Screening techniques for sweet potato drought tolerance

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Introduction

- CIP – ARC collaboration (2009-2012
- Methods for screening – fast, reliable?
- Identify parameter/s early growth stage

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Plant physiology

- Use mechanisms to adapt to environment
- Identify mechanisms, survival, enhance
- Lack of knowledge, 75% yield lost – environmental impact

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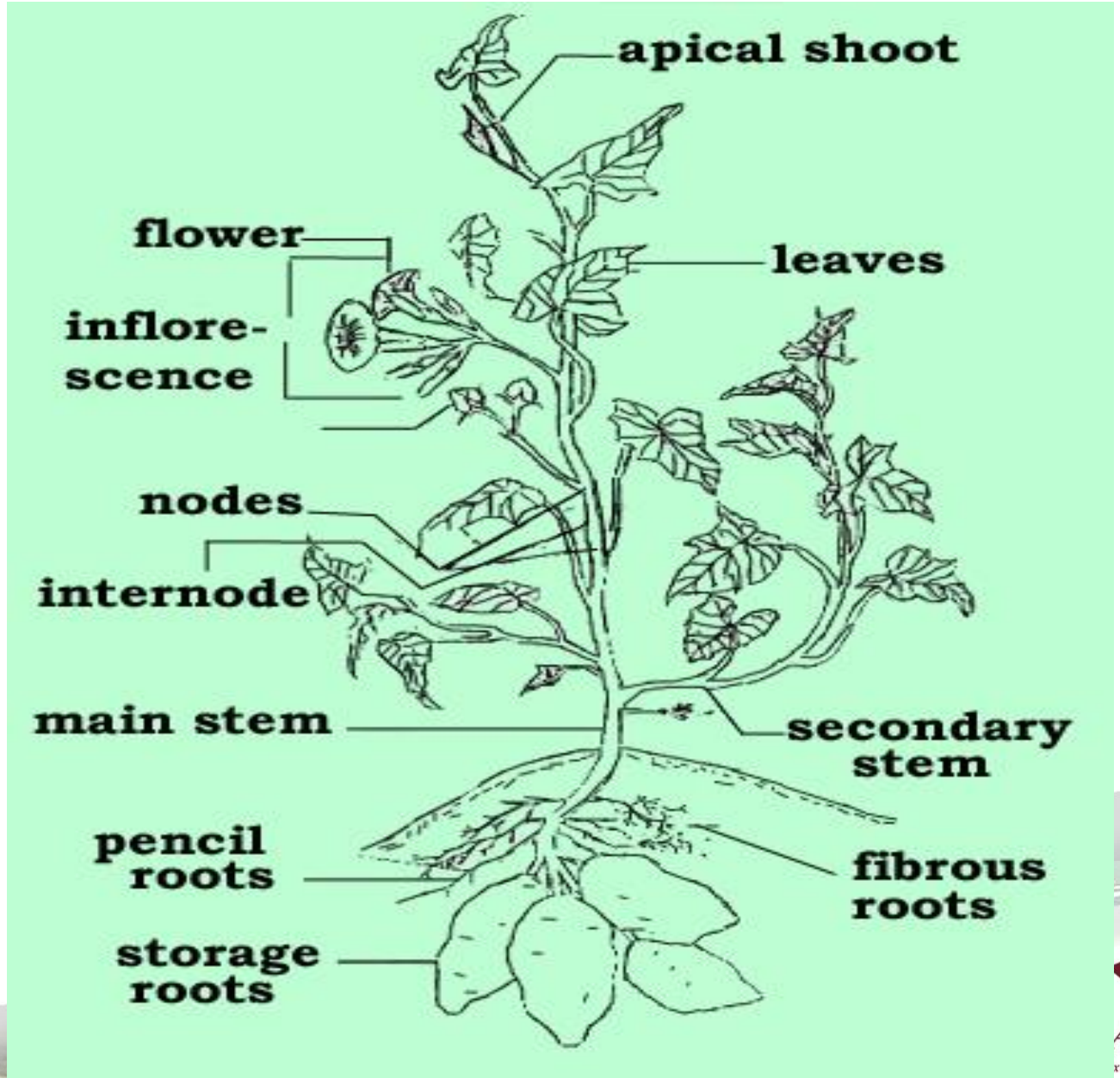
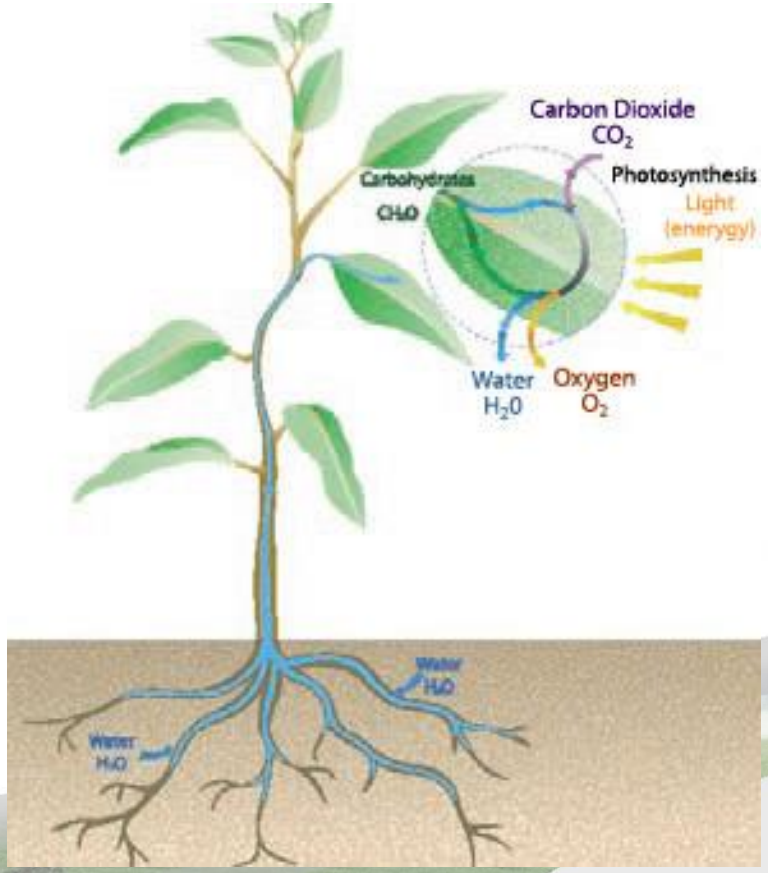
Plant physiology

- Tolerance – high metabolism(mild stress) reduced activity(severe stress)
- Avoidance – reduction in metabolic activity, dormant state, exposure to stress

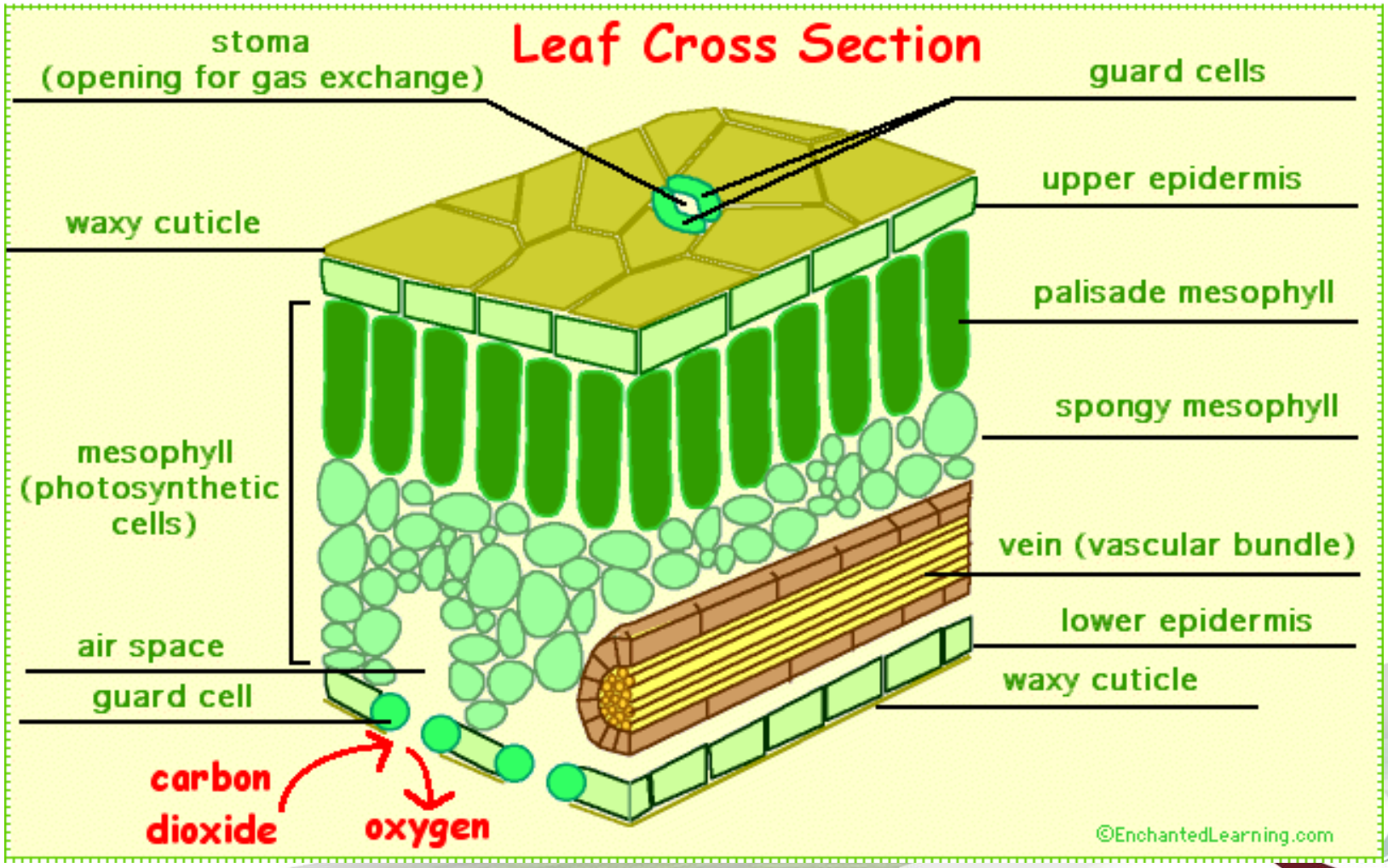
Morphology



Plant physiology



Leaf cross section



Materials and Methods

Choice of methods?

Choice of locations?

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Trial set up

- different locations – focus ARC-Roodeplaat
- 4, 8, 35 genotypes
- 100%, 60%, 30% treatments

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Location


Oncangwa
Katima Mulilo
Tsumeb
Grootfontein
Outjio
Otjiwarongo
NAMIBIA
ZIMBABWE
BOTSWANA
MOZAMBIQUE
Usakos
Okahandja
Gobabis
Swakopmund
Walvis Bay
WINDHOEK
Rehoboth
Beitbridge
Messina
Pietersburg
Phalaborwa
Rustenburg
PRETORIA
Nelspruit
Mmabatho
Witbank
Mafikeng
Krugersdorp
JOHANNESBURG
MBABANE
SWAZILAND
Vereeniging
Ermelo
Aus
Keetmanshoop
Lüderitz
Klerksdorp
Levurrisa
Hotazel
Kuruman
Kroonstad
Newcastle
Sishen
Upington
Welkom
Vryheid
Karasburg
Bethelehem
Oranjemund
Alexander Bay
Kimberley
Ladysmith
Richards Bay
Port Nolloth
BLOEMFONTEIN
MASERU
LESOTHO
Pietermaritzburg
Springbok
Prieska
Durban
De Aar
Kokstad
Bitterfontein
Aliwal North
Port Shepstone
Victoria West
Calvinia
ATLANTIC OCEAN
Umtata
Port St. Johns
Beaufort West
Graaf Reinet
INDIAN OCEAN
Bisho
Saldanha
East London
Grahamstown
Oudtshoorn
Paarl
CAPE TOWN
Worcester
George
Knysna
Port Elizabeth
Stellenbosch
Mossel Bay

Location

Treatments

Water management

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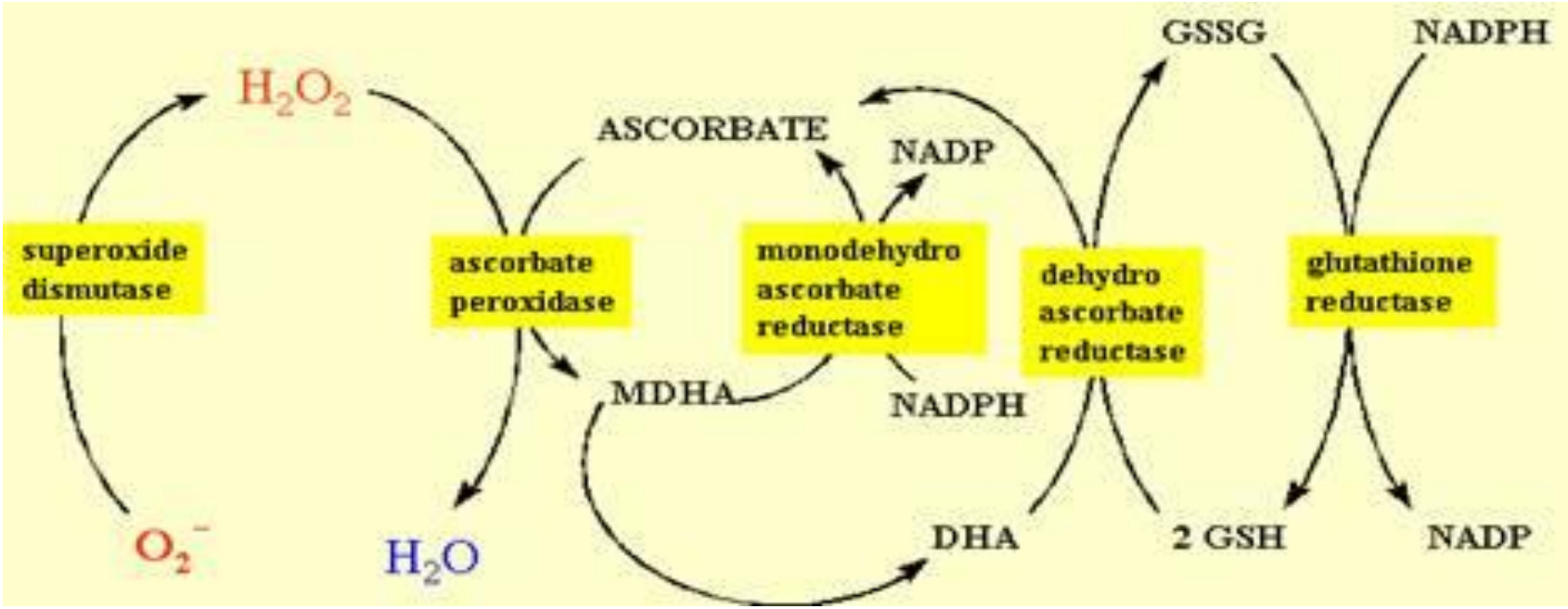
Methodology

Leaf material

- Harvest before sunrise – steady state
- Keep cold – enzymes labile

Antioxidants



Reductase – produce NADP accept e from PS I
Dismutase – neutralize O radical
Peroxidase – disposes of hydrogen peroxide

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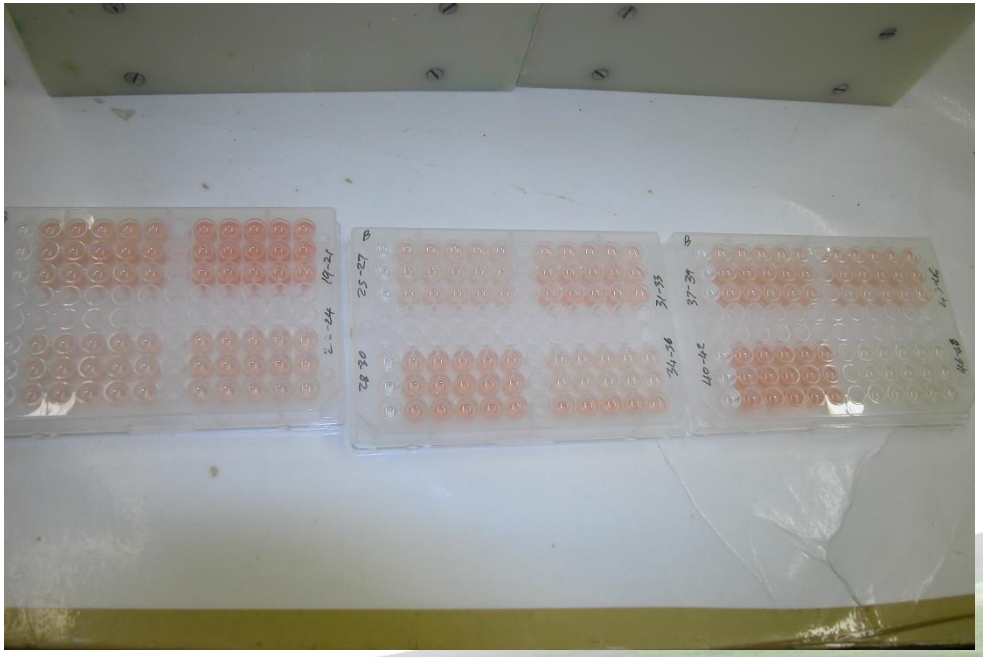
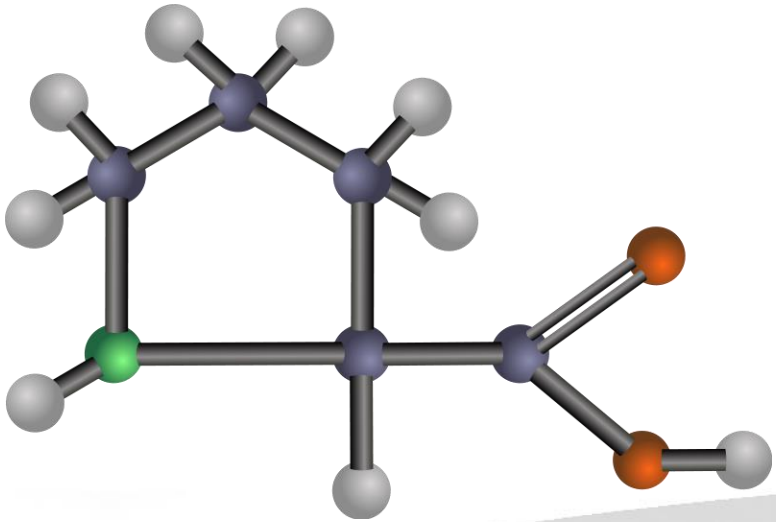
Free Proline

- Stabilize membrane structure
- Maintain osmotic balance





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Chlorophyll content measurement

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LA measurement

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Stem length

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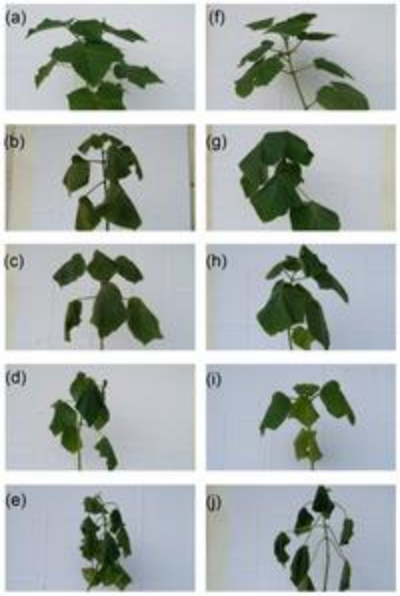
Relative water content



$$RWC(\%) = \frac{FW\text{-}DW}{TW\text{-}DW} \times 100$$

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Stomatal conductance

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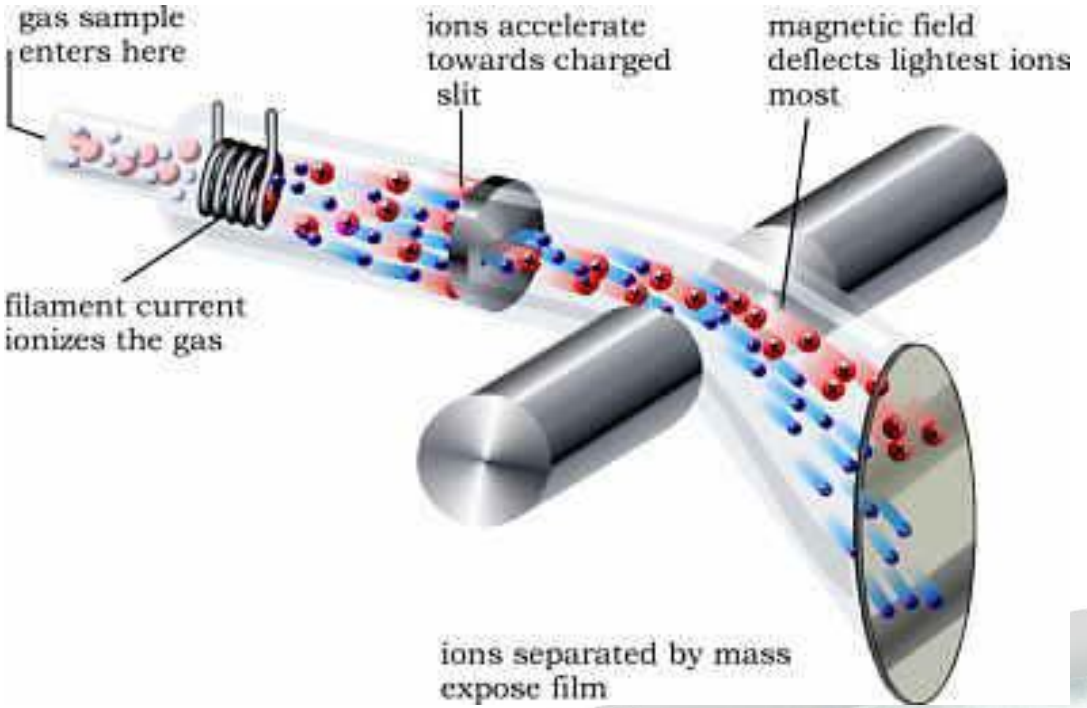
C^{13} discrimination analysis

- 98% atm CO_2 is C12
- Rubisco preference for 12C
- During drought 13C↔12C ratio ↑ more positive as conductance ↓: Leads to Δ ↓
- Possible relation Δ to g_s, Δ to t/ha, Δ to WUE

C13 determination



Yield

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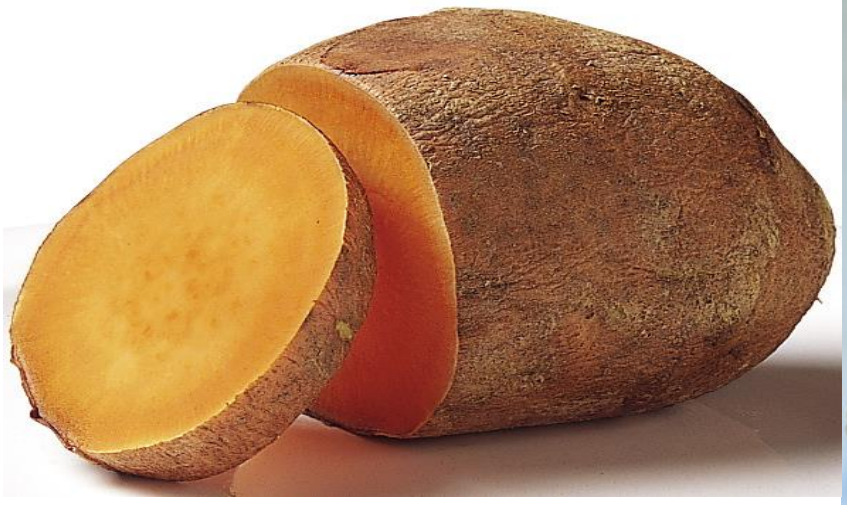
Carotenoid



Results

Pearson Correlation Analysis

Matrix

(-)

(+)

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Correlation (4 genotypes)

Variables	LAI	cm	GR	SOD	AP	CCI	discr	cond	RWC	NR	proline	t/ha	WUE	carotene
LAI	**1**	0.251	**-0.469**	0.218	-0.308	0.263	**0.388**	**0.690**	0.245	0.239	**-0.423**	0.326	**0.487**	-0.238
cm	0.251	**1**	**-0.548**	0.121	**-0.472**	-0.037	-0.213	**0.414**	**0.578**	**0.465**	**-0.598**	**0.526**	**0.587**	-0.101
GR	**-0.469**	**-0.548**	**1**	**-0.405**	**0.392**	-0.320	-0.092	**-0.552**	**-0.647**	**-0.578**	**0.621**	**-0.633**	**-0.728**	0.300
SOD	0.218	0.121	**-0.405**	**1**	0.150	0.334	0.231	**0.479**	0.224	**0.425**	-0.178	**0.374**	**0.395**	-0.073
AP	-0.308	**-0.472**	**0.392**	0.150	**1**	-0.053	0.117	-0.264	**-0.408**	**-0.483**	**0.448**	**-0.417**	**-0.445**	0.021
CCI	0.263	-0.037	-0.320	0.334	-0.053	**1**	0.140	0.272	0.249	**0.420**	-0.184	0.169	0.308	**-0.658**
discr	**0.388**	-0.213	-0.092	0.231	0.117	0.140	**1**	**0.382**	-0.130	-0.027	0.032	-0.017	0.076	-0.145
cond	**0.690**	**0.414**	**-0.552**	**0.479**	-0.264	0.272	**0.382**	**1**	0.251	**0.365**	**-0.434**	**0.501**	**0.608**	-0.112
RWC	0.245	**0.578**	**-0.647**	0.224	**-0.408**	0.249	-0.130	0.251	**1**	**0.644**	**-0.558**	**0.512**	**0.664**	-0.249
NR	0.239	**0.465**	**-0.578**	**0.425**	**-0.483**	**0.420**	-0.027	**0.365**	**0.644**	**1**	**-0.440**	**0.633**	**0.611**	-0.126
proline	**-0.423**	**-0.598**	**0.621**	-0.178	**0.448**	-0.184	0.032	**-0.434**	**-0.558**	**-0.440**	**1**	**-0.503**	**-0.680**	0.295
t/ha	0.326	**0.526**	**-0.633**	**0.374**	**-0.417**	0.169	-0.017	**0.501**	**0.512**	**0.633**	**-0.503**	**1**	**0.698**	0.082
WUE	**0.487**	**0.587**	**-0.728**	**0.395**	**-0.445**	0.308	0.076	**0.608**	**0.664**	**0.611**	**-0.680**	**0.698**	**1**	-0.247
carotene	-0.238	-0.101	0.300	-0.073	0.021	**-0.658**	-0.145	-0.112	-0.249	-0.126	0.295	0.082	-0.247	**1**

LAI – leaf cover, cm – stem length, GR – glutathione reductase, SOD – superoxide dismutase, AP – ascorbate peroxidase, CCI – chlorophyll index, Δ - 13C discrimination, NR – nitrate reductase, proline, g_s – stomatal conductance, RWC – relative water content, t/ha – yield, WUE – water use efficiency, carotene – carotene content

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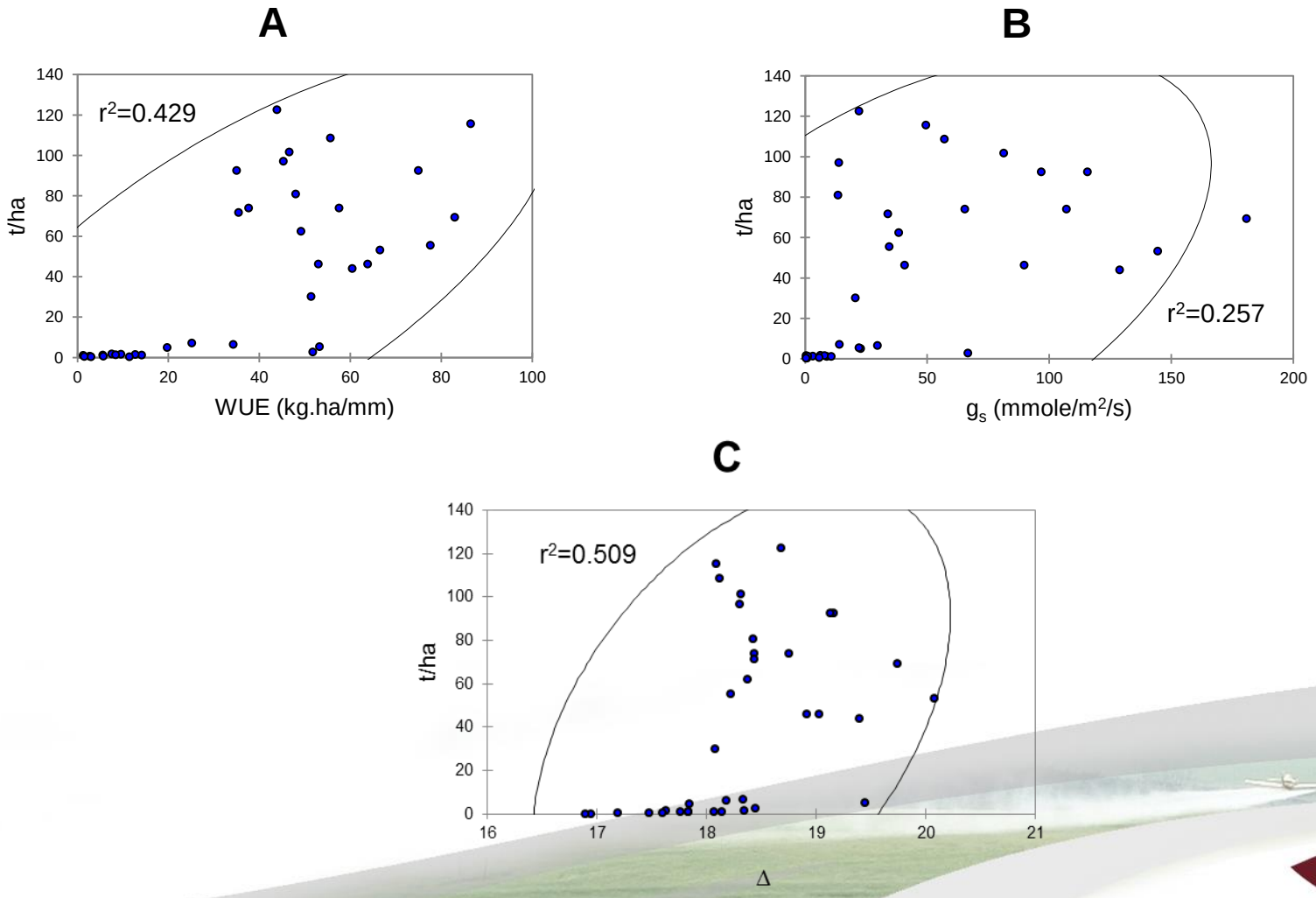
Correlation with yield



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Correlation (35 genotypes)

Variables	LAI	GR	SOD	AP	CCI	discr	cond	NR	prolien	t/ha	WUE	carotene
LAI	**1**	**-0.642**	**-0.512**	**-0.176**	-0.075	**0.538**	**0.703**	**0.849**	**-0.807**	**0.812**	**0.621**	-0.080
GR	**-0.642**	**1**	**0.392**	**0.177**	0.032	**-0.482**	**-0.614**	**-0.719**	**0.637**	**-0.699**	**-0.528**	**0.261**
SOD	**-0.512**	**0.392**	**1**	**0.375**	0.043	**-0.350**	**-0.566**	**-0.632**	**0.509**	**-0.600**	**-0.457**	-0.002
AP	**-0.176**	**0.177**	**0.375**	**1**	-0.060	**-0.223**	**-0.266**	**-0.266**	**0.187**	**-0.205**	-0.107	-0.108
CCI	-0.075	0.032	0.043	-0.060	**1**	-0.094	-0.049	-0.045	0.037	-0.150	-0.165	**0.182**
discr	**0.538**	**-0.482**	**-0.350**	**-0.223**	-0.094	**1**	**0.585**	**0.561**	**-0.496**	**0.561**	**0.448**	-0.057
cond	**0.703**	**-0.614**	**-0.566**	**-0.266**	-0.049	**0.585**	**1**	**0.793**	**-0.692**	**0.765**	**0.602**	**-0.186**
NR	**0.849**	**-0.719**	**-0.632**	**-0.266**	-0.045	**0.561**	**0.793**	**1**	**-0.891**	**0.885**	**0.591**	-0.144
prolien	**-0.807**	**0.637**	**0.509**	**0.187**	0.037	**-0.496**	**-0.692**	**-0.891**	**1**	**-0.798**	**-0.481**	0.160
t/ha	**0.812**	**-0.699**	**-0.600**	**-0.205**	-0.150	**0.561**	**0.765**	**0.885**	**-0.798**	**1**	**0.835**	**-0.220**
WUE	**0.621**	**-0.528**	**-0.457**	-0.107	-0.165	**0.448**	**0.602**	**0.591**	**-0.481**	**0.835**	**1**	**-0.206**
carotene	-0.080	**0.261**	-0.002	-0.108	**0.182**	-0.057	**-0.186**	-0.144	0.160	**-0.220**	**-0.206**	**1**

LAI – leaf cover, GR – glutathione reductase, SOD – superoxide dismutase, AP – ascorbate peroxidase, CCI – chlorophyll index, Δ - 13C discrimination, NR – nitrate reductase, proline, g_s – stomatal conductance, RWC – relative water content, t/ha – yield, WUE – water use efficiency, carotene – carotene content

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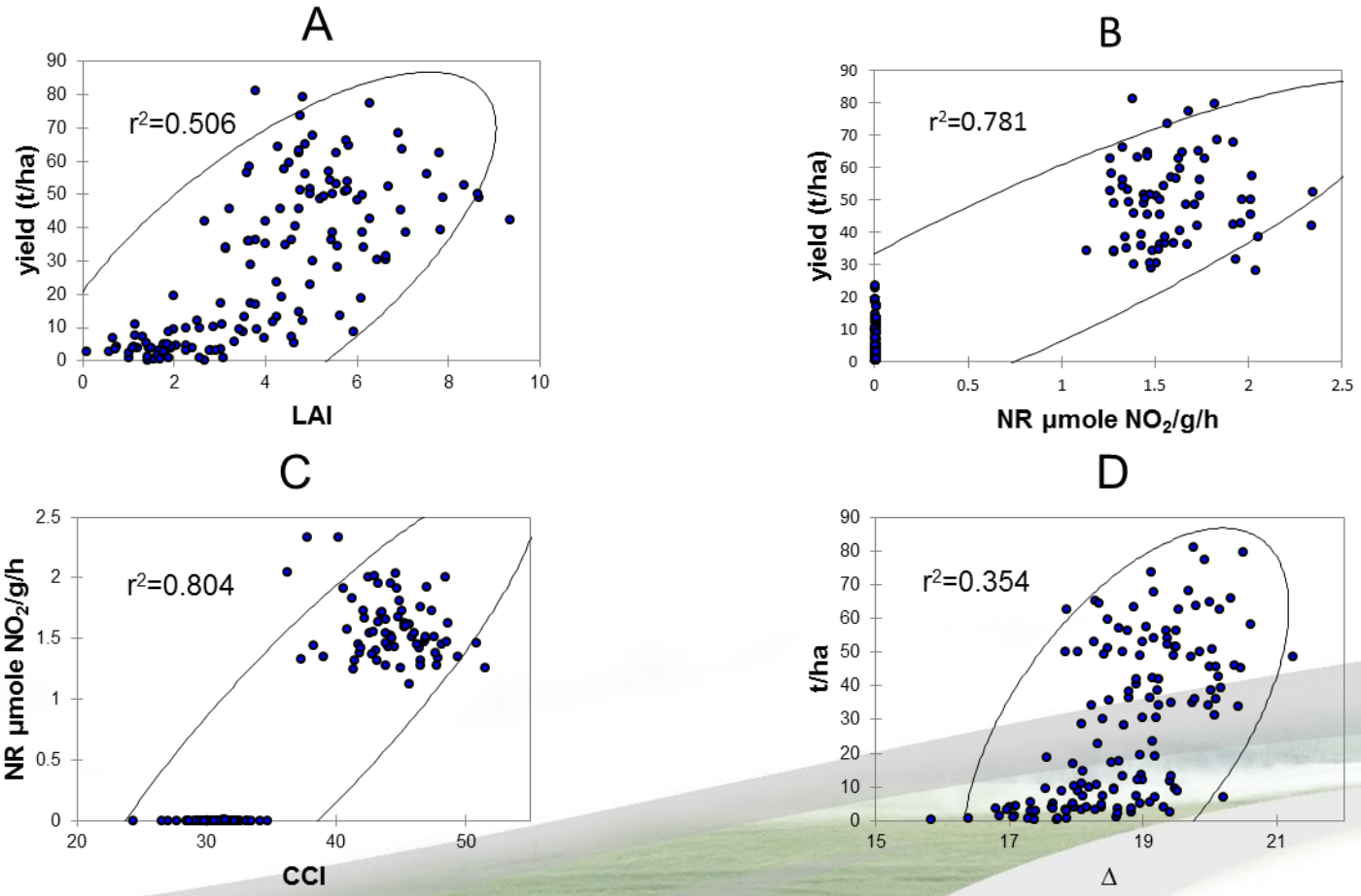
Correlation (35 genotypes)



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Correlation (8 genotypes)

Variables	LAI	cm	GR	SOD	AP	CCI	discr	NR	proline	t/ha	WUE	carotene	cond	RWC
LAI	**1**	**0.447**	**-0.554**	-0.124	**-0.555**	0.258	**0.665**	**0.774**	**-0.803**	**0.738**	**0.388**	-0.049	**0.801**	-0.226
cm	**0.447**	**1**	**-0.379**	**-0.337**	-0.169	-0.276	**0.623**	**0.542**	**-0.517**	0.276	0.068	-0.280	**0.439**	-0.177
GR	**-0.554**	**-0.379**	**1**	0.042	0.241	**-0.337**	**-0.357**	**-0.535**	**0.500**	**-0.391**	-0.080	-0.166	**-0.524**	0.128
SOD	-0.124	**-0.337**	0.042	**1**	0.216	0.041	-0.242	-0.247	0.183	-0.229	-0.231	**0.329**	-0.183	0.166
AP	**-0.555**	-0.169	0.241	0.216	**1**	**-0.314**	**-0.479**	**-0.574**	**0.705**	**-0.561**	**-0.337**	0.203	**-0.603**	**0.301**
CCI	0.258	-0.276	**-0.337**	0.041	**-0.314**	**1**	-0.025	0.079	-0.121	**0.300**	0.152	**0.323**	0.124	0.014
discr	**0.665**	**0.623**	**-0.357**	-0.242	**-0.479**	-0.025	**1**	**0.679**	**-0.665**	**0.583**	0.255	**-0.293**	**0.636**	**-0.308**
NR	**0.774**	**0.542**	**-0.535**	-0.247	**-0.574**	0.079	**0.679**	**1**	**-0.793**	**0.669**	**0.294**	-0.252	**0.871**	-0.253
proline	**-0.803**	**-0.517**	**0.500**	0.183	**0.705**	-0.121	**-0.665**	**-0.793**	**1**	**-0.696**	**-0.378**	0.247	**-0.823**	**0.386**
t/ha	**0.738**	0.276	**-0.391**	-0.229	**-0.561**	**0.300**	**0.583**	**0.669**	**-0.696**	**1**	**0.796**	-0.259	**0.700**	0.013
WUE	**0.388**	0.068	-0.080	-0.231	**-0.337**	0.152	0.255	**0.294**	**-0.378**	**0.796**	**1**	**-0.307**	**0.328**	0.150
carotene	-0.049	-0.280	-0.166	**0.329**	0.203	**0.323**	**-0.293**	-0.252	0.247	-0.259	**-0.307**	**1**	-0.131	0.098
cond	**0.801**	**0.439**	**-0.524**	-0.183	**-0.603**	0.124	**0.636**	**0.871**	**-0.823**	**0.700**	**0.328**	-0.131	**1**	-0.270
RWC	-0.226	-0.177	0.128	0.166	**0.301**	0.014	**-0.308**	-0.253	**0.386**	0.013	0.150	0.098	-0.270	**1**

LAI – leaf cover, cm – stem length, GR – glutathione reductase, SOD – superoxide dismutase, AP – ascorbate peroxidase, CCI – chlorophyll index, Δ - 13C discrimination, NR – nitrate reductase, proline, g_s – stomatal conductance, RWC – relative water content, t/ha – yield, WUE – water use efficiency, carotene – carotene content

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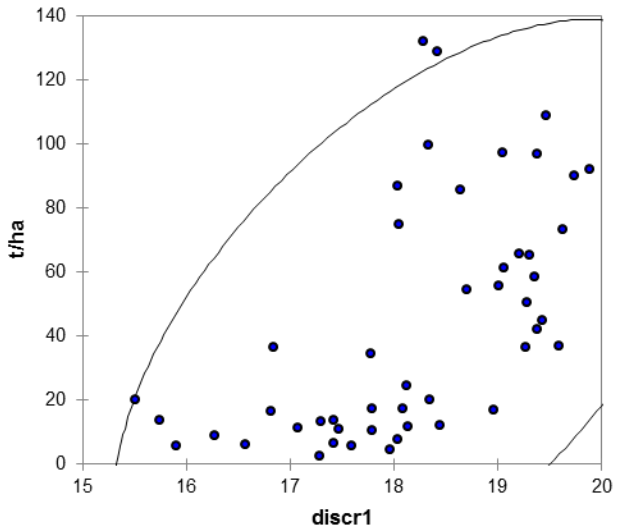
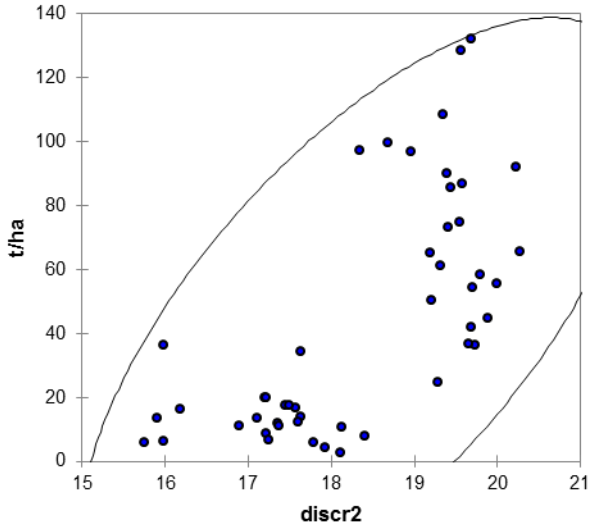
Correlation (8 genotypes)





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Conclusion

- 14 repeatable correlations
- GR:proline +, GR:Δ -, GR:NR -, g_s:GR -, Δ:NR +, Δ:proline, g_s:Δ +, NR:proline -, g_s:NR +, t/ha:NR +, proline:g_s -, g_s:t/ha +, WUE:t/ha +, Δ:t/ha +
- Correlations between early/late parameters

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Conclusion

- Correlation between Δ and t/ha not very strong – better with more genotypes more repeats?
- Large variation measurements: ie. stomatal conductance, chlorophyll content – could become smaller with more repeats

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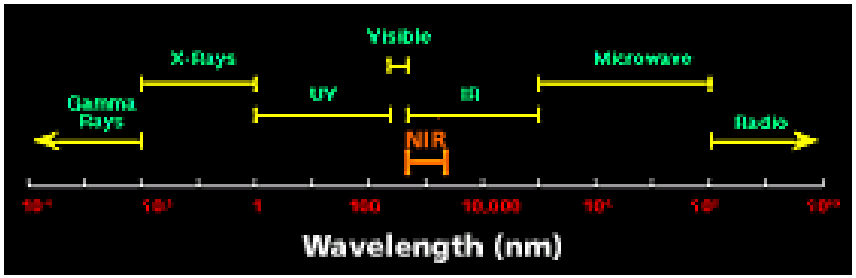
NIRS calibration

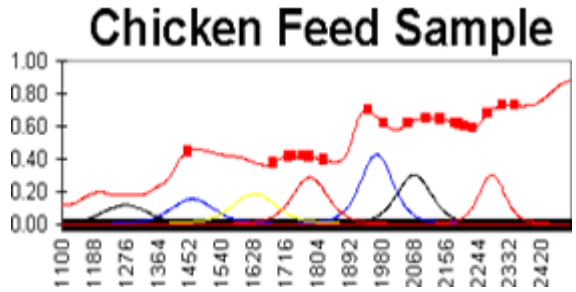
NIRS (Near-InfraRed Spectroscopy) is the technique of using a sample's NIR absorbance characteristics to predict parameters of interest. NIR is a region of the Electromagnetic spectrum. Click the chart below to enlarge the graphic.



NIRS exploits the fact that many natural products absorb NIR radiation at specific regions or wavelengths. Specifically, N-H, O-H and C-H bonds are strongly absorbed by NIR radiation, with other molecular bonds less so. Thus, samples high in proteins (many N-H bonds) will absorb more in the amine (N-H) bond regions than samples low in protein. Samples high in moisture and or / sugars will have higher adsorptions in regions associated with hydroxyl (OH) bonds. A sample's NIR spectrum will be a composite of all the absorbances from all of the molecular bonds in the sample.

NIRS calibration

A simplified diagram:



To create a calibration, samples are chosen which are representative to the samples that will be analyzed. There may be as few as 60 samples or there could be several thousand. These samples are analyzed in the NIR, and the spectra stored. The samples are then sent to have the reference analysis performed on them. These are termed calibration samples. We will use 60 sample analyzed for protein content and scanned on a NIRSystems model 5000 for this example.

When the samples come back, the spectroscopist has:

60 sample spectra consisting of 700 datapoints (1100 - 2500 nm)

60 sample reference data consisting of protein content values

To create a calibration, a mathematical relationship can be established between these two sets of data.

This relationship can then be used to predict the parameter value in unknown samples.

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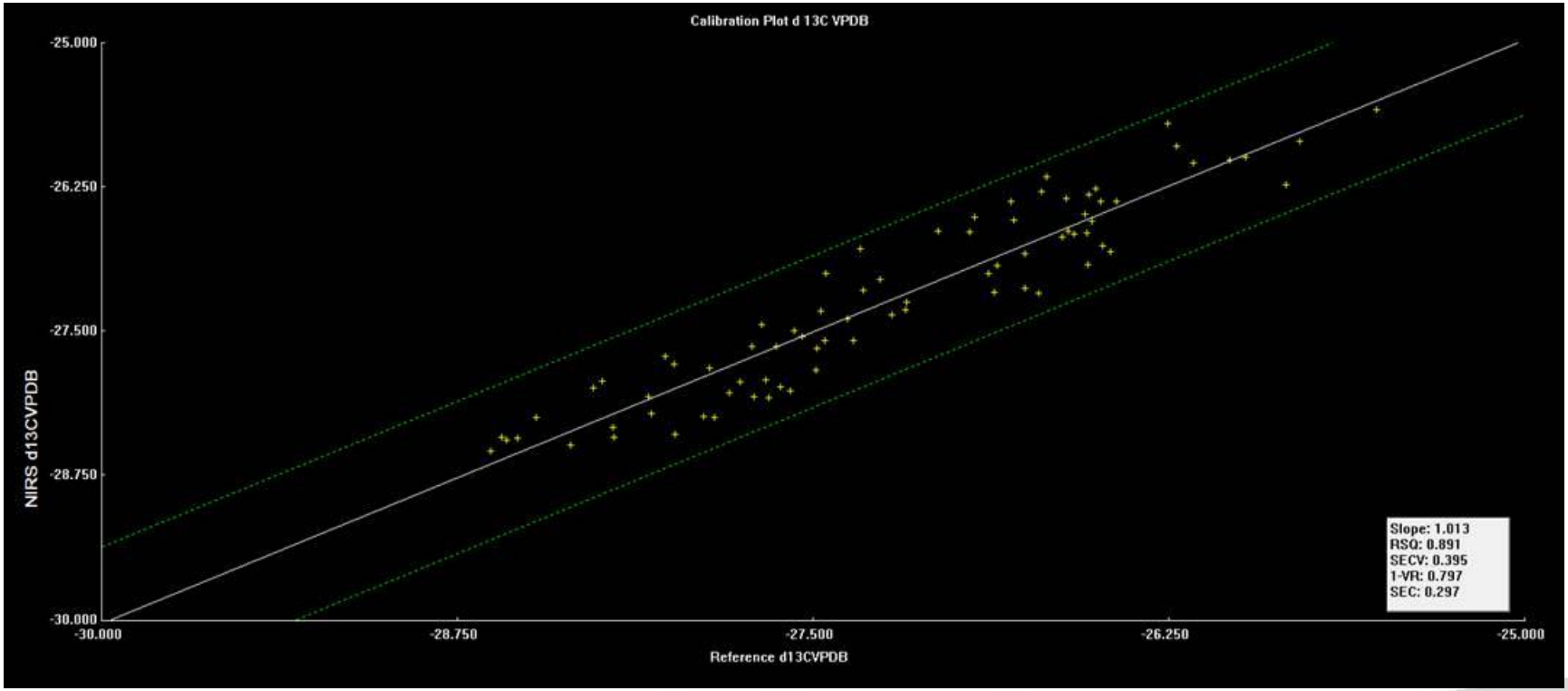

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Recommendations

- More repeats for screening difference between genotypes
- 100% treatment - too much water – difference to 60% small

Thank you

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