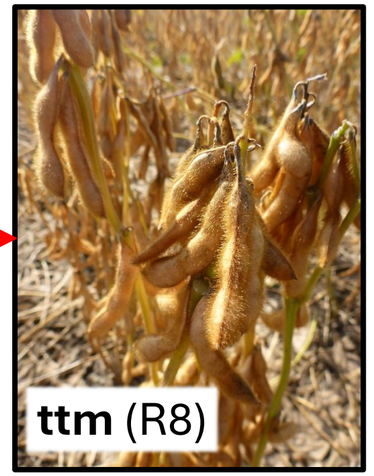
Methods

1) Scoring of **phenotyping properties**:

- Time to flowering (**ttf**) R1 stage
- Time to mature (**ttm**) R8 stage

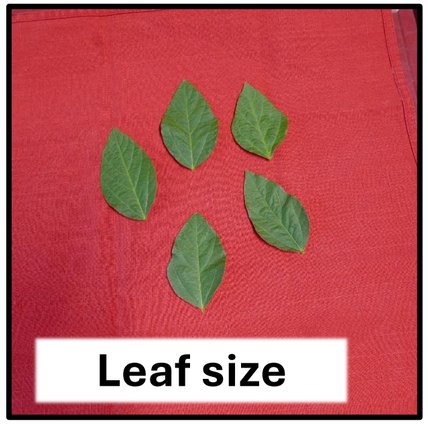


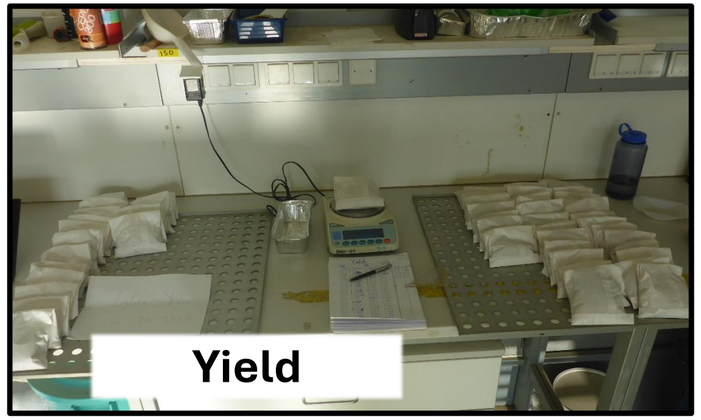
Reproductive time

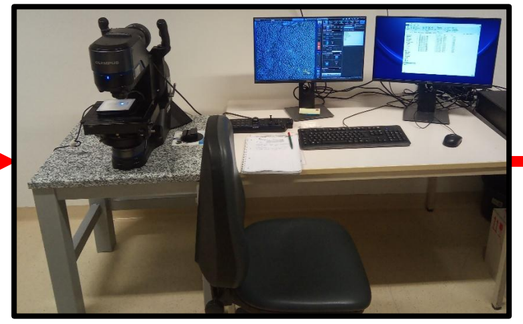


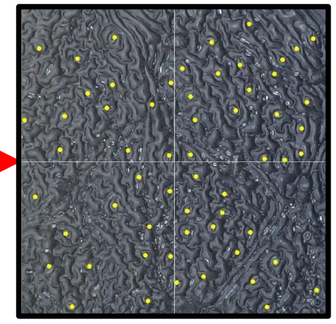
2) Measuring/**Leaf sampling:**

- **Leaf size** (5 middle leaflets)
- **Yield** (harvested seeds of 0.5m^2, converted to dt/ha)
- **Stomatal density** (abaxial leaf surface, only MG 00)









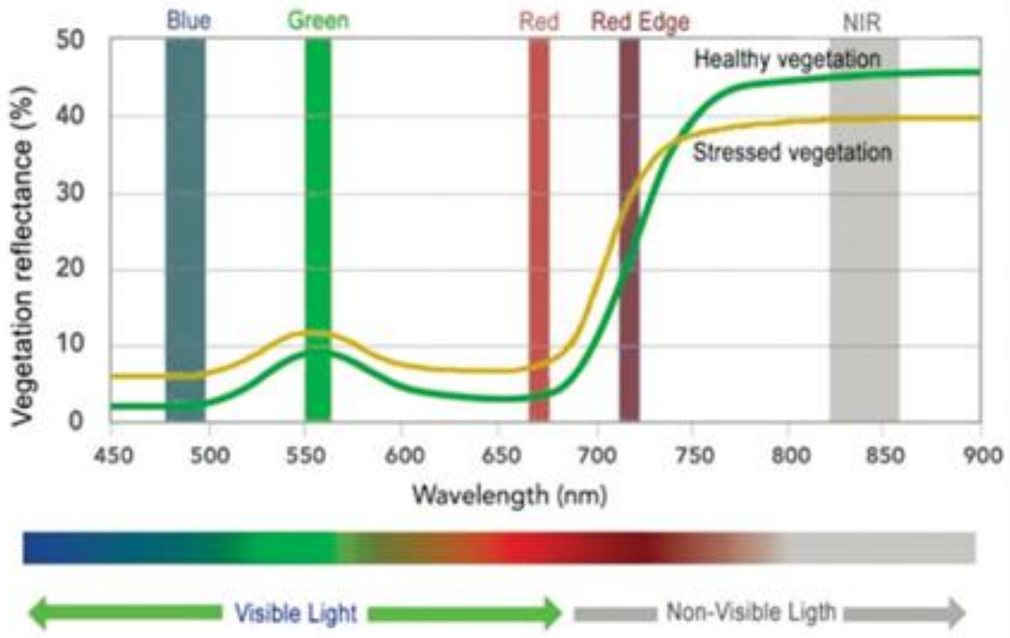
Stomatal density – workflow

Methods

3) **Hyperspectral reflectance**

- Handheld approach, wavelength range: 325 - 1075 nm (VNIR)
- Screenings on sunny days during solar noon (between 11:00 and 14:00), screening period: 14. July to 13. August 2024
- 16 hyperspectral indices chosen:

Abbr.	Index name	Index formula	Reference
Water indices			
WI	Reflectance water index	$\frac{R_{970}}{R_{900}}$	Peñuelas et al. (1993)
NWI-1	Norm. water index 1	$\frac{R_{970}-R_{900}}{R_{970}+R_{900}}$	Babar et al. (2006)
NWI-2	Norm. water index 2	$\frac{R_{970}-R_{850}}{R_{970}+R_{850}}$	Babar et al. (2006)
NWI-3	Norm. water index 3	$\frac{R_{970}-R_{920}}{R_{970}+R_{920}}$	Prasad et al. (2007)
NWI-4	Norm. water index 4	$\frac{R_{970}-R_{880}}{R_{970}+R_{880}}$	Prasad et al. (2007)
NWI-5	Norm. water index 5	$\frac{R_{970}-R_{930}}{R_{970}+R_{930}}$	Prey et al. (2020)
WI-1	Water index 1	$\frac{R_{940}}{R_{915}}$	Christenson et al. (2016)
WI-2	Water index 2	$\frac{R_{990}}{R_{915}}$	Christenson et al. (2016)
WI-3	Water index 3	$\frac{R_{990}}{R_{940}}$	Christenson et al. (2016)
Vegetation, chlorophyll and nitrogen indices			
NDVI	Normalized difference vegetation index	$\frac{R_{NIR}-R_{Red}}{R_{NIR}+R_{Red}}$	Rouse et al. (1973)
MA1-R	RVI, Ratio vegetation index (for soybean)	$\frac{R_{638}}{R_{674}}$	Zhang et al. (2019)
PSSRa	Pigment specific simple ratio (chloroph. a)	$\frac{R_{NIR}}{R_{675}}$	Blackburn (1998)
PSSRb	Pigment specific simple ratio (chloroph. b)	$\frac{R_{NIR}}{R_{650}}$	Blackburn (1998)
CI	Chlorophyll index (red edge)	$\frac{R_{NIR}}{R_{RE}} - 1$	Gitelson et al. (2005)
NRI	Nitrogen reflectance index	$\frac{R_{NIR}}{R_{Green}}$	Cao et al. (2015)
REIP	Red edge inflection point (N-content)	$R_{700} + 40 * \frac{\frac{R_{670}+R_{780}}{2} - R_{700}}{R_{740}-R_{700}}$	Prey et al. (2020)



Methods

4) **Thermography**:

– Handheld approach, wavelength range: 7 – 14 µm (SWIR)

– Testing material: 11 varieties at trial Y2

– Measurements for daytime at 12:00 and at 15:00 (CEST)

– Thermal indices:

Drought stress level	VPD [kPa]
Low	< 1.5
Mid	1.5 – 2.0
High	>2.0

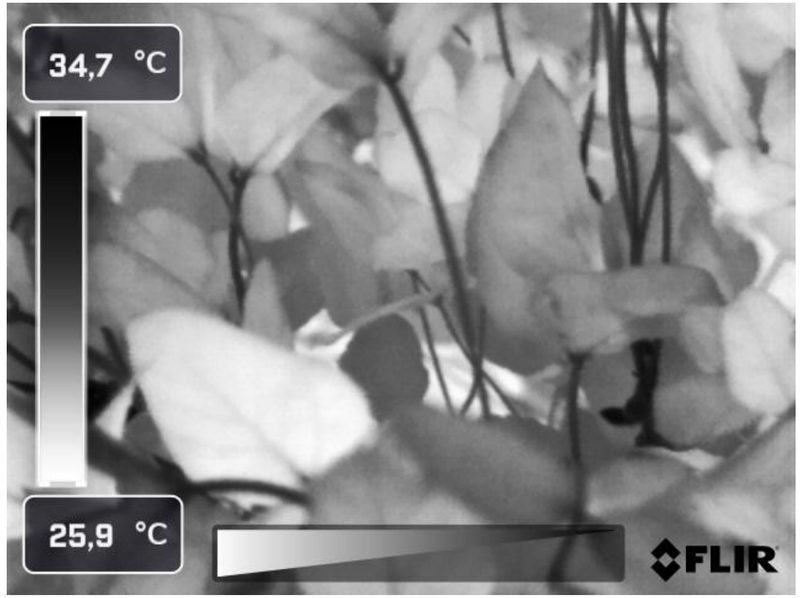
- **Vapor pressure deficit** (VPD) in [kPa]:

- **Air canopy temperature difference** in [°C]:

$$dT_c = T_c - T_a$$

- **Simplified Crop Water Stress Index** (siCWSI):

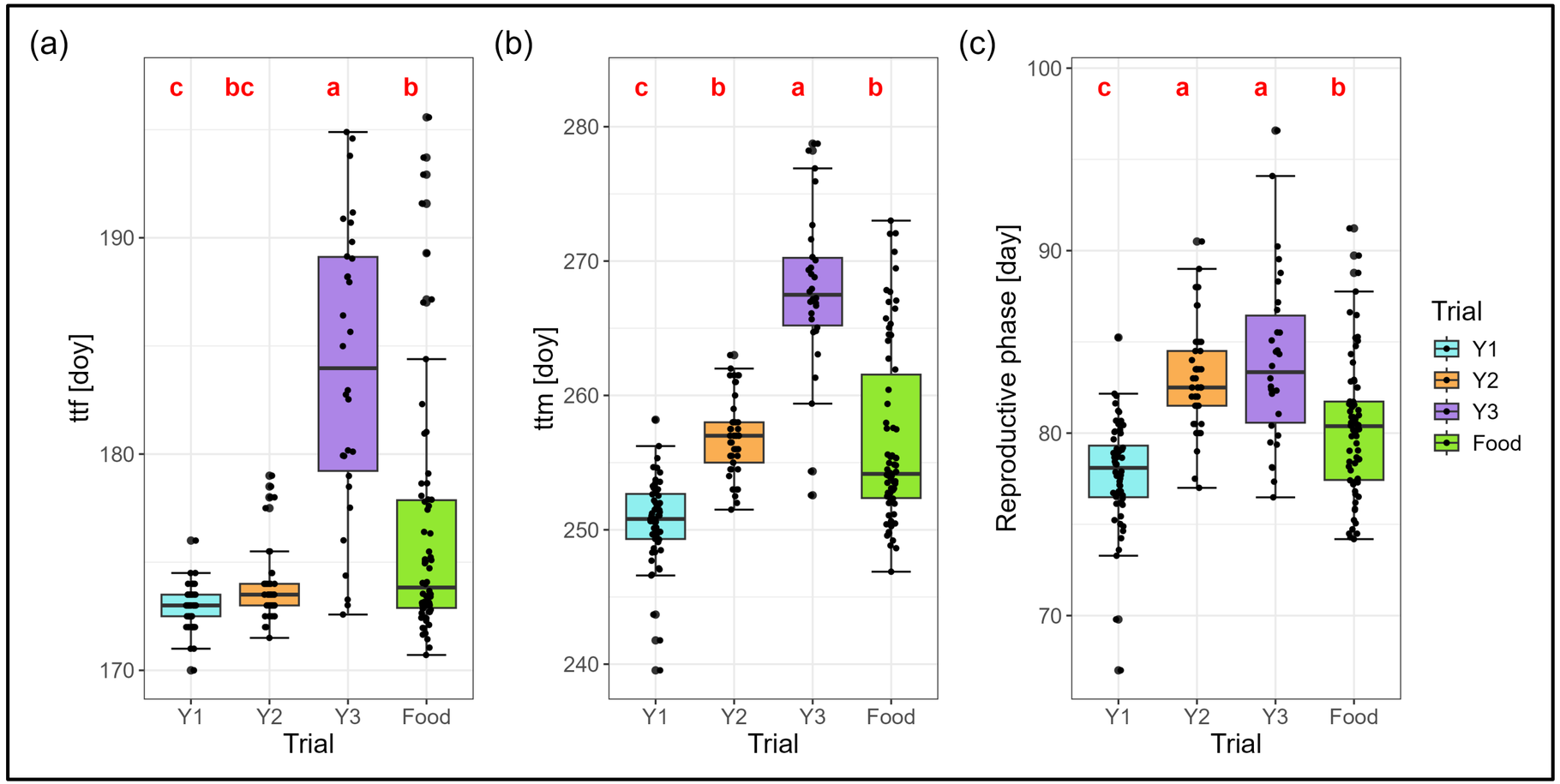
$$siCWSI = \frac{T_c - T_{min}}{T_{max} - T_{min}}$$



Results – Scoring traits

Trials

Y1	= early genotypes	(MG 000)
Y2	= mid early genotypes	(MG 00)
Y3	= late genotypes	(MG 0/I)
Food	= genotypes for food use	(MG 000 – I)



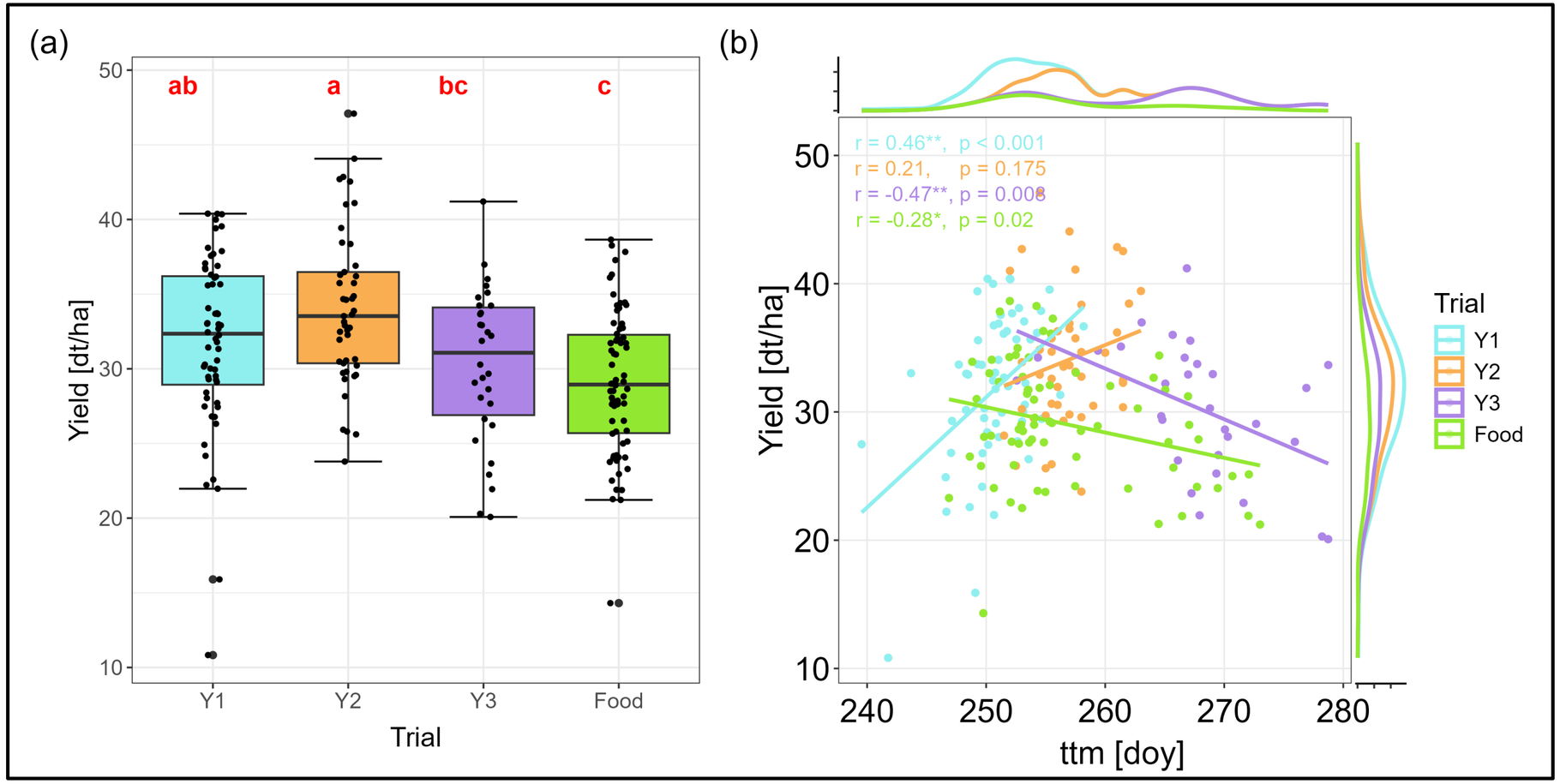
ttf = time to flowering
ttm = time to maturity
doy = day of the year

Sowing date = 131 doy

Results – Yield

Trials

Y1	= early genotypes	(MG 000)
Y2	= mid early genotypes	(MG 00)
Y3	= late genotypes	(MG 0/I)
Food	= genotypes for food use	(MG 000 – I)



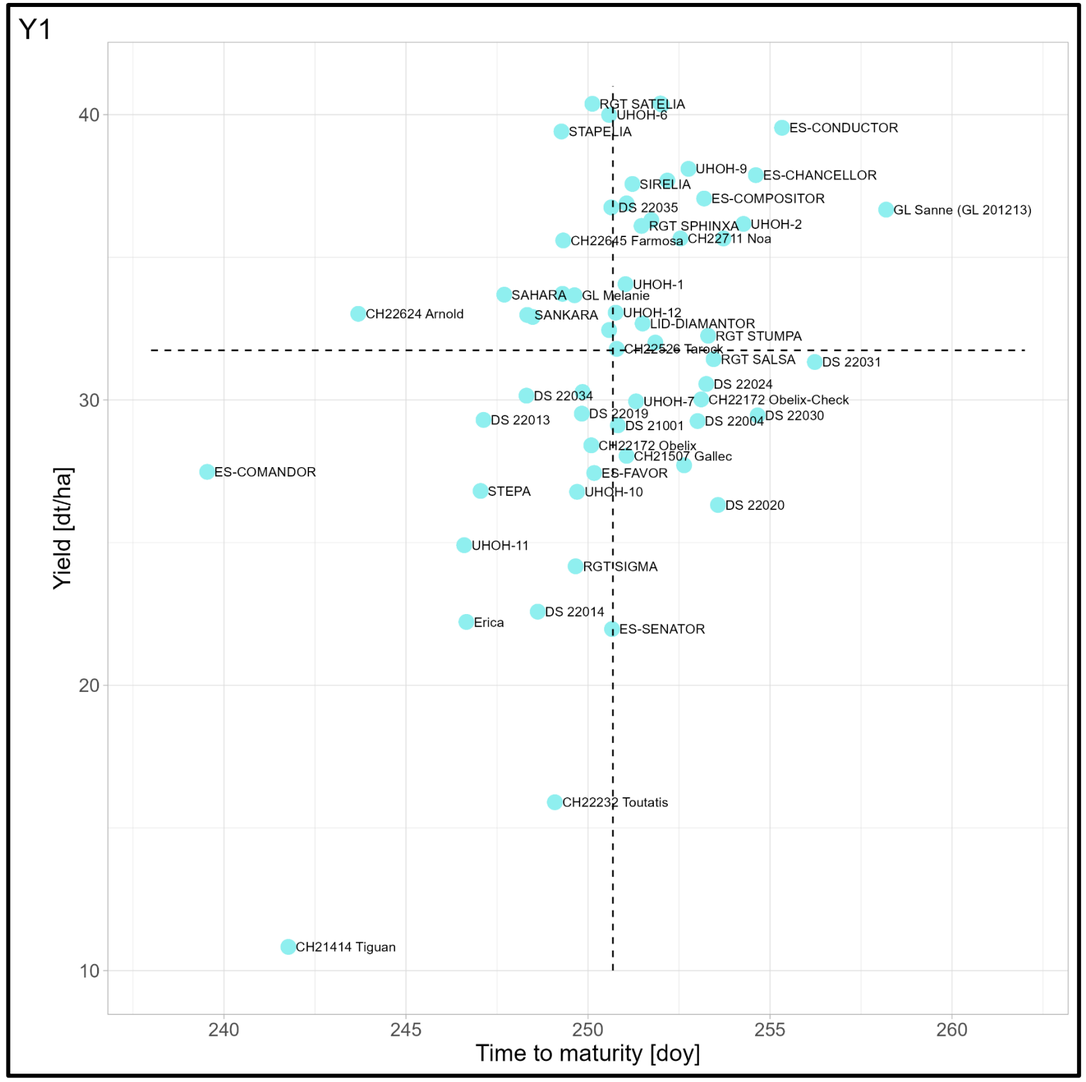
ttm = time to maturity
doy = day of the year

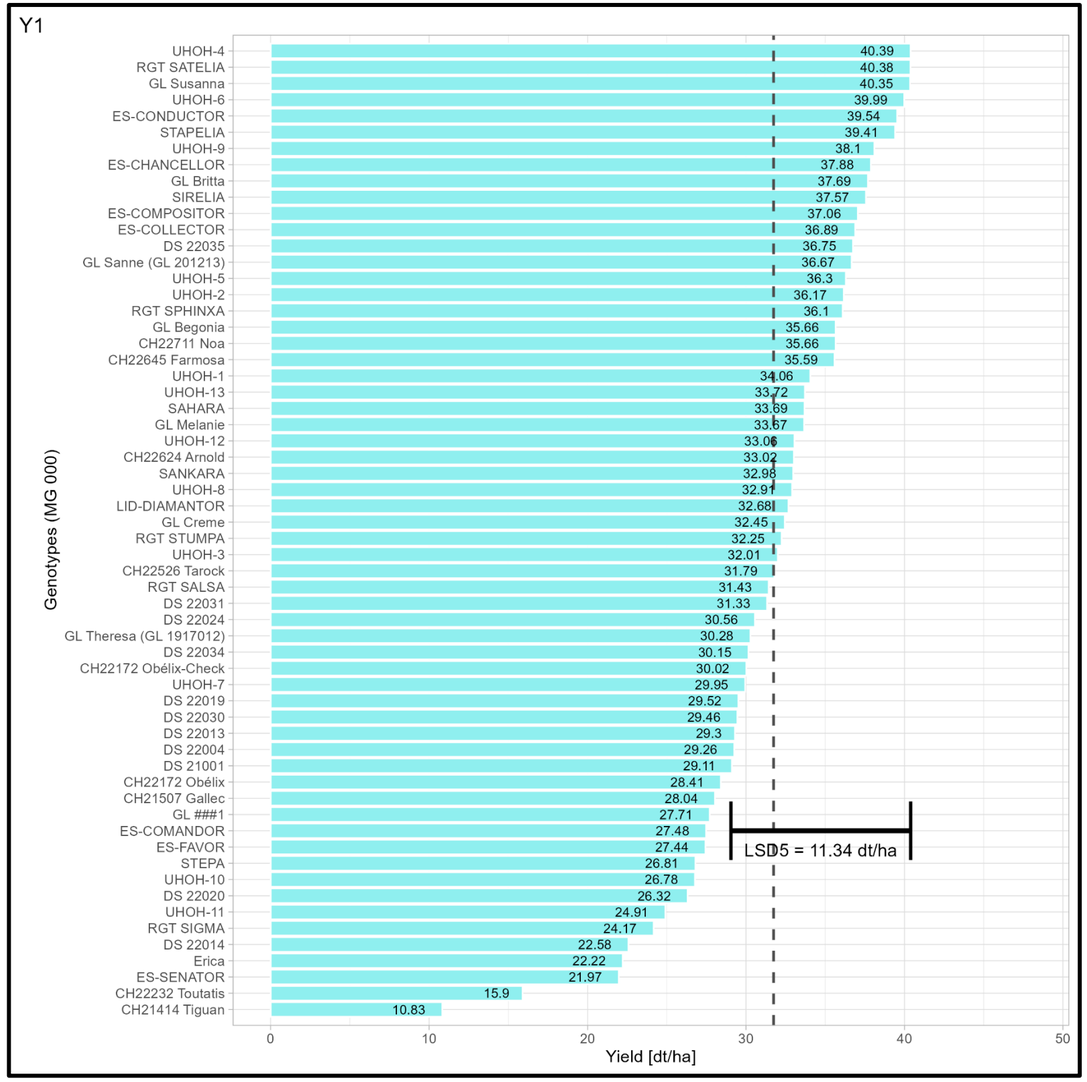
Sowing date = 131 doy

Results – Yield vs. ttm

Y1 = early genotypes (MG 000)

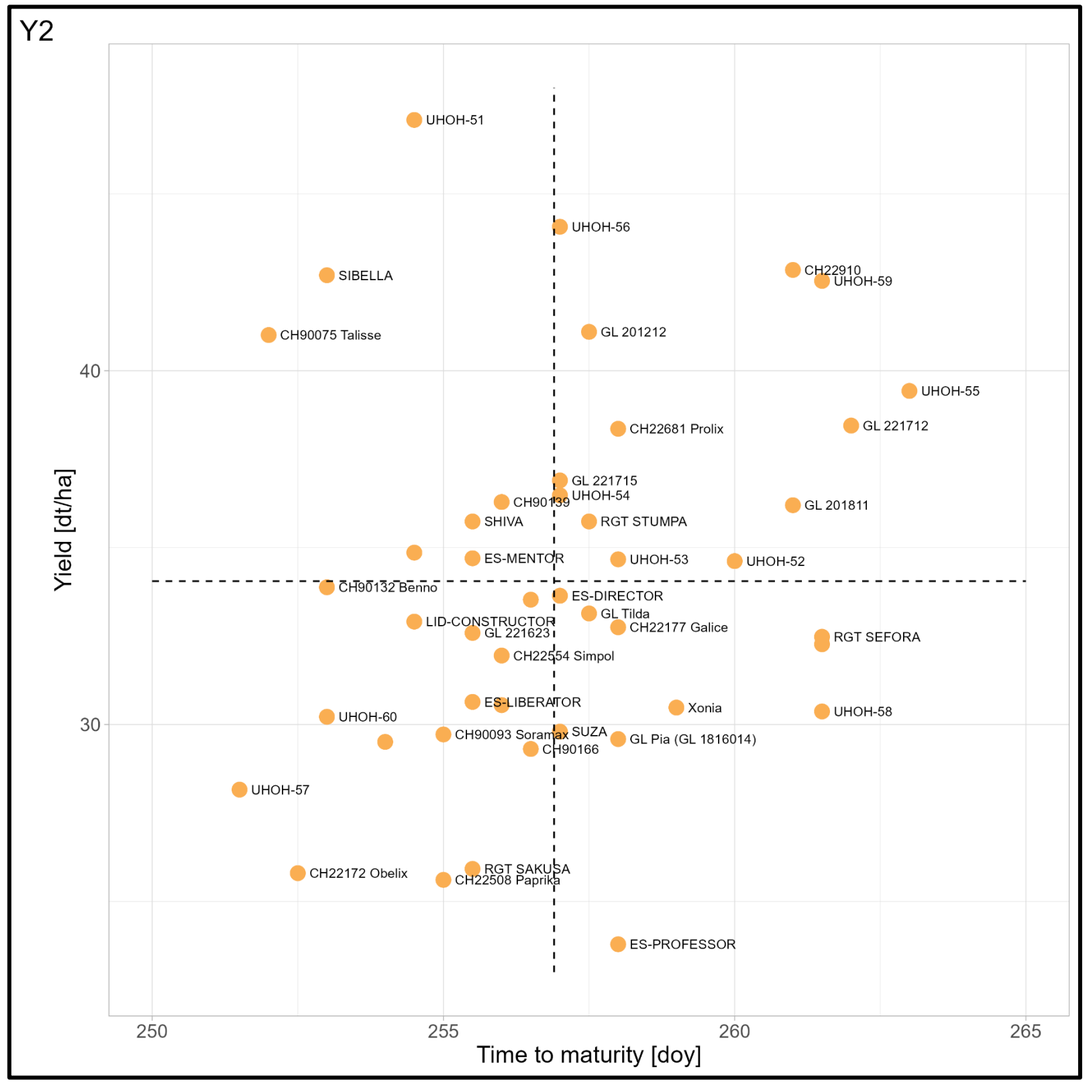
LSD5 = least significant difference at 5% probability

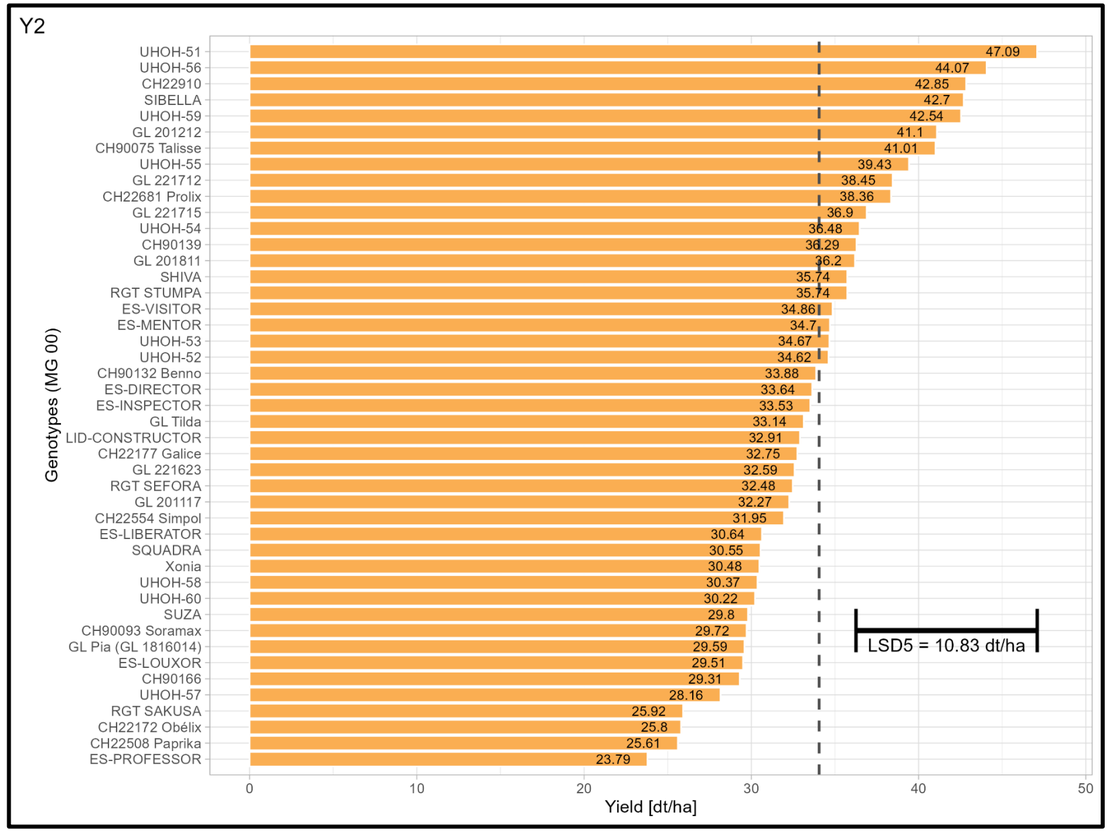




Results – Yield vs. ttm

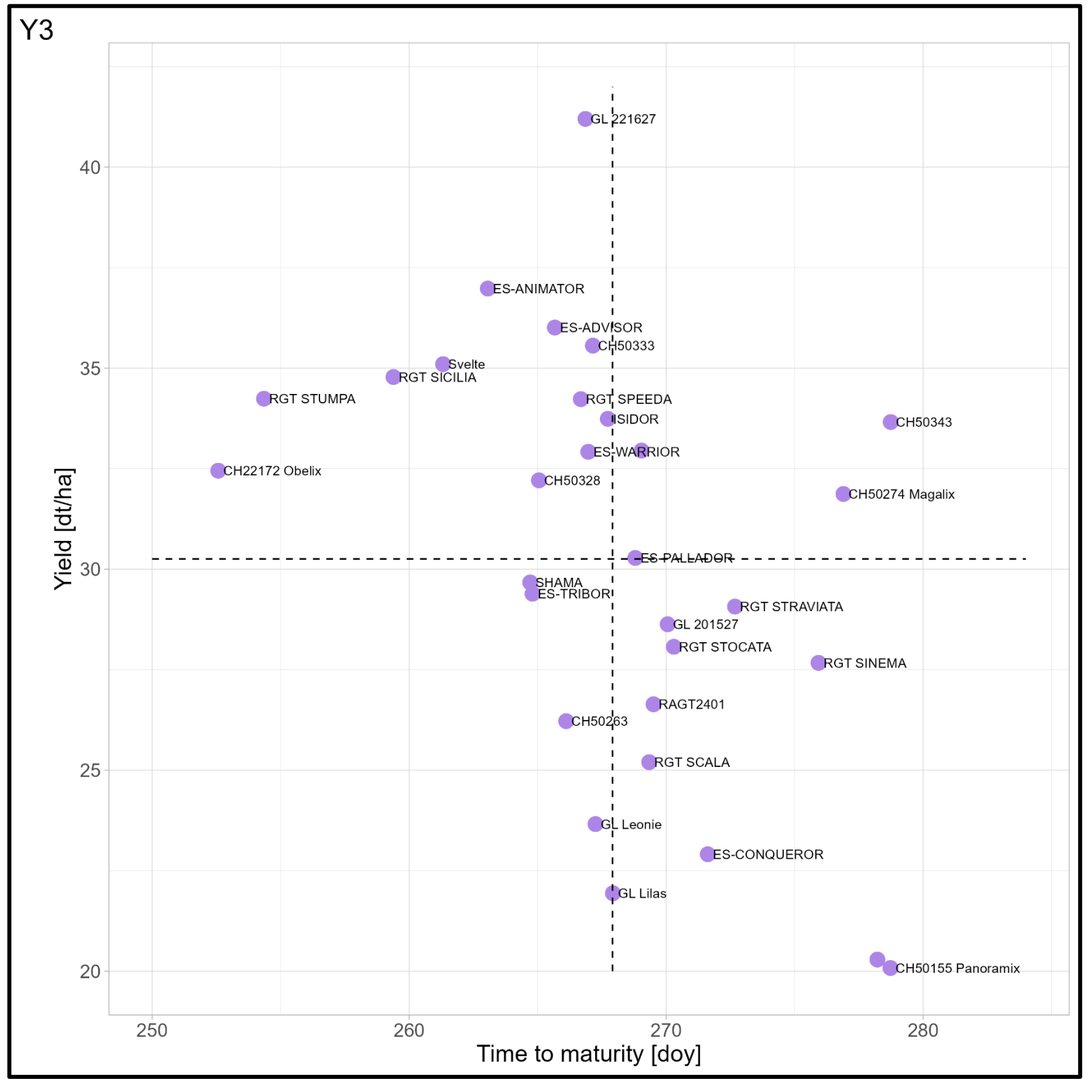
Y2	= mid early genotypes	(MG 00)
LSD5	= least significant difference at 5% probability	

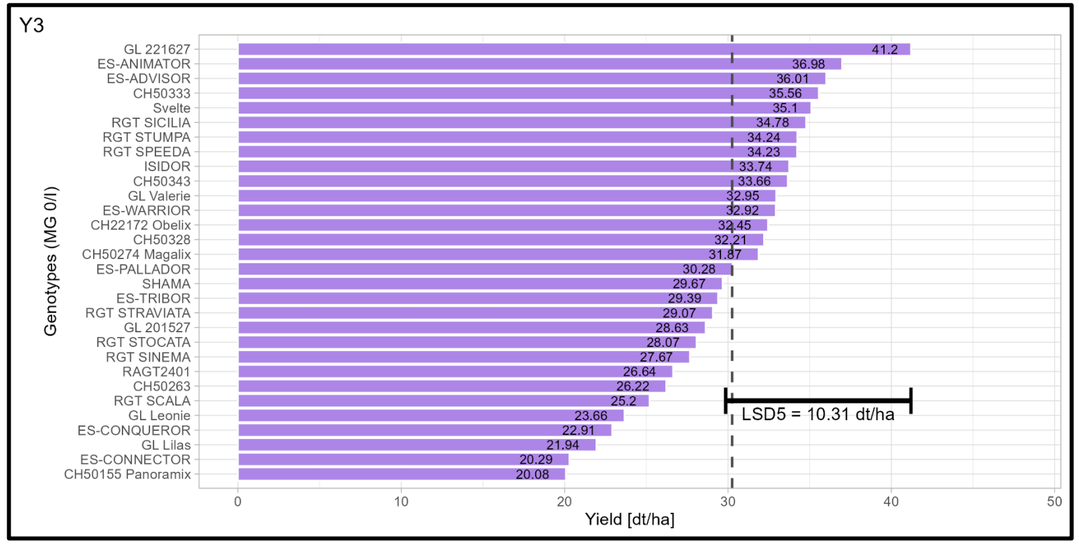




Results – Yield vs. ttm

Y3	= late genotypes	(MG 0/I)
LSD5	= least significant difference at 5% probability	

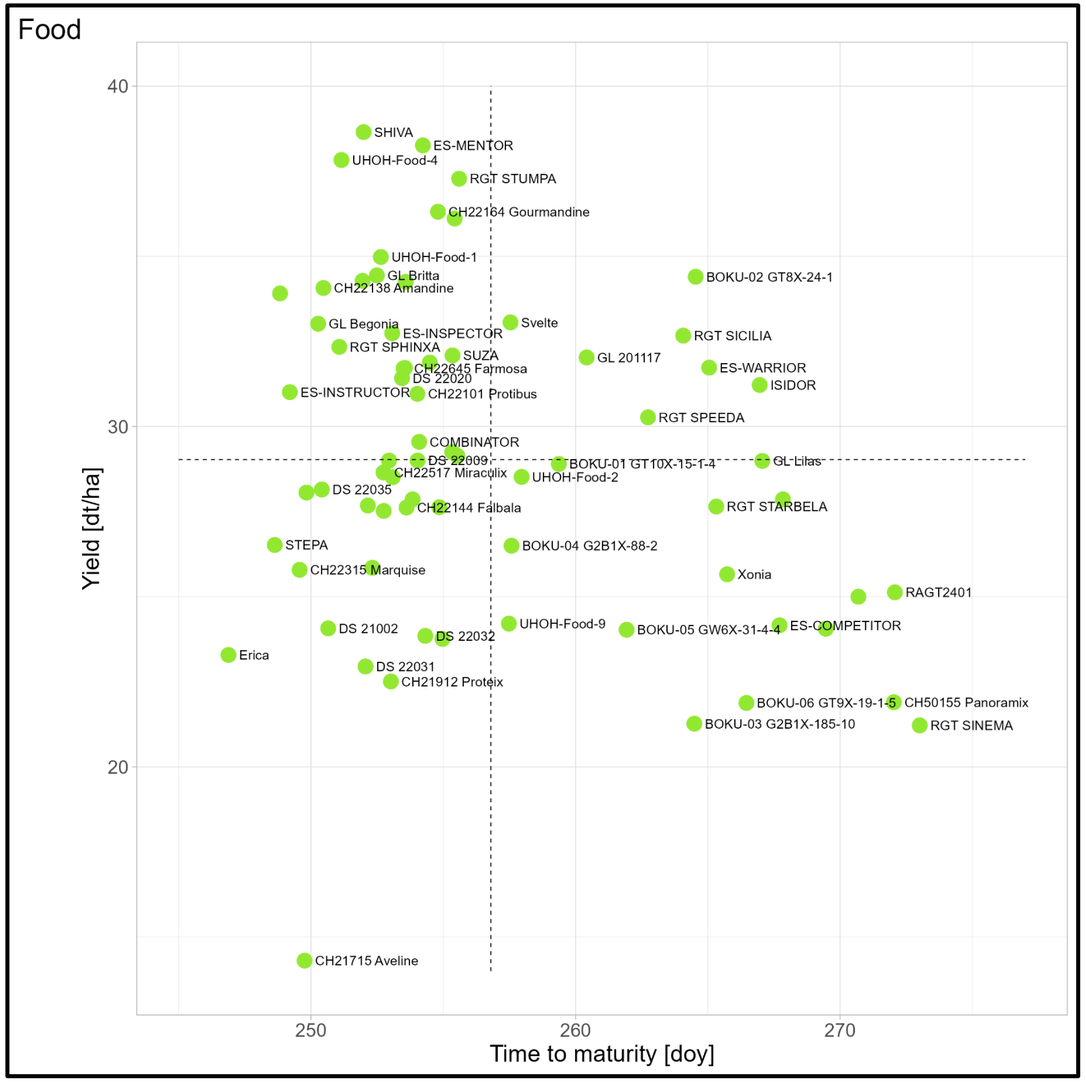


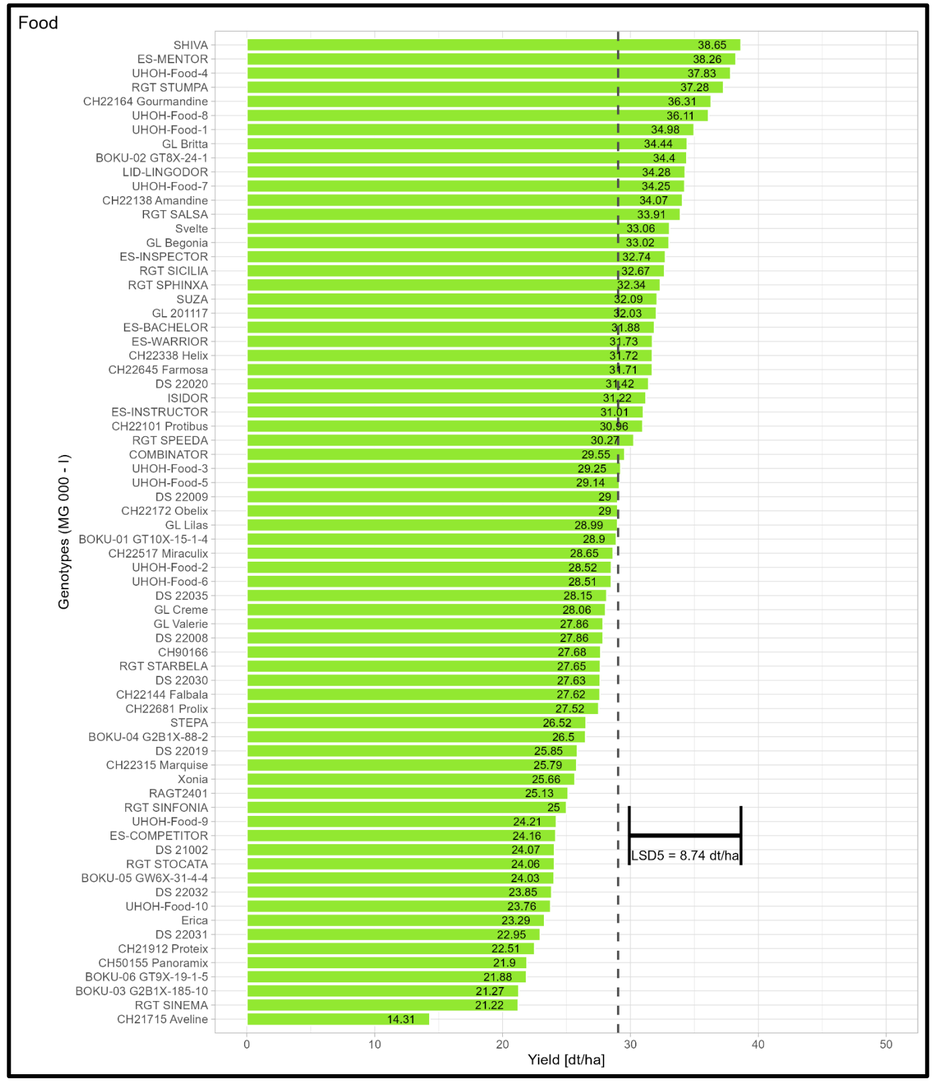


Results – Yield vs. ttm

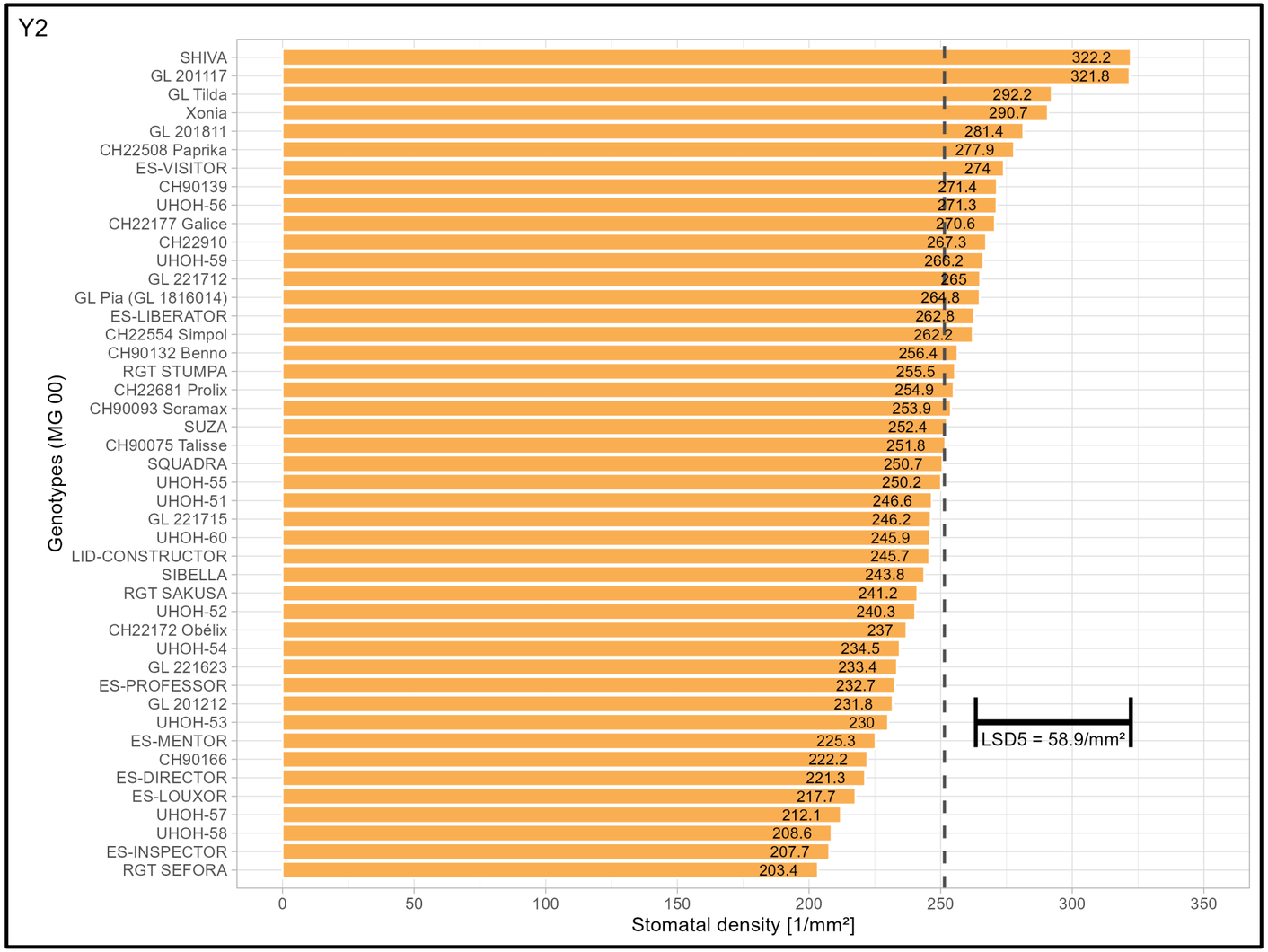
Food = genotypes for food use (MG 000 – I)

LSD5 = least significant difference at 5% probability





Results – Stomatal density



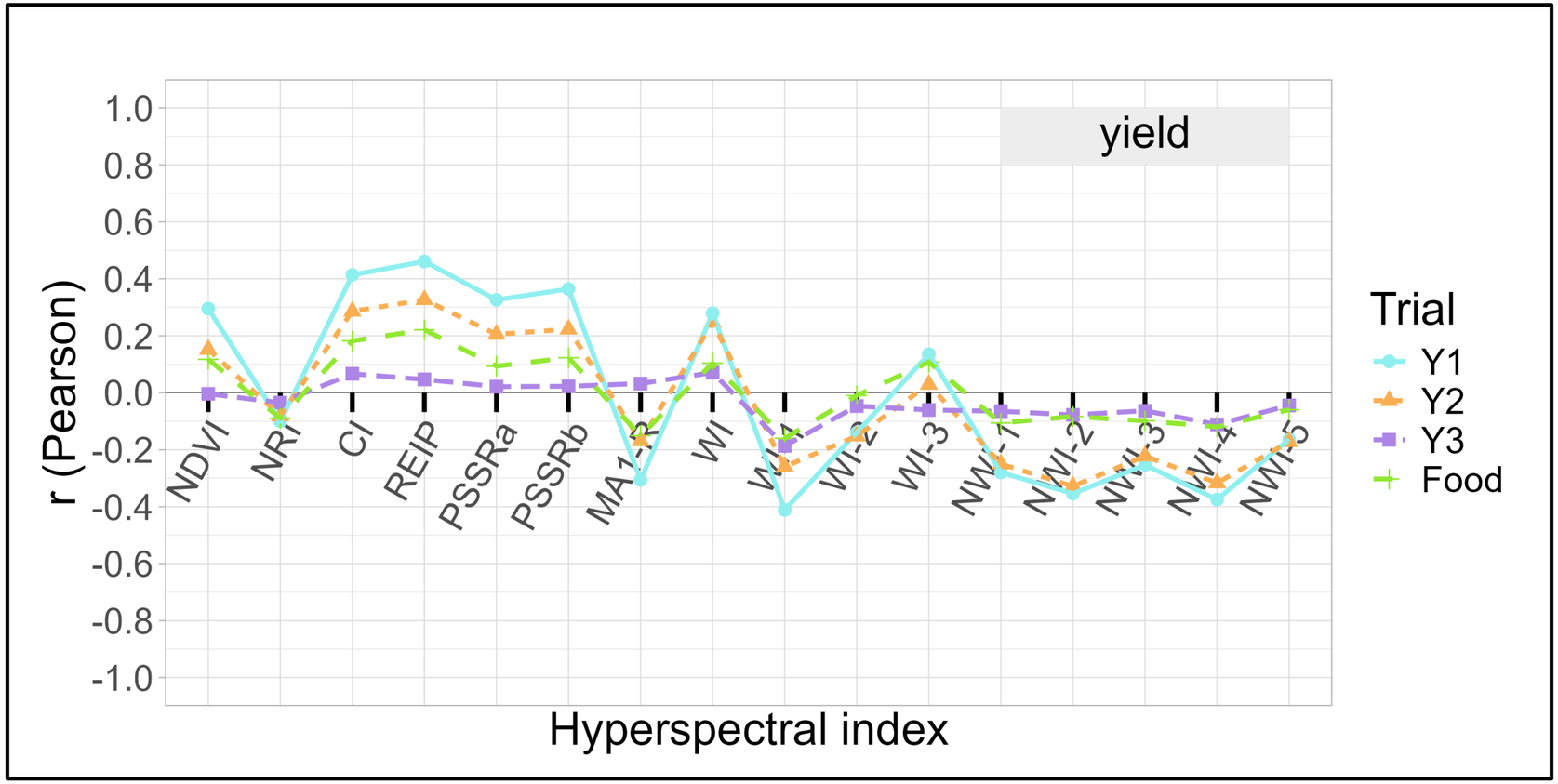
Y2 = mid early genotypes (MG 00)
LSD5 = least significant difference at 5% probability

Results –

Hyperspectral reflectance vs. Yield

Trials

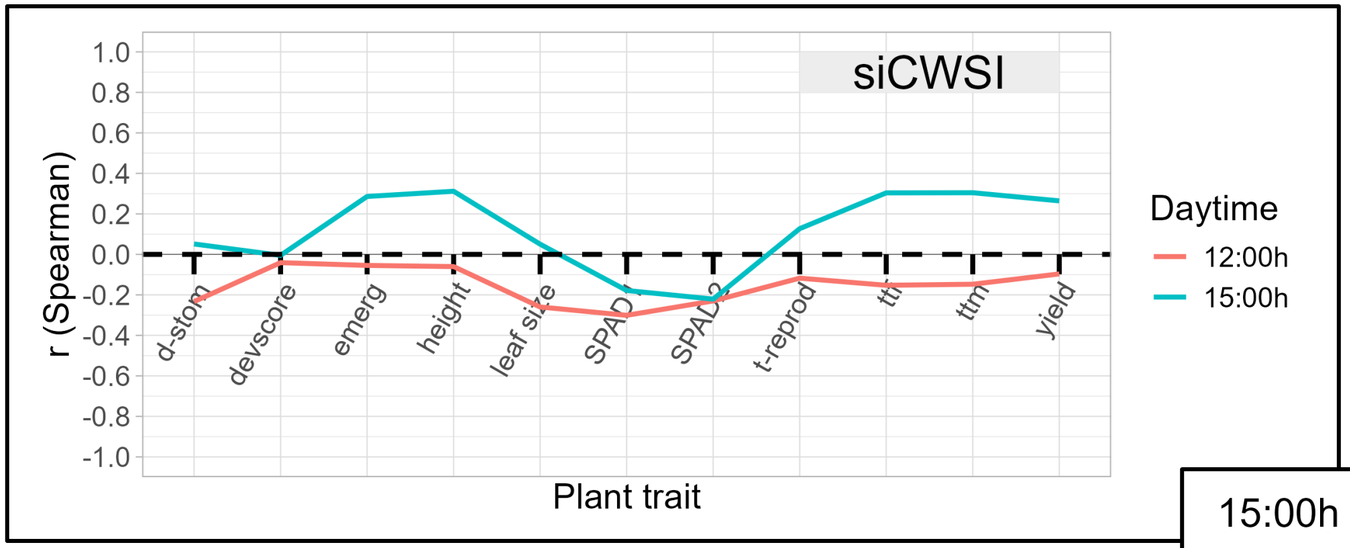
Y1	= early genotypes	(MG 000)
Y2	= mid early genotypes	(MG 00)
Y3	= late genotypes	(MG 0/I)
Food	= genotypes for food use	(MG 000 – I)

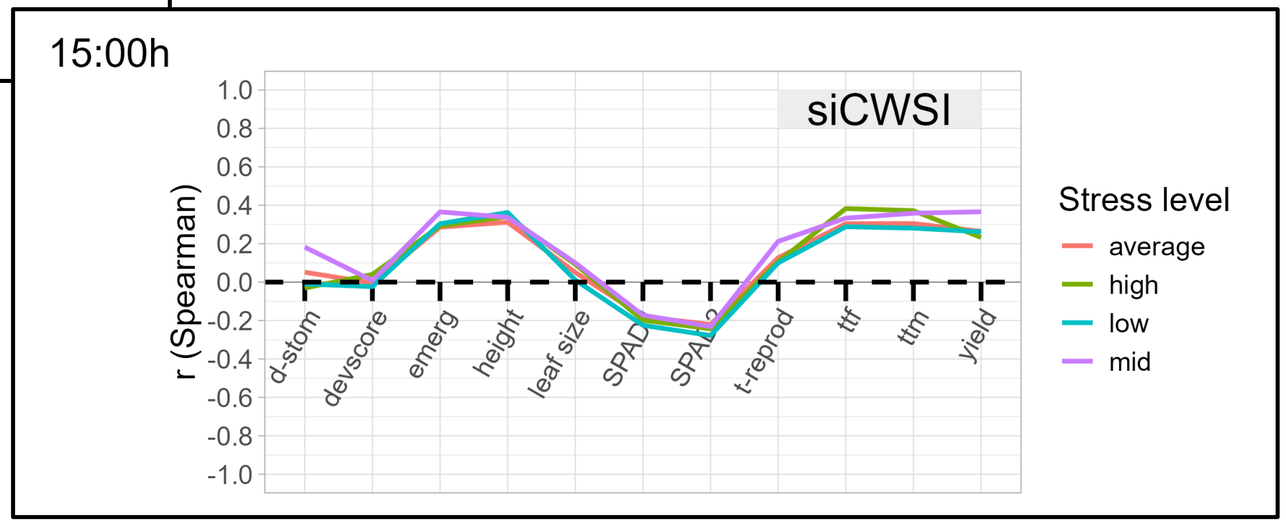


siCWSI = simplified Crop Water Stress Index

Results –

Thermography





Discussion & Conclusion

1) **Grain yield:** significant differences between and within maturity groups.
Highest yield measured for mid-early varieties (MG 00).

2) **Stomatal density:** differences measureable with nail-polish-method,
BUT highly time-consuming during sampling and stomata counting.

3) **Hyperspectroscopy:** Highest correlation with grain yield was shown for chlorophyll indices CI, REIP and water indices WI-1, NWI-2 and NWI-4.

4) **Thermography:** Screening in the afternoon at 15:00 more reliable than at 12:00.
Time-consuming screening technique for handheld approach.
-> Screening with drones will be more useful for future breeding research.

Legume Generation

Legume Generation (Boosting innovation in breeding for the next generation of legume crops for Europe) has received funding from the European Union through Horizon Europe under grant agreement number 101081329

[Merci BOKU]

Results – Plant height

Trials

Y1	= early genotypes	(MG 000)
Y2	= mid early genotypes	(MG 00)
Y3	= late genotypes	(MG 0/I)
Food	= genotypes for food use	(MG 000 – I)