



Smart technologies for phenotyping - Milan, 28 June 2018

Smartphone-based application for plant phenotyping

Roberto Confalonieri, Livia Paleari, Ermes Movedi, Marco Foi

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- Hundreds of genotypes under evaluation could turn into long time for measuring traits (White et al., 2011)
 - ✓ Often, few instruments available (costly)
 - ✓ Phenotyping on many genotypes should be "synchronous"
 - Time needed for phenotyping could lead to use "small" sample sizes (to save time)
- A-synchronous phenotyping on different genotypes and/or suboptimal sample sizes could generate uncertainty
 - ✓ that could be larger than differences between genotypes



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• Greenhouse and field platforms, robots











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• Greenhouse and field platforms, robots





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- Greenhouse and field platforms, robots
 - ✓ Cost?
 - Which kind of traits can be actually quantified

...what do we mean by "trait"?



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- "Trait":
 - ✓ First time defined by Darwin (1859)?
 - ✓ Development of disciplines
 - quantitative genetics
 - ecophysiology
 - functional ecology

Solutions:

 Classification frameworks based on the trait role in determining individual fitness (e.g., Arnold, 1983; Violle et al., 2007)

Dialects"? he term assumed a variety of connotations... ...the underlying concept is sometimes (often?) unclear

(Violle et al., 2007)



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Solutions:

 Classification frameworks based on the trait role in determining individual fitness (e.g., Arnold, 1983; Violle et al., 2007)



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 - ✓ Development of disciplines
 - quantitative genetics
 - ecophysiology
 - functional ecology

Solutions:

- Classification frameworks based on the trait role in determining individual fitness (e.g., Arnold, 1983; Violle et al., 2007)
- Trait ontologies...

Dialects"? he term assumed a variety of connotations... ...the underlying concept is sometimes (often?) unclear e et al., 2007)



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• Trait ontologies:

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🛛 🗖 plant_trait_ontolo	gy Term "leaf angle" (TO:00	000206)						
Term Name	leaf angle							
Term Accession	TO:0000206							
Aspect	plant_trait_ontology							
Synonyms (2)	LBP, LFAG							
Definition	A leaf attitude (TO:0000206) which is associated with the angle of a leaf (PO:0025034) measured against the stem.							
Comment	iment None							
Derivation								
 plant trait (T0:0000387) #0 E (i) anatomy and morphology trait (T0:000017) #0 E (i) plant structure anatomy and morphology trait (T0:0000839) #0 E (i) multi-tissue plant structure anatomy and morphology trait (T0:0000736) #0 E (i) plant organ anatomy and morphology trait (T0:0000747) #0 E (i) phyllome anatomy and morphology trait (T0:0000748) #0 E (i) leaf anatomy and morphology trait (T0:0000748) #0 E (i) leaf attitude (T0:0000824) #0 E (i) leaf angle (T0:0000206) #41 								
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• Trait ontologies:





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- Trait ontologies:
 - They appear as good solutions but unfortunately they are not as good as they would like to be
 - Definitions are not completely unambiguous
 - Sometimes traits are not described in a quantitative way
- ...Breeders have to
 - $\checkmark\,$ phenotype **hundreds** of **lines**
 - ✓ in a short interval of time

Trait = "something that can be measured easily and rapidly"

This is a **potential source** of **misunderstandings** and **uncertainty**



- Greenhouse and field platforms, robots
 - ✓ Cost?
 - \checkmark Which kind of traits can be actually quantified
 - ✓ $G \times E$ interaction?
 - o Greenhouse platforms
 - \rightarrow plants in pots
 - → "environmental" conditions...
 - \rightarrow gradients inside the greenhouse
 - Field platforms
 - \rightarrow transport them?
 - → how many?



- We are traveling along a different road:
 - ✓ Low cost → many instruments → parallel work
 - ✓ Clear what a trait is
 - ✓ Integrated ecosystem of tools to support
 - phenotyping
 - "tagging" measurements
 - storing/pre-processing/exporting data



Which traits?

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We propose **a suite of apps for smartphone** for estimating the following traits:

Trait	App name	References (examples of studies where the traits was estimated)
Leaf area index (LAI)	PocketLAI	e.g., Royo et al. (2004)
Leaf angle/leaf angle distribution	PocketPlant3D	e.g., Li et al. (2015)
Leaf N content (or greenness)	PocketN	e.g., Graziani et al. (2010)
Resistance to pathogen/% tissue affected	PocketDisease	e.g., Kongprakhon et al. (2009)



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plant_trait_ontology Te	erm "leaf area index" (TO:001200	1)					
Term Name	leaf area index						
Term Accession	TO:0012001						
Aspect	plant_trait_ontology						
Synonyms (0)	None.		6 JL 1				
Definition	Leaf Area Index (LAI) is the ratio of total upp which the vegetation grows.	per leaf surface of vegetation divided by the surface area	a of the la	nd on			
Comment	None						
Derivation							
 plant trait (T0:0000387) #0 ⊞ [i] biochemical trait (T0:0000277) #1 ⊞ [i] biological process related trait (T0:0000283) #0 ⊞ [i] physiological process related trait (T0:0000349) #0 ⊞ [i] leaf area index (T0:0012001) #0 							
Database Cross-References (1)						
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÷ Infertile tiller number ia_a Identifier CO 321:0000362 Ξ Leaf area index is a Ξ LAI Computation method_of Formula (LAI = leaf area / ground area, m2 / m2) index soale o Ŧ Lodging incidence is a ÷ Peduncle neck break ia_a Method class Computation Ŧ Plant early vigour is a Ŧ Plant growth habit Method LAI can be determined directly by taking a sample + Plant height description of canopy from a plant canopy, measuring the leaf area per sample plot with an area meter or image Ŧ Plant hybrid necrosis incidence is a scanner and dividing it by the plot land surface ŧ Plant stand is a area. Indirect methods use ceptometer to measure canopy geometry or light extinction and relate it to + Plant vigour is_a LAI. (LAI = leaf area / ground area, m2 / m2).



Type of measurement	Instrument (examples)	References (e.g.)
Direct	Planimeter	e.g., Borrel et al. (2000)
Indirect-proximal	Digital/hemispherical photography	e.g., Casedeus and Villegas (2014)
Indirect-remote	Reflectance (relationships with vegetation indices)	e.g., Haboudane et al. (2004)



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Total one-sided area of leaf tissue per unit ground surface





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- It can be **measured**
 - 1. collection of leaves
 - 2. measurement of their area
 - ✓ dedicated instruments



 \checkmark acquiring and processing leaf images





- It can be estimated (indirect methods)
 - o allometric relationships
 - o inversion of light transmittance models
 - ✓ LAI-2000, LAI-2200
 - ✓ ceptometers (AccuPAR, SUNSCAN)
 - ✓ hemispherical camera











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LAI-2000 (now LAI-2200) and ceptometers

- quite **expensive** (4000 more than 10000 \$)
- characterized by low portability (12×24×109 65×14×43 cm; 4.15 - 6.5 kg – with cases)
- long and expensive maintenance services in case of damages

...field campaigns can be interrupted!





ATTESTATO DI BREVETTO PER INVENZIONE INDUSTRIALE



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START

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CAPTURE:	
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PROCESSING:	
Sky Conditions	Cloudy >
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automatically acquired at 57.5° while the user is rotating the device











How many readings?





How many readings?





PocketLAI – Tests (1)

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Computers and Electronics in Agriculture 96 (2013) 67-74



Development of an app for estimating leaf area index using a smartphone. Trueness and precision determination and comparison with other indirect methods

CrossMark

R. Confalonieri^{a,*}, M. Foi^b, R. Casa^c, S. Aquaro^d, E. Tona^d, M. Peterle^d, A. Boldini^d, G. De Carli^d, A. Ferrari^d, G. Finotto^d, T. Guarneri^d, V. Manzoni^d, E. Movedi^d, A. Nisoli^d, L. Paleari^d, I. Radici^d, M. Suardi^d, D. Veronesi^d, S. Bregaglio^a, G. Cappelli^a, M.E. Chiodini^a, P. Dominoni^a, C. Francone^a, N. Frasso^a, T. Stella^a, M. Acutis^a



PocketLAI – Tests (1)

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The **ISO 5725-2 protocol** was **adapted** for in vivo field methods, to derive Accuracy, i.e., Trueness & Precision (Repeatability & Reproducibility)



Confalonieri et al. (2014) Field Crop. Res. 161, 128-136





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Leaf area index (m² m⁻²) - destructive method

- The methods present similar performances
- They have the same tendency to underestimate high LAI values



PocketLAI – Tests (1)

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	Date	Plot	Method	Mean LAI (m ² m ⁻²)		Repeata	Repeatability		lucibility
	-			Destructive	Estimated	rª	RSD _r ^b	R°	RSD _R ^d
Pocketl Al.	11/6/2012	D1	PocketLAI	0.49	0.20	0.16	28.33	0.18	31.04
I OCKEILAI.			AccuPAR		0.24	0.48 ^f	70.03 ^f	0.48	70.03
l one of the			LAI-2000 5R		0.60°	1.04 ^f	63.30 ^f	1.04	63.30
			LAI-2000 4R		0.54	0.88	54.79	0.91	56.99
most precise,		D2	PocketLAI	0.25	0.09	0.15	57.76	0.15	57.94
for both			AccuPAR		0.13	0.28 ^f	75.00 ^f	0.28	75.00
			LAI-2000 5R		0.47	0.89 ^f	68.14 ^f	0.89	68.14
- repeatability			LAI-2000 4R		0.39	1.04 ^t	95.60 [±]	1.04	95.60
ranzaduaibility	9/7/2012	D1	PocketLAI	3.11	4.13	1.45 ^t	12.50 [±]	1.45	12.50
			AccuPAR		3.37	1.16	12.30	1.19	12.56
	-		LAI-2000 5R		3.16	0.83 ^t	9.43 ¹	0.83	9.43
			LAI-2000 4R		3.56	0.98 ^r	9.85 ^r	0.98	9.85
		D2	PocketLAI	2.02	2.55	1.11 ^r	15.54 ^r	1.11	15.54
			AccuPAR		1.84	0.84 ^r	16.36 ^r	0.84	16.36
			LAI-2000 5R		2.11	1.42 ¹	24.04 ^t	1.42	24.04
			LAI-2000 4R		2.32	1.67 ^t	25.70 [±]	1.67	25.70
	30/7/2012	D1	PocketLAI	6.10	4.03	0.97	8.56	1.03	9.12
			AccuPAR		4.37	0.79	6.43	0.95	7.74
			LAI-2000 5R		3.51	1.51 ^r	15.39 ^r	1.51	15.39
			LAI-2000 4R		3.98	1.78 ^t	16.01 ^r	1.78	16.01
		D2	PocketLAI	2.92	3.80	0.96	9.03	1.02	9.58
			AccuPAR		2.84	0.84	10.62	1.17	14.74
			LAI-2000 5R		2.69	1.58	20.93	1.70	22.59
a. rapatability limit			LAI-2000 4R		3.02	1 86	22.05	2.01	23 79

^a: repeatability limit. ^b: relative standard deviation of repeatability.

^c: reproducibility limit.

^d: relative standard deviation of reproducibility.

^e: laboratory 3 is an outlier according to the Cochran test.

^f: corrected value (s_r set equal to s_R in case $s_r > s_R$; Orwitz, 1995; Scaglia et al., 2011).



Article



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Multitemporal Monitoring of Plant Area Index in the Valencia Rice District with PocketLAI

Manuel Campos-Taberner^{1,*}, Franciso Javier García-Haro¹, Roberto Confalonieri², Beatriz Martínez¹, Álvaro Moreno¹, Sergio Sánchez-Ruiz¹, María Amparo Gilabert¹, Fernando Camacho³, Mirco Boschetti⁴ and Lorenzo Busetto⁴





PocketLAI – Tests (3, 4)

CrossMark

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Field Crops Research 155 (2014) 38-41



Short communication

Comparison of leaf area index estimates by ceptometer and PocketLAI smart app in canopies with different structures \ddagger

C. Francone^a, V. Pagani^a, M. Foi^b, G. Cappelli^a, R. Confalonieri^{a,*}



Applied Vegetation Science 18 (2015) 716-723

Estimating leaf area index in tree species using the PocketLAI smart app

Francesca Orlando, Ermes Movedi, Livia Paleari, Carlo Gilardelli, Marco Foi, Michele Dell'Oro & Roberto Confalonieri



Leaf angle/distribution

Plant_trait_ontology Term ×								
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Term Name	leaf angle							
Term Accession	TO:0000206							
Aspect	plant_trait_ontology							
Synonyms (2)	LBP, LFAG							
Definition	A leaf attitude (TO:0000206) which is assoc	iated with the angle of a leaf (PO:0025034) measured a	gainst th	ie stem	1.			
Comment	None							
Derivation								
 plant trait (TO:0000387) #0 := plant trait (TO:0000387) #0 := [i] anatomy and morphology trait (TO:0000839) #0 := [i] plant structure anatomy and morphology trait (TO:0000836) #0 := [i] plant organ anatomy and morphology trait (TO:0000736) #0 := [i] plant organ anatomy and morphology trait (TO:0000747) #0 := [i] plant organ anatomy and morphology trait (TO:0000748) #0 := [i] leaf anatomy and morphology trait (TO:0000748) #0 := [i] leaf attitude (TO:0000824) #0 := [i] leaf angle (TO:0000206) #41 								



+

+

leaf blade anthocyanin

leaf blade greenness

leaf blade pubescence

is_a

leaf length

فر عا

is a

12.2

Leaf angle/distribution

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Category 5 Horizontal

Category 9 Drooping



Leaf angle/distribution

Type of measurement	Instrument (examples)	References (e.g.)
Direct	Inclinometer	e.g., Deckmyn et al (2000)
Indirect-proximal	LAI-2000	e.g., Zou et al. (2014)
Indirect-remote	Stereo imaging	e.g., Biskhup et al. (2007)



Leaf angle/distribution

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• E.g., Pioneer





Figure 2. Leaf angle measurements were taken using a clinometer smartphone app.

• This does not represent the angle of photosynthetic tissues...



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- We developed a smart app for
 - ✓ Measuring angle of leaf insertion
 - ✓ Measuring angles of photosynthetic tissues
 - Deriving synthetic parameters of distributions of the angles of photosynthetic tissues (3D canopy scan)



Leaves can bend!



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Research Paper

PocketPlant3D: Analysing canopy structure using a smartphone

CrossMark

Roberto Confalonieri ^{a,*}, Livia Paleari ^a, Marco Foi ^b, Ermes Movedi ^c, Fosco M. Vesely ^a, William Thoelke ^{a,d}, Cristina Agape ^d, Giulia Borlini ^d, Irene Ferri ^d, Federico Massara ^d, Roberto Motta ^d, Riccardo A. Ravasi ^d, Sofia Tartarini ^d, Camilla Zoppolato ^d, Luca M. Baia ^d, Andrea Brumana ^d, Davide Colombo ^d, Antonio Curatolo ^d, Valerio Fauda ^d, Denise Gaia ^d, Andrea Gerosa ^d, Antonio Ghilardi ^d, Enrico Grassi ^d, Andrea Magarini ^d, Francesco Novelli ^d, Fatima B. Perez Garcia ^d, Andrea Rota Graziosi ^d, Michele Salvan ^d, Tommaso Tadiello ^d, Laura Rossini ^e

^a University of Milan, DESP, Cassandra Lab, Italy

^b University of Milan, Ardito Desio Earth Sciences Department, Cassandra Lab, Italy

^c University of Milan, DiSAA, Cassandra Lab, Italy

^d University of Milan, Cropping Systems MS Course, Italy

^e University of Milan, DiSAA, Italy



BIOSYSTEMS ENGINEERING 164 (2017) 1-12



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• Campbell's ellipsoidal distribution (Campbell, 1986)



 $A \approx \chi + 1.774 (\chi + 1.182)^{-0.733}$



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Campbell's ellipsoidal distribution (Campbell, 1986)







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• β-distribution (Goel and Strebel, 1984; Pisek et al., 2011)

$$f(t) = \frac{1}{\beta \mu \nu} (1-t)^{\mu} f(t)^{-1}$$

$$t = 2 \cdot \theta_{\rm L} / \pi$$

$$\beta \mu \nu = \frac{\Gamma \mu \Gamma \nu}{\Gamma \mu + \nu}$$

$$\mu = (1 - \overline{t}) \begin{pmatrix} \sigma_0^2 \\ \sigma_t^2 \\ \sigma_t^2 \end{pmatrix}$$

$$\nu = \overline{t} \left(\frac{\sigma_0^2}{\sigma_t^2} - 1 \right)$$

$$\sigma_0^2 = \overline{t}(1 - \overline{t})$$



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• β-distribution (Goel and Strebel, 1984; Pisek et al., 2011)





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• Phenotyping bean lines







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	E.g., flower or TO:0000303.		E	.g., d1 or	waxy			
🗉 🖬 plant_trait_ontology Te	erm "stay green trait" (TO:000271	.2)						
Term Name	stay green trait					П		
Term Accession	TO:0002712					П		
Aspect	plant_trait_ontology					U		
Synonyms (0)	None.					Н		
Definition	The color of plant and the photosynthetic org of senescing.	ans remain green compared to turning yellow and	or brown	at the ti	me	П		
Comment	None					H		
Derivation						H		
 plant trait (T0:0000387) #0 :: [i] anatomy and morphology trait (T0:0000017) #0 ::								
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Traits, methods and scales

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ŧ	culm node underlying colour	^				
ŧ	culm number		Identifier CO_320:000057	77		
ŧ	flag leaf angle					
(±	grain thickness		Catagory 0, 000- Not visible	under anthocyanin		
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ŧ	leaf angle i.a					
	leaf blade anthocyanin		Category 3 061= Light green	n		
Ē	leaf blade greenness					
	Leaf blade greenness method_of		Category 3 060= Medium G	àreen		
	im leaf blade greenness scale scale_of					
+	leaf blade pubescence		Category 7 063= Dark green	n		

Term information



Type of measurement	Instrument (examples)	References (e.g.)
Direct	Elemental analyzer	e.g., Vigneau et al. (2011)
Indirect-proximal	SPAD	e.g., Cabangon et al. (2011)
Indirect-remote	Reflectance (relationships with vegetation indices)	e.g., Babar et al., (2006)



- Leaf color chart (LCC)
- KONICA MINOLTA SPAD-502
- Force-A Dualex 4









PocketN

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- Image processing (greenness)
- Background panel to guarantee flat reflectance under a wide range of light conditions





Research Paper

Improving in vivo plant nitrogen content estimates from digital images: Trueness and precision of a new approach as compared to other methods and commercial devices

Roberto Confalonieri ^{a,*}, Livia Paleari ^a, Ermes Movedi ^a, Valentina Pagani ^a, Francesca Orlando ^a, Marco Foi ^b, Michela Barbieri ^c, Michele Pesenti ^c, Oliver Cairati ^c, Marco S. La Sala ^c, Riccardo Besana ^c, Sara Minoli ^c, Eleonora Bellocchio ^c, Silvia Croci ^c, Silvia Mocchi ^c, Francesca Lampugnani ^c, Alberto Lubatti ^c, Andrea Quarteroni ^c, Daniele De Min ^c, Alessandro Signorelli ^c, Alessandro Ferri ^c, Giordano Ruggeri ^c, Simone Locatelli ^d, Matteo Bertoglio ^a, Paolo Dominoni ^a, Stefano Bocchi ^a, Gian Attilio Sacchi ^a, Marco Acutis ^a



BIOSYSTEMS ENGINEERING 135 (2015) 21-30







Cassandra Disease resistance/% tissue dis.

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Ontology Search		Annotation Search					
Find: TO:0000468		Find:	4				
Ontology Type: All Ontology Types		Annotation Type: All Object Types V					
	E.g., flower or TO:0000303.		E.g., (d1 or wa	ky.		
plant_trait_ontology Term "leaf blast disease resistance" (TO:0000468)							
Term Name	leaf blast disease resistance						
Term Accession	TO:0000468						
Aspect	plant_trait_ontology						
Synonyms (2)	BI, LFBLRS						
Definition	Causal agent: Magnaporthe grisea. Symptoms: Can affect either or both the leaf and panicles.						
Comment	None						
Derivation							
 plant trait (T0:0000387) #0 ⊞ (i) stress trait (T0:0000164) #8 ⊞ (i) biotic stress trait (T0:0000179) #0 ⊞ (i) crop damage resistance (T0:0000236) #0 ⊞ (i) disease resistance (T0:0000112) #3 ⊞ (i) fungal disease resistance (T0:0000439) #4 ⊞ (i) blast disease resistance (T0:000074) #191 ⊞ (i) leaf blast disease resistance (T0:0000488) #80 							

Cassandra Disease resistance/% tissue dis.

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Traits, methods and scales

DOWNLOAD SHOW OBSOLETE TERMS English V leaf blast damage scale Permalink . General 0 Comments Rice traits Identifier CO_320:0000341 Abiotic stress is_a Agronomical is a + Category 0 No lesions observed Biochemical ia_a Biotic stress is_a Leaf blast damage is_a Category 9 More than 75% leaf area affected ÷ leaf blast damage amount method_of leaf blast damage estimation method of Category 1 Small brown specks of pin-point size or larger leaf blast damage scale scale_of brown specks without sporulating center ÷ leaf blast damage type + bacterial blight damage Category 2 Small roundish to slightly elongated, necrotic gray anotal about 4.0 mm in diamater, with a distinct

Term information



Type of measurement	Instrument (examples)	References (e.g.)
Direct	"Visual"	e.g., Faivre- Rampant et al. (2010)
Indirect-proximal	Spectral reflectance	e.g., Huang et al. (2007)
Indirect-remote	Hyper-spectral imaging	e.g., Huang et al. (2007)



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• Often, **visual assessments** are carried out, by means of **reference panels** like the one below:







PocketDisease

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Leaf pictures taken on a **dedicated background panel**:

- "flat reflectance" on a wide range of light conditions
- weft to speed focus
- QR codes for normalizing picture size regardless of the distance from the panel/angle

Automatic recognition of the **region** where the **leaf** is





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Refining the detection of the image **region of interest**



Detection of **leaf borders** (marked in white)

5

Source image: Collobiano_5.jpg; Sample# 1. Leaf area: 1282.9 mm²; Lesion area: 157.52 mm² (12.28% of leaf area). Identification of lesions and measure of their area



PocketDisease

- It is based on **neural networks** for image processing
- Currently under development
 - Transferring the network on the device to avoid backend services (sometimes no Wi-Fi in field)



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Simplicity is complexity resolved (C. Brâncuşi)





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