

International Seminar:

'Strengthening Agroforestry Programs in Higher Education for Food Security In Sub-Saharan Africa – SAPHE'

Analysis of adaptive genetic variation

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Outline

Introduction





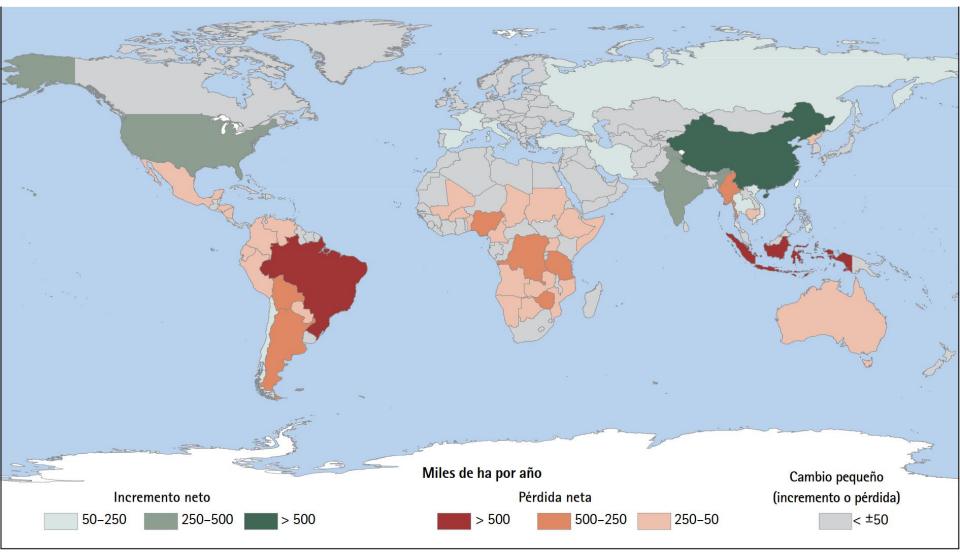
- Methods and tools
- Examples
- [Practical issues/Discussion...]

Pre-Introduction

Usefulness Exportable methodology Practical cases



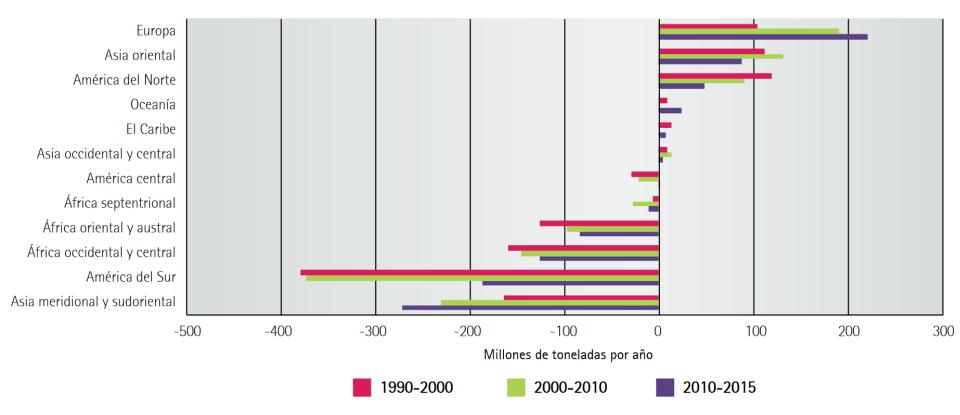
Net annual gain/lost 1990-2015



ÁFRICA								
(58 países y territorios)								
Variable (unidad, año)ª	Total	Dirección del cambio ^b	Cambio anual ^ь (%)	Disponibilidad de datos ^e (situación/ tendencia)				
Área de bosque (millones de ha, 2015)	624	\downarrow	-0,49	A/A				
Bosque natural (millones de ha, 2015)⁴	600	\checkmark	-0,54	A/A				
Bosque plantado (millones de ha, 2015)	16	\uparrow	1,34	A/A				
Cambio neto anual del bosque (millones de ha, 2010–2015)	-2,8			A/*				
Cambio neto anual del bosque natural (millones de ha, 2010–2015) ^ª	-3,1			A/*				
Cambio neto anual del bosque plantado (millones de ha, 2010–2015)	0,2			A/*				
Existencias en formación en los bosques (miles de millones de m ³ , 2015) ^e	79	\downarrow	-0,37	A/A				
Existencias en formación en los bosques (m ³ por ha, 2015) ^e	128	\uparrow	0,13	A/A				
Carbono en la biomasa por encima y por debajo del suelo (Gt, 2015) ^e	60	\downarrow	-0,43	A/A				
Carbono en la biomasa por encima y por debajo del suelo (toneladas por ha, 2015) ^e	96	\uparrow	0,07	A/A				

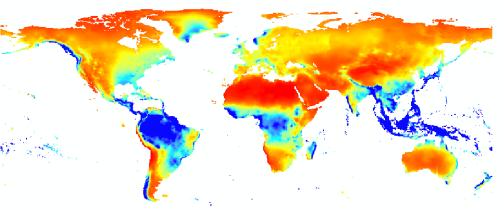
Intl. Seminar 'Strengthening Agroforesuly Flogranis in Figure Education for Food Security in Sub-Sanaran Anica – SAFTE Madrid, 27 Feb-03 Mar 2017

During the last 25 years the forest biomass Carbon stock has been reduced 11,1 Gt, It equivalents a reduction of 442 millions of tons at year

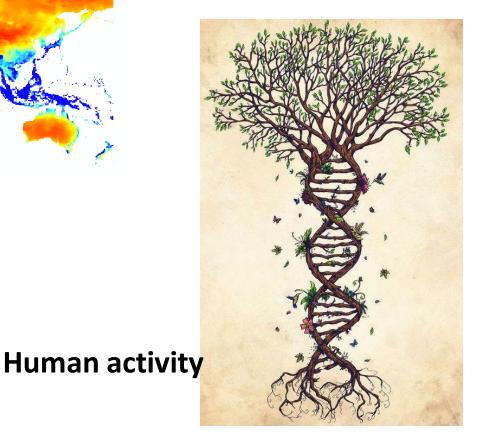


Introduction

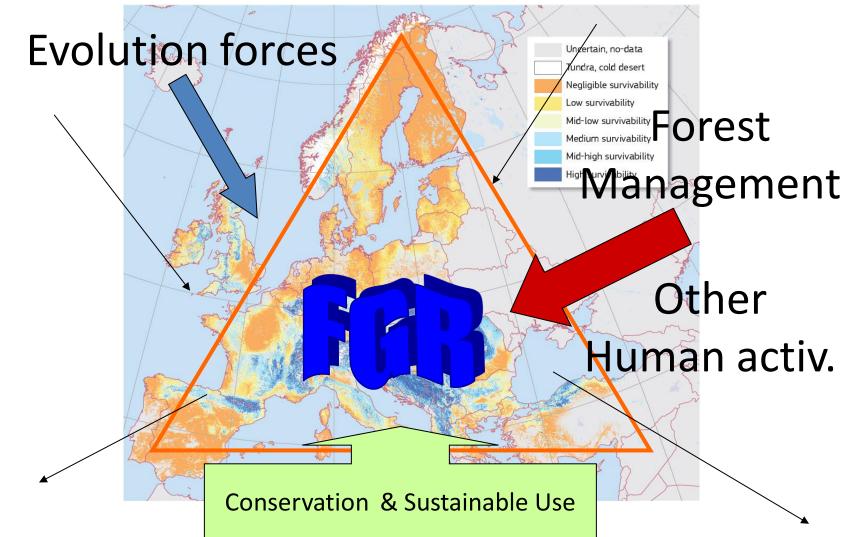
Climate dynamics



Genetics (adaptation)



DIFFERENT TIME SPAN BUT SIMILAR EFFECTS



Genetics: Evolution Forces

- Genetic drift: non directional / random changes in frequency between generations / small pops.
- Natural Selection: Adaptive changes (fitness, reprod. success)
- Migration: Gen exchange among populations.
- Reproductive Systems Gene recombination in successive generations
- Mutation: Gene changes at molecular level

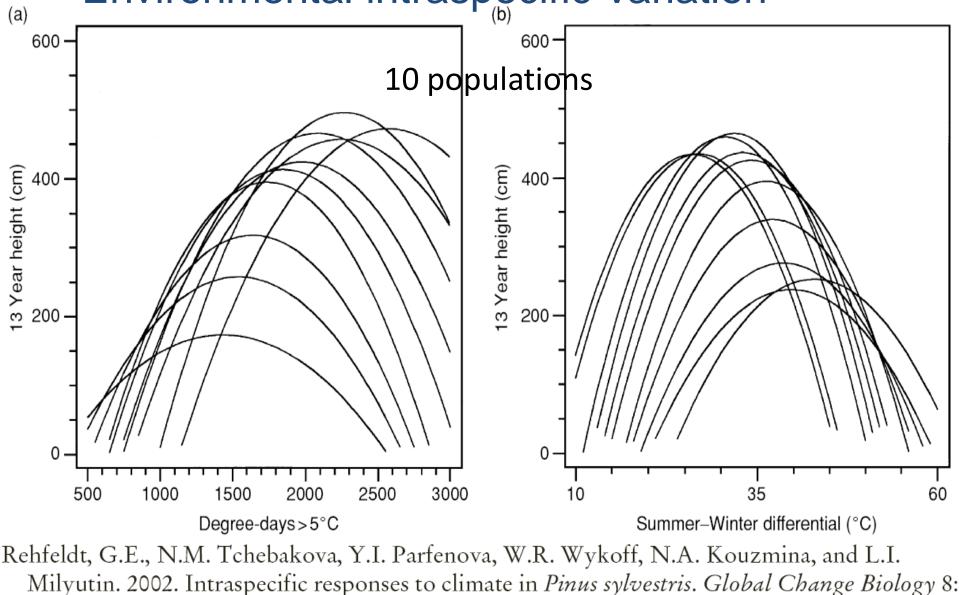
• "Life's little mysteries" Gene flexibility (Phen-Plas) Autorganizative , novelties,

Genetic diversity

- Existing variation among "populations"
- As consequence of evolution processes & ...

• One of the most powerful resource for fighting against global change

Environmental intraspecific Variation



1-18.

Adaptive Variation

Contrary to "neutral" variation Environmental adaptation related traits Survival Growth (Low: suboptimal or drought defense) Water use efficiency

Low economical importance but evolutive/adaptive Reproductive potential (flowering, mating synchr., seed ripening, etc)

$$P=G+E \qquad V_p=V_G+V_E=V_A+V_{NA}+V_E$$

Use for Conservation FGR

Structure, distribution & patterns of

Adapt. variation inter/intra pop.

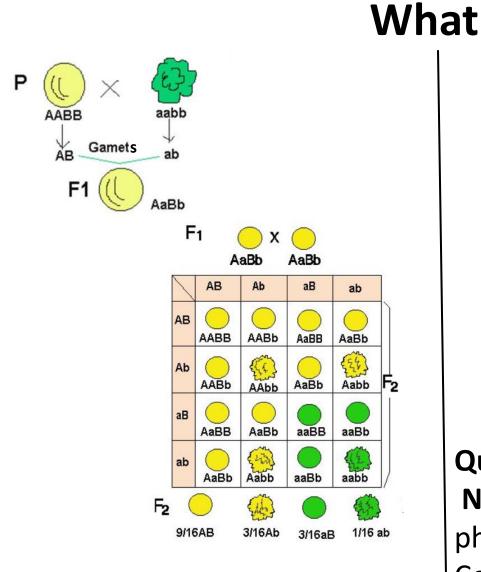
Variation in metapopulations

Genetic diversity based on neutral markers can NOT substitute direct measurements of quantitative variation in adaptive traits and particularly for forest genetic resources conservation

Basic information in reduced N_e populations

Info about threatening level

.



Mendel Laws





AAAAAAA





AaAaAaAa

Quantitative Genetics: NO direct relation phenotype & genotype Continuous distribution genotypes

What /How to

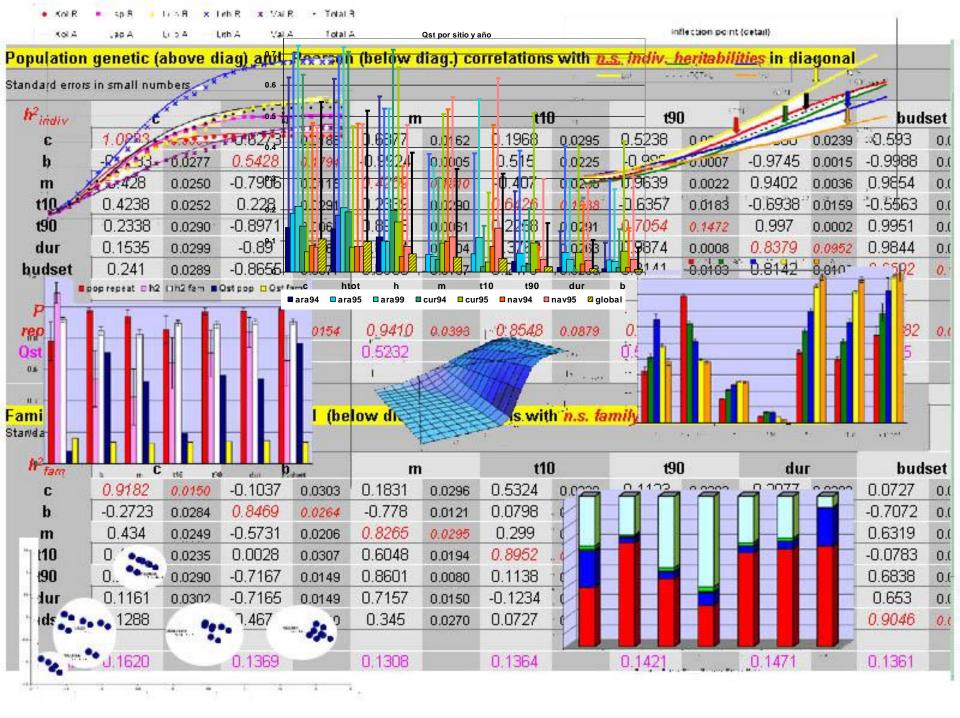


Tools for addressing analytical problems without fixed laws

Variability

- Existence
- Dealing and understanding (adaptiveness?)
- Modeling and controlling

experimental error





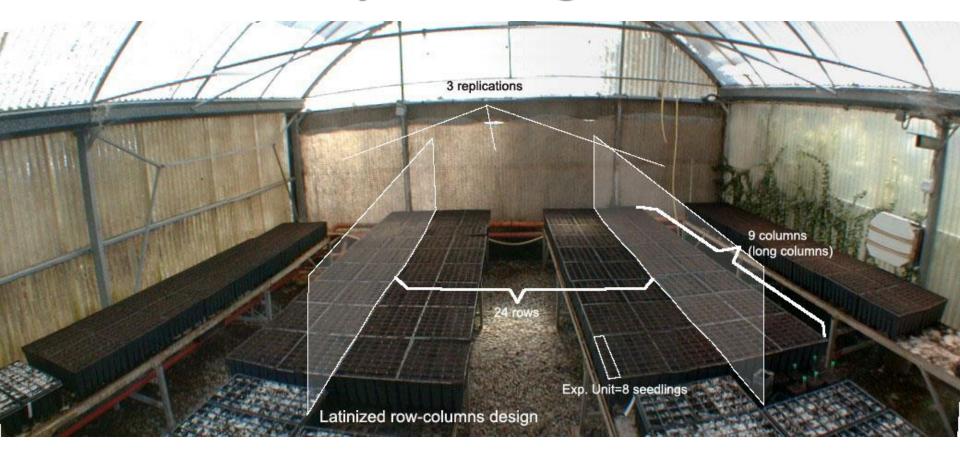
Provenance Trials



 $x_{ijklmn} = \mu + repl_i + block_i(repl_i) + plot_k + pop_i + seedlot_m(pop_i) + \varepsilon_n$ Intil Seminar 'Strengthening Agroforestry Programs in Higher Education for Food Security in Sub-Saharan Africa – SAPHE' Madrid, 27 Feb-03 Mar 2017

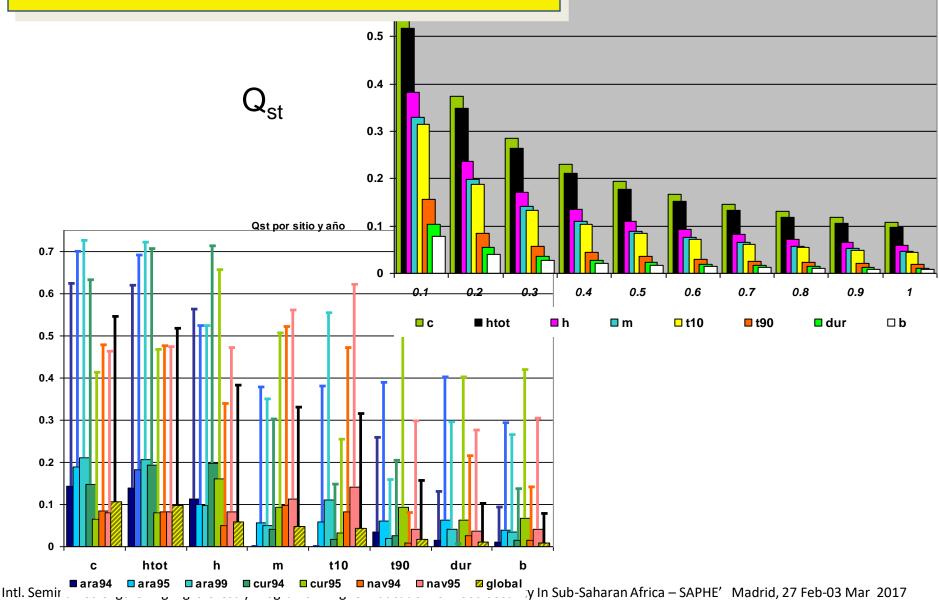
TXed mode REMEBLUP 8

Layout designs

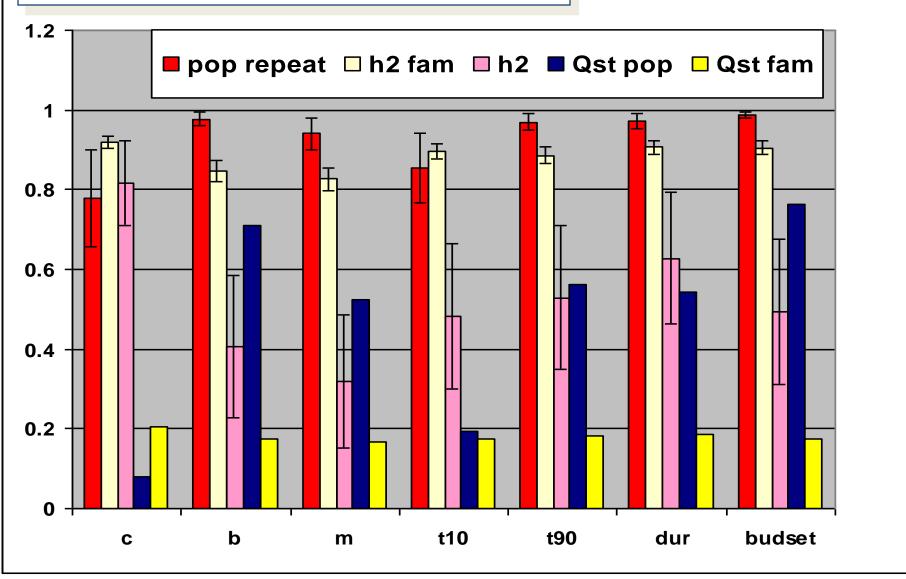


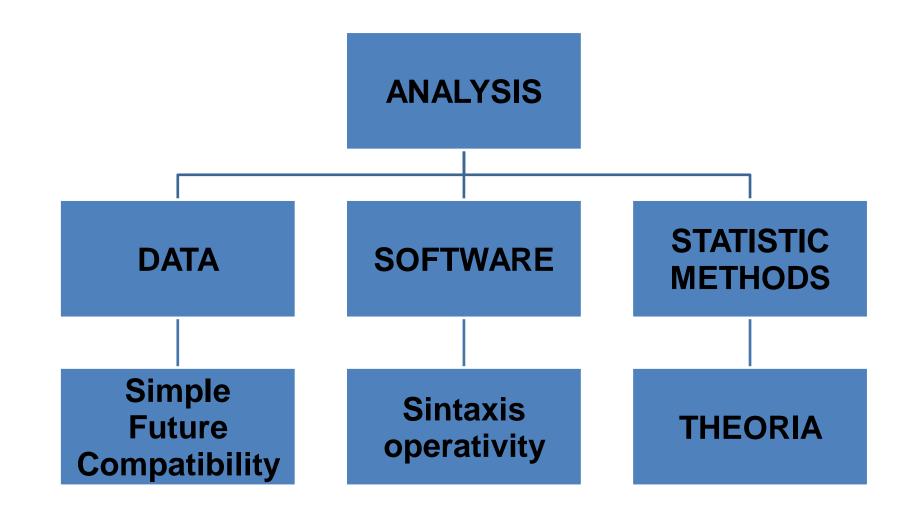
Provenance Trials $\sigma^2_{prov} + \sum_i \frac{\sigma^2_{provx\alpha_i}}{n_i}$ $Q_{st} = \frac{\sigma^2_{between}}{\sigma^2_{between} + 2\sigma^2_{inside}}$ $\left(\sigma_{prov}^{2} + \sum_{i} \frac{\sigma_{provx\alpha_{i}}^{2}}{n_{i}}\right) + 2h^{2}\sigma_{error}^{2}$ $r_{pop}^{2} = \frac{\sigma_{pop}^{2}}{\sigma_{pop}^{2} + \frac{\sigma_{colum}^{2}}{b.r/f} + \frac{\sigma_{block}^{2}}{b/p} + \frac{\sigma_{e}^{2}}{p.n}} \xrightarrow{\prime} \frac{\tau}{p.n}$ $h_{fam}^{2} = \frac{\sigma_{fam}^{2}}{\sigma_{fam}^{2} + \frac{\sigma_{colum}^{2}}{b.r} + \frac{\sigma_{block}^{2}}{c} + \frac{\sigma_{e}^{2}}{f.n}} \qquad Q_{st} pop = \frac{\sigma_{pop}^{2}}{\sigma_{pop}^{2} + 2(4\sigma_{fam}^{2})}$

Provenance Trials



Progeny Trials





Genetic evaluation

Design and Analysis of experiments

Basic Principles

Conventional and current (IB) designs

➢Software

HOW to



Genetic evaluation

Adaptive variation

Trials (Spp /prov /prog /clon)

Conservation Utility

Planning of an experiment (i)

- Definition of objectives
- Definition of all sources of variation
 - Treatments and their levels
 - Experimental units
 - Nuisance factors: blocking, noise & covs
- Setting up the experimental units & treatments

Planning of an experiment (ii)

Definition of response variable, experimental process and issues foresight

- Set up the model
- Scheme of analysis steps
- Set up sampling size

Review all foregoing points and modifying if necessary

Stages (i)

- Definition of the problem
- Definition of objectives
- Selection of treatments to test (interactions)
- Selection of the material to test
- Selection of the experimental design (simple)
- Selection of the experimental unit size and number of replications

Stages (ii)

- Control of "surroundings" effects
- Kind of data to be taken
- Selection of statistical tests
- Accomplishment of the experiment
- Analysis and interpretation of results
 Final reporting (conclusions)

Principles (i)

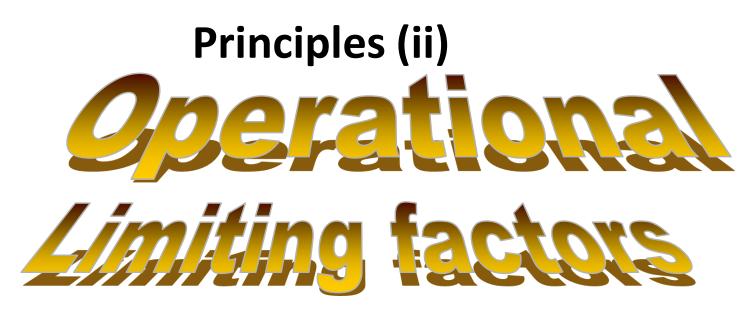
1.Replications. (experimental error basis) Standard Error of Difference Agronomic trails SED<1/3 diff Material selection SED<1/6 diff Knowing s² & d ==> n

$$v = \sqrt{\frac{2\sigma^2}{(n)}}$$

2. Treatment (broad sense) Randomization

3. Local control of existing variation in trial site (Blocking or spatial analysis)

"typical numbers & expressions" 25 plants per plot A replications Atreatments randomization 3 sites 2border lines latinization **RCB** 25 genotypes 5x5m spacing single tree plot



Number of available effectives Site constraints (topography, surface ...) Technical limitations (machinery,) Measurements Competence, specific needs, Future treatments, thinnings,... Spacing, density

Experimental design

Initial assumptions or constraints:

Additivity Normality Homocedasticity.

Different treatment errors are independient & distributed N(0,σ²)

Statistic tests:

N: Shapiro-Wilks, graphs distrib, freq acum., res * pred H: Barlett, Levenne, ratios variances

Transformations No parametric methods

Elementary Designs



Model:
$$y_{ij} = \mu + t_i + \varepsilon_{ij}$$

	dof	SS	MS	F	EMS
Total	rt-1	а	a/rt-1		
Treat	t-1	b	b/t-1	MS _T / MS _E	σ_{e}^{2} +r σ_{t}^{2}
Error	t(r-1)	С	c/t(r-1)		σ² _e

Model y = treat;

Elementary Designs



Model:
$$y_{ijk} = \mu + t_i + b_j + \varepsilon_{ijk}$$

	dof	SS	MS	F	EMS
Total	rb-1	а	a/rb-1		
Treat	t-1	b	b/t-1	MS _T / MS _E	σ_{e}^{2} +b σ_{t}^{2}
Blq	b-1	С	c/b-1	MS _B /MS _E	σ_{e}^{2} +t σ_{b}^{2}
Error	t-1)(r-1)	d	d/t(r-1)		σ² _e

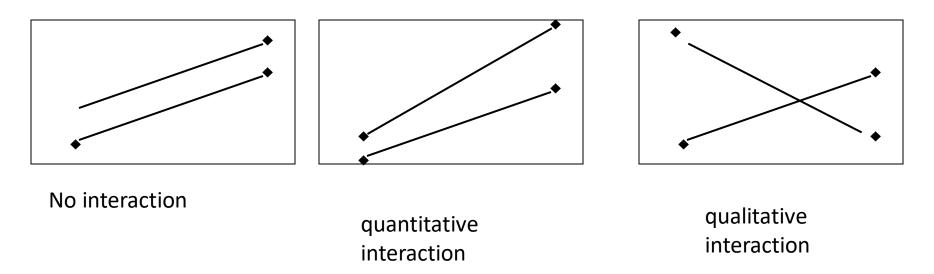
Model y = treat blq;

Experimental design

Possible structure of treatments Factorial: total combination all x all Possibility interactions study (GxE)

Reaction norms

$$y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_{ij} + \varepsilon_{ijk}$$



Experimental design

Possible structure of treatments Hierarquical or nested: Impossible combination $y_{ijk} = \mu + \alpha_i + \beta_j (\alpha_i) + \varepsilon_{ijk}$

Model: y = pop fam(pop);

Is it important the treatment structure ?

HN

other structure + important.....

¿Fixed o Random?



- 1. Critical decision
- 2. Not well documented on texts
- 3. Usually based on subjective statistic agreements

<u>Fixed</u>: Levels of factor clearly targeted or selected *Results & conclusions* from anova are for these levels *Main aim*: Mean estimation of the variable for each level (BLUE)

<u>**Random</u></u>: Levels are a random sample from all possible.** *Results & conclusions* **from anova can be extrapolated + level** *Main aim***: Variability estimation of the variable or factor or perhaps prediction at a given level</u>**

	dof	MS	A y B fixed	A y B rand	A:fix B:rand
Total	abr-1				
А	a-1	MS_A	MS _A / MS _E	MS_A / MS_{AB}	MS_A / MS_{AB}
В	b-1	MS _B	MS _B / MS _E	MS _B / MS _{AB}	MS _B / MS _E
AxB	(a-1)(b-1)	MS _{AB}	MS _{AB} / MS _E	MS _{AB} / MS _E	MS_{AB}/MS_{E}
Error	ab(r-1)	MS_{E}			

$$\sigma_e^2 + c_1 \Phi_\alpha$$
 $\sigma_e^2 + n \sigma_{ab}^2 + nb \sigma_a^2$

¿Fixed o Random?

How to asses? A PRIORI

Scientific Criteria :

 is it possible to repeat the factor levels in other site or year?
 has it meaning this replication?
 Yes + Yes = Fixed

Statistic Criteria :

"Random" few levels (3-5) =>weak variance estimation, Better setting as fixed and use the results only at these levels

"Fixed" with many levels (>10) without structure, better setting as random and estimating means by BLUPs (

E.M.S. Numeric difficulty

¿Fixed o Random?

GLM

Model y =loc blq(loc) var var*loc; Random loc blq(loc) var*loc /test;

- 1º Calculation as fixed
- 2º Calculation EMS
- 3º Repeat F-tests with proper denominators

MIXED MODELS

Incomplete Blocks

Evaluation : high nº genotypes limited material

'Many genotypes' means huge blocks # no control

I.B. Not all treat by block, so several blocks are needed for a complete replication

Based on Additivity: B-C= $(B-C)_3$ = $(B-A)_1$ - $(C-A)_2$

		В	Α	•	1
-	•	А	С	•	2
В	•	-	-	С	3

Experimental error independent of treatment

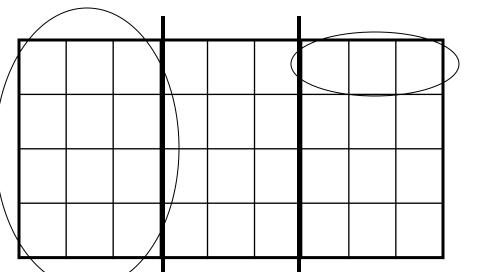
Incomplete Blocks

- Coexist direct & indirect comparisons
- Lost of accuracy on indirect comparisons but experimental error reduction

Resolvable designs

i.e.: g=k bi

 α -latice, latinized, row-columns,...



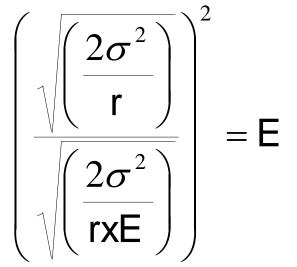
Complex specific Software

interblock info

I.B. design Efficiency

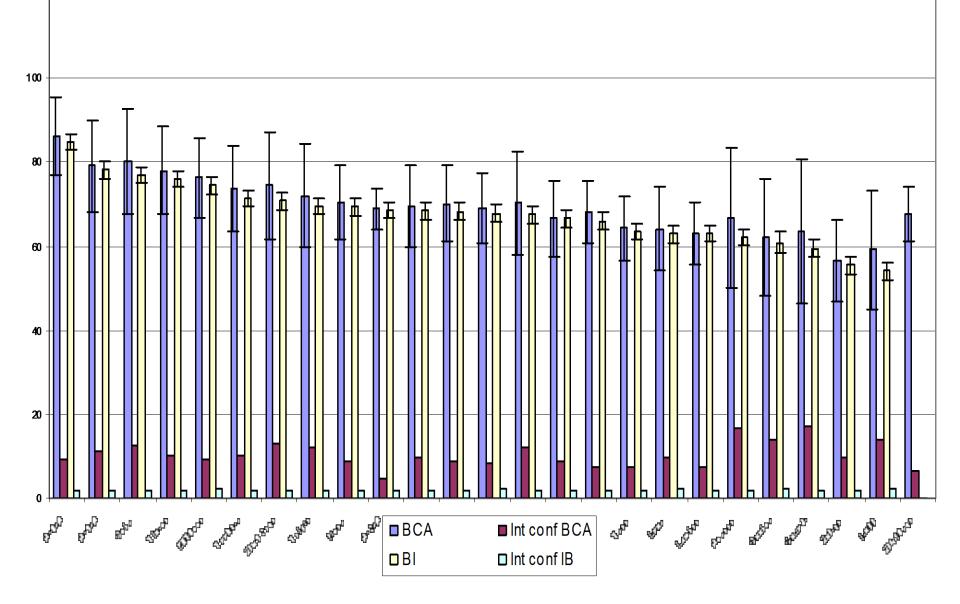
Objective: To compare genotypes highest accuracy

E = (SED_{RCB}/SED_{IB})² n^o of extra replications in a RCB to get same accuracy level



A IB with 4 reps y E=1.5 equals to a RCB with 4x1.5=6 CB

"Efficiency" ~ costs



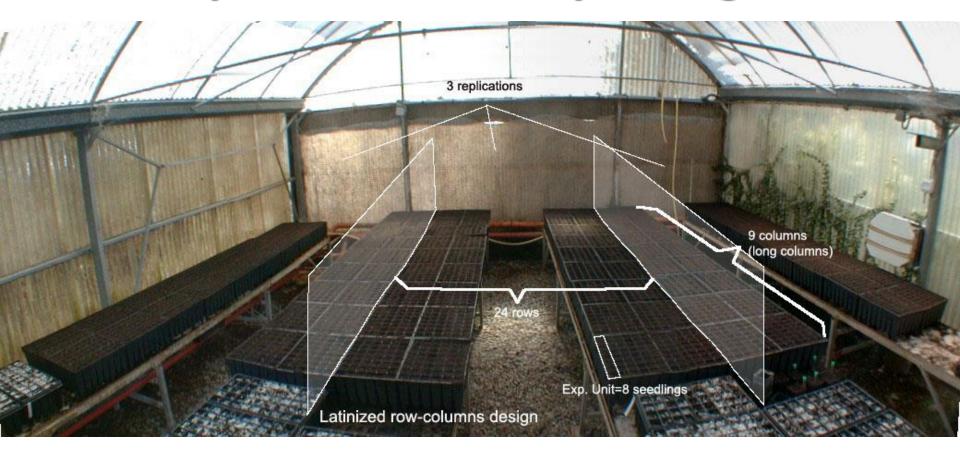
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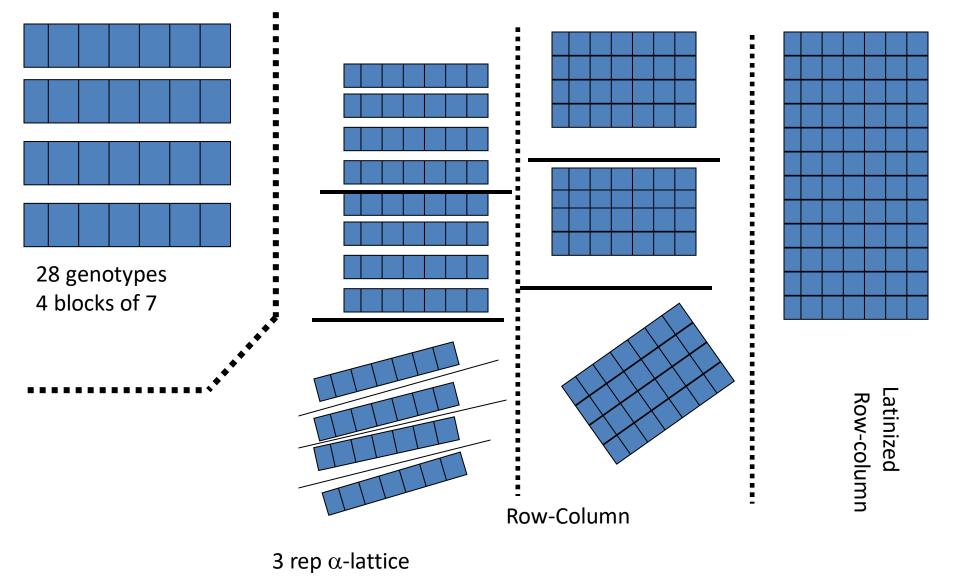
120

For simple and RCB AZARsXXI.exe



Layout software: CyCDesigN





http://www.vsni.co.uk/software/cycdesign/

VSNi



CycDesigN is a computer package for the generation of optimal or near-optimal experimental designs. It comprises

three modules: CycDesigN is a computer package fc It provides the most comprehensive design generati involved in field, glasshouse and laboratory trials.

The designs include cyclic, alpha and factorial design designs, t-latinized and partially-latinized designs as a result, the algorithms incorporate the most recent CycXover is a sub-system, generates optimal or near crossover experiment involves the application of sec periods.

The observations made on each subject at the end o applied in the current period, and the carry-over effe designs are also known as change-over or carry-ove your output from a CycDesigN or CycXOver session i generate either GenStat or SAS code for the analysis CycAnalysis manual. So CycDesigN's focus is on the treatments, as in variety trials.

In contrast, GenStat concentrates on the more tradit and strip plots, criss-cross designs, Latin and Graeco designs. CycDesigN is written in Visual C++ and runs

Current users can download it here.



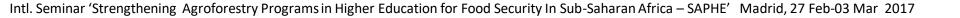
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CSIRO Forestry and Forest Products Canberra Australia

CycDesigN

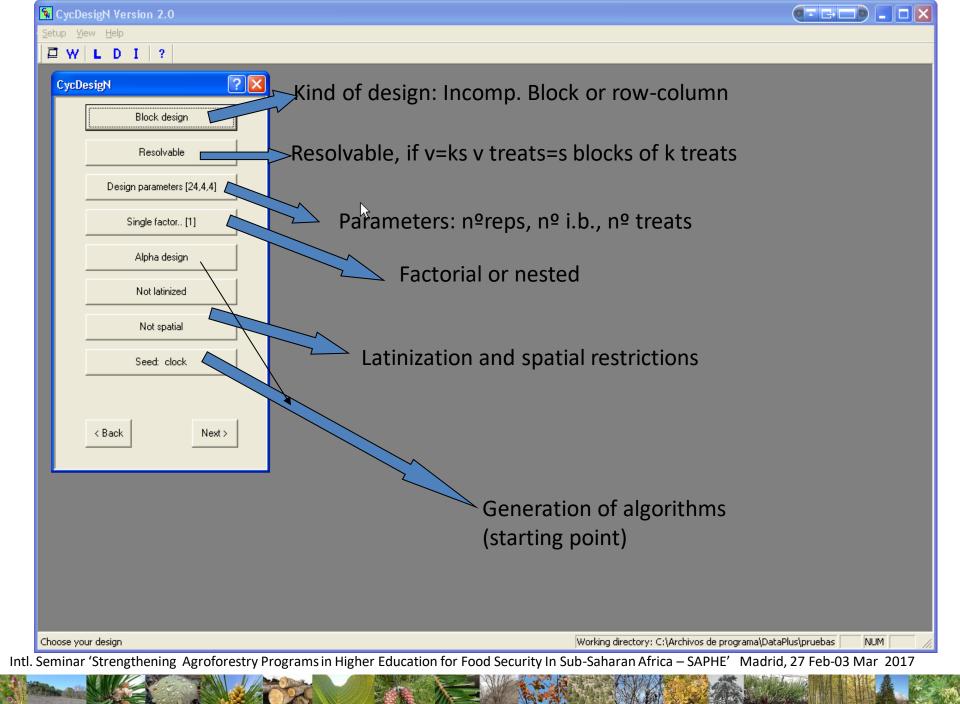
The University of Waikato Hamilton New Zealand

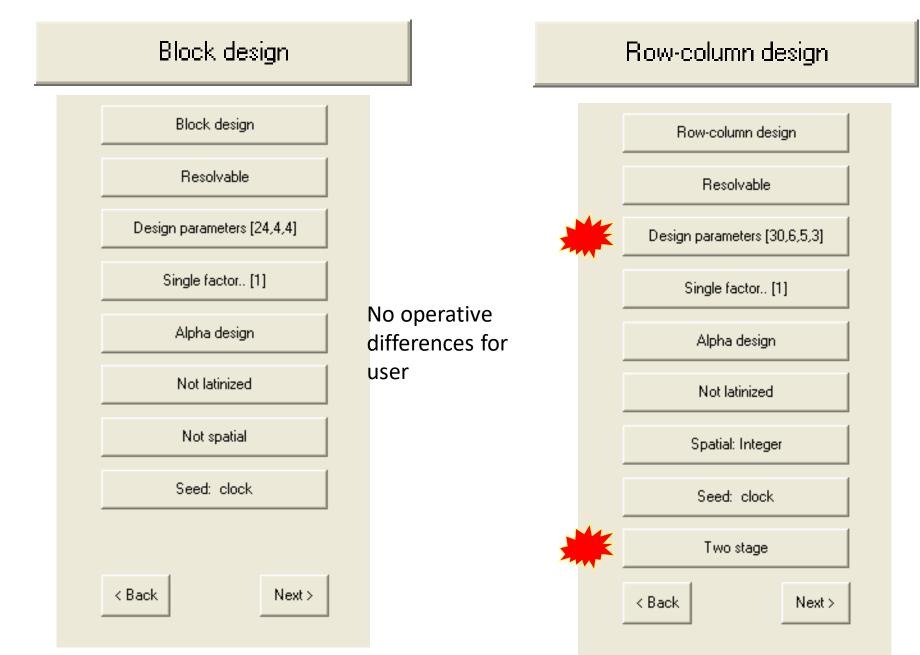
Version 2.0



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		Ro	w-column	design			
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		Design p	arameters	[120,10,12,3]			
		S	ingle facto	vr [1]		Working directory Enter your new working directory	
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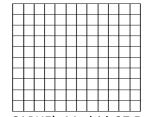
Design parameters [120,10,3]

Block design

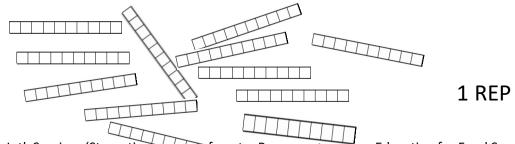
Design parameters 🛛 💽 🔀
Number of treatments 120
Number of units/block 10
Number of replicates 3
< Back Next >

Row-column design

Design parameters	? 🗙
Number of treatments	120
Number of rows	10 🔹
Number of columns	12 •
Number of replicates	3 🔹
< Back	Next >



12 I.B. of 10 treat.



Single factor., [5]

NESTED 5 POPULATIONS: 30, 20, 24, 26 y 20 Families respectiv.

Treatment structure	? ×
Single factor	
•	
Number of treatment groups 5	÷
Factorial	
C	
< Back Next >	

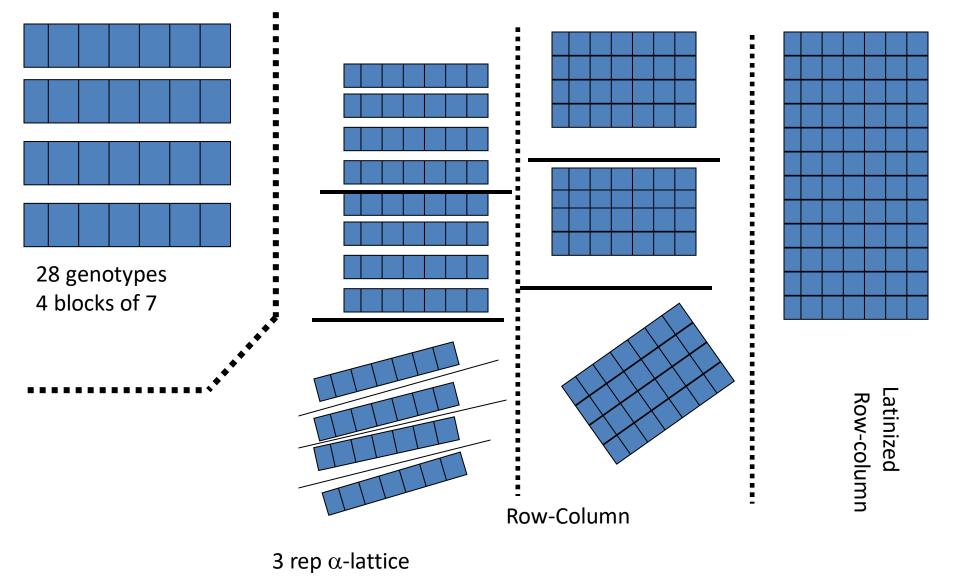
Treatment group sizes		? 🗙
1 30 -	2	26
3 20 🔸	4	20
5 24 🛨		
< Back		Next >

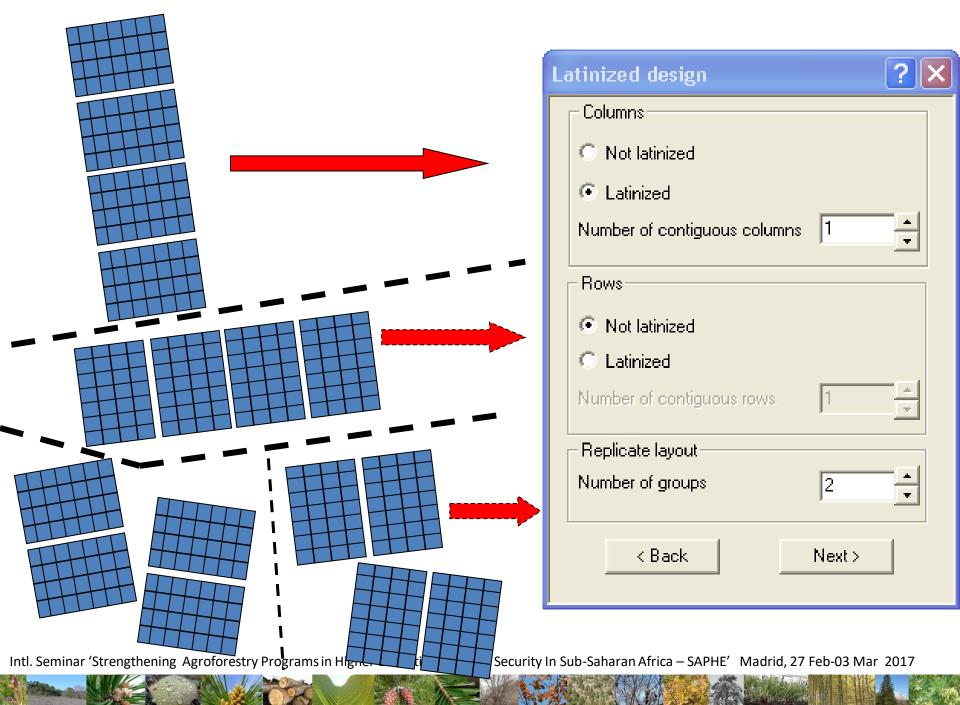
FACTORIAL:

2 Factors 10 levels in factor 1 12 levels in factor 2 10 x 12= 120 treat.

Treatment structure	? 🛛
Single factor	
C	
Factorial	
•	
Number of factors	2 +
< Back	Next>

Factor levels	? 🛛
1 10 +	2 12
< Back	Next >





S CycDesigN Version 2.0		
· Setup View Help		Randomization/Output 🛛 🕐 🗙
CycDesigN ? × Row-column design Resolvable Design parameters [120,10,12,3] Single factor [1] Alpha design Latinized[1,0,1] Not spatial Seed: clock Two stage < Back Next >	Working directory Image: Concelered state Enter your new working directory Image: Concelered state Cancel Image: Concelered state Operation of the conding our levels (treats o genotypes)	Randomize Number of randomizations 1 Seed: clock Default levels Default levels Rows by columns Index file: design.ind Design file: design.out Log file: design.log < Back
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Row-column design		column	1	2	3	0	5	e	7	8	9	10		12
Resolvable				4	3	4	5	6		0	Э	10	11	12
Design parameters	row +													
Number of treatments = 120	1	86	70	92	54	107	96	6	106	101	20	13	114	
Number of rows = 10						9	115	93	82	14	94	18	103	60
Number of columns = 12		3	2	59	102	53	15	71	116	76	78	77	75	1
Number of replicates = 3 T		4 I	49	72	73	89	50	43	5	51	87	22	8	79
Latinized by columns		5 1	67	28	85	68	84	21	46	80	11		105	23
Lacinized by columns		6 1	110	104	37	40	3	98	100	33	117	111	57	16
Random number seed for design generation = 46		- 1					-							
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Two stage		8	90	55	31	42	66	88	62	91	19	29	61	97
-		9	38	32	36	109	44	112	83	45	118	120	81	17
Average efficiency factors (Upper bounds)		10	24	7	34	63	74	39	25	41	65	69	56	35
Row 0.895737 (0.896446)														
Column 0.875536 (0.875563)		rep 2												
Row-Column 0.789671 (0.795142)		-				4	-					10		10
	=	column	1	2	3	4	5	6	7	8	9	10	11	12
	===	row +	+											
Concurrence Row Column	=	1	17	58	18	70	102	63	67	61	116	80	117	50
0 5229 5520 1 1842 1620		2	9	15	81	85	54	19	26	24	106	43	12	100
2 69 0		3	92	97	33	75	49	111	53	28	74	30	82	118
2 05 0		4 1	71	36	35	21	94	90	57	89	32	52	51	20
Randomization 1	design.log	5 1	3	40	86	27	23		115	47	8	25	29	93
		6 1	72	14	59			107	65		-	110	68	31
Random number seed for randomization = 203		- 1								_				
		7	56			45	104	83	79	64	37	60	96	2
Treatment randomization:		8	77	46	87	66	41	103	112	98	114	55	34	109
Group 1:		9	62	13	84	5	39	95	91	78	22	4	38	48
98 100 102 97 103 36 105 108 110 112 115 70		10	99	69	88	113	10	73	7	44	42	101	1	105
	114 28 117													
30 26 8 118 119 21 40 24 64 91 104 5		rep 3												
82 60 9 79 14 94 6 80 34 84 86 59 20 46 101 38 37 111 65 52 107 17 32 56		column	1	2	3	4	5	6	7	8	9	10	11	12
20 46 101 38 37 111 65 52 107 17 32 56 55 11 106 2 69 29 7 15 53 87 43 71				4	5	т	5	0		0	9	10	ТТ	12
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83 45 50 44 51 85 89 33 22 35 68 73		1	10	85	17	96	86	78	109	55	57	14	73	74
		2	98	30	50	100	76	66	11	54	18	38	32	7
Replicate randomization:		3	84	117	41	59	42	20	113	56	43	47	97	83
3 1 2		4 1	103	89	108	92	118	25	1	67	15	104	5	29
		5 1	69	62	75	94	28	79	106	107	23	112	119	110
Column randomization:		6 1		120		91	71	37	52	82		105	24	87
11 4 8 12 1 10 2 3 5 6 7 9)													
		7	53	2	12	8	16	99	39	36	6	19	80	21
Row randomization:		8	60	33	90	44	26	68	72	27		116	93	13
2 9 8 10 3 4 5 6 1 7		9	51	114	64	58	4	81	111	88	48	3	65	102
6 4 2 8 5 3 1 9 7 10 10 6 7 8 9 9 7 1 1 9		10	31	115	101	95	46	49	61	22	45	35	77	9
10 6 7 9 8 2 5 4 1 3														

🚺 desi	ign.ind	- Bloc	d	G		
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1	1	1	2	86 70		
1	1	1	4	54		
1	1 1	1 1	5	92 54 107 96	≡ 1	
1	1	1	7	6 106		
1	1	1	9	101		
1	1	1	11	13		
1	1 1	1 2	12 1	114 113		
1	1	2	22	12		
1	1	ž	4	9		
1	1 1	2	5	115 93		
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Ind. Seminar Strengthening Agronorestry Hograms in Higher Education for Food Security In Sub-

Data collection

Maps (file: "design.out")

Forms (file: "design.ind")

□ Specialized software

Imagination

*Word & Excel !!!

Templates for data recording From file: "design.ind" Open with MS Office Word Return code: ^p Replace ^p by ^p ^p ^p ^p □^p n-times n:number of plants per experimental unit

□ Save as .txt (unformated text)

Templates for data recording (ii)

- Attention to specifications when saving as .txt:
 - Windows default
 - Unchecked boxes
- After saving change the extension to .csv

Templates for data recording (iii)

Open with MS Office Excel

Click in the first column

Data / text in columns

Delimited / tab, space & consider consec. sep. as one



Use formulas & regular excel tips

Thanks for your attention