



$$x_{ijklmn} = \mu + repl_i + block_j(repl_i) + plot_k + pop_l + seedlot_m(pop_l) + \varepsilon_n$$

“Training session” on design of experiments (an outline of)

Replications: 1-4

Block: 1-18 (by replication)

Experimental
unit: 5 seedlings

Plot: 1-5

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 GOBIERNO
DE ARAGON

 cita
CENTRO DE INVESTIGACIÓN Y TECNOLOGÍA
AGROALIMENTARIA DE ARAGÓN

Ethiopia-Spain PCI project Training session on design of experiments. Madrid 25-26/04/2012

Sketch out

- ❖ Introduction
- ❖ Stages
- ❖ Principles
- ❖ Designs and analysis theory
- ❖ Operational tools for design
- ❖ [Analysis of experiments]

Introduction

❖ Goal

Tool for addressing analytical problems without fixed laws

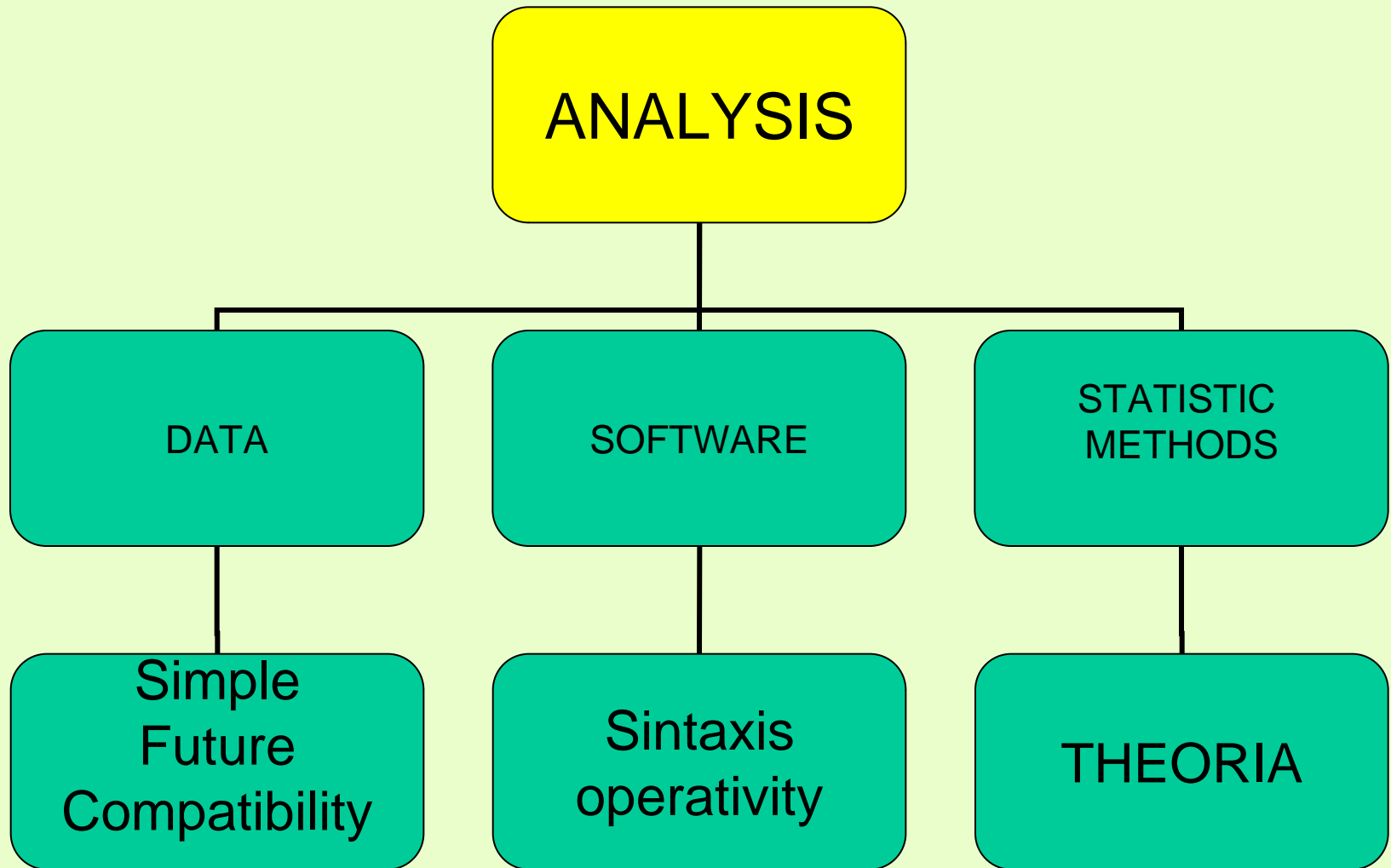
❖ Variability

- Existence
- Dealing and understanding
- Modeling and controlling

experimental error

Plant material or treatment evaluation

- ❖ Design of experiments
- ❖ Analysis of experiments
 - Basic Principles
 - Conventional and current (IB) designs
 - Software



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. Replication **n**s. (experimental error basis)

Standard Error of Difference

Agronomic trials $SED < 1/3$ diff

Material selection $SED < 1/6$ diff

Knowing s^2 & $d \implies n$

$$SED = \sqrt{\frac{2\sigma^2}{n}}$$

2. Treatment (broad sense) **R**andomization

3. Local control of existing variation in trial site
(**B**locking or spatial analysis)

ANDVA / SDA / Robust methods

“typical numbers & expressions”

4 replications
randomization
25 plants per plot
4 treatments
latinization
3 sites
2 border lines
RCB
single tree plot
5 x 5 m spacing
25 genotypes

Principles (ii)

Operational Limiting factors

Number of available effectives

Site constraints (topography, surface ...)

Technical limitations (machinery,)

Measurements

Competence, specific needs,

Future treatments, thinings,...

Spacing, density

Experimental design

Initial hypotheses or constraints:

Additive Model

Normality

Homocedasticity.

Different treatment errors
are independent
& distributed $N(0, \sigma^2)$

Statistic tests:

N: Shapiro-Wilks, graphs distrib,
freq acum., res * pred

H: Barlett, Levenne, ratios variances

Transformations

No parametric methods

Elementary Designs

R.D.

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

	dof	SS	MS	F	EMS
Total	rt-1	a	a/rt-1		
Treat	t-1	b	b/t-1	MS_T / MS_E	$\sigma_e^2 + r \sigma_t^2$
Error	t(r-1)	c	c/t(r-1)		σ_e^2



```
Data.....;
Proc GLM;
      Class treat;
      Model y = treat;
Run;
```

Elementary Designs

R.G.B

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

	dof	SS	MS	F	EMS
Total	rb-1	a	a/rb-1		
Treat	t-1	b	b/t-1	MS_T / MS_E	$\sigma_e^2 + b \sigma_t^2$
Blq	b-1	c	c/b-1	MS_B / MS_E	$\sigma_e^2 + t \sigma_b^2$
Error	t-1)(r-1)	d	d/t(r-1)		σ_e^2



```
Data.....;
Proc GLM;
    Class treat blq;
    Model y = treat blq;
Run;
```

Experimental design

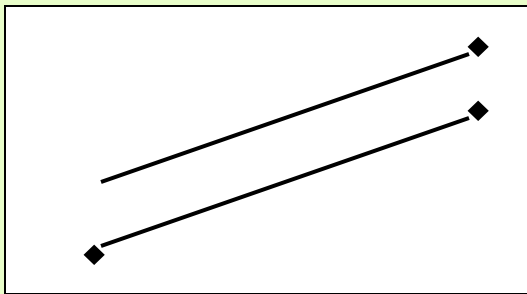
Possible structure of treatments

Factorial: total combination all x all

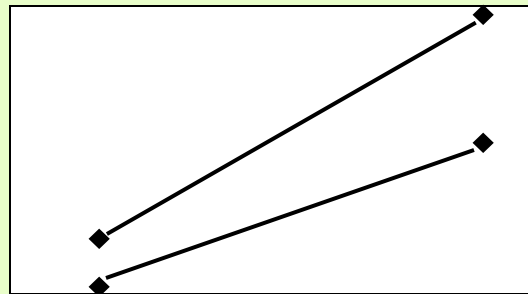
Possibility interactions study (GxE)

Reaction norms

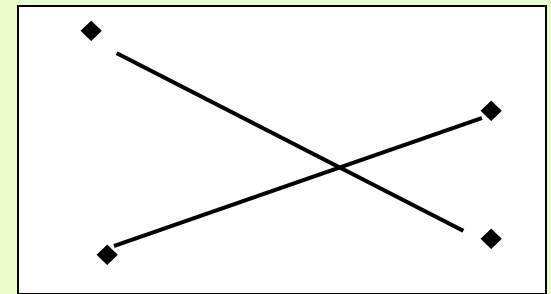
$$y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_{ij} + \varepsilon_{ijk}$$



No interaction



quantitative
interaction



qualitative
interaction

Experimental design

Possible structure of treatments

Hierarchical or nested: Impossible combination

$$y_{ijk} = \mu + \alpha_i + \beta_j(\alpha_i) + \varepsilon_{ijk}$$



```
Data.....;
```

```
Proc GLM;
```

```
Class pop fam;
```

```
Model y = pop fam(pop);
```

```
Run;
```

Is it important the treatment structure ?

F

E.M.S.

other structure + important.....

¿Fixed o Random?

F E.M.S.

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

Fixed: 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)

Random: Levels are a random sample from all posible.

Results & conclusions from anova can be extrapolated + levels

Main aim: Variability estimation of the variable or factor
or perhaps prediction at a given level

(BLUP)

	dof	MS	A y B fixed	A y B rand	A:fix B:rand
Total	abr-1				
A	a-1	MS_A	MS_A / MS_E	MS_A / MS_{AB}	MS_A / MS_{AB}
B	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 :

- 1) is it possible to repeat the factor levels in other site or year?
- 2) 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?

Data.....;

Proc GLM;

Class loc var blq;

Model y =loc blq(loc) var var*loc;

Random loc blq(loc) var*loc /test;

Run;

1º Calculation as fixed

2º Calculation EMS

3º Repeat F-tests with proper denominators

PROC MIXED

Incomplete Blocks

Evaluation : high n^0 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

.	.	B	A	.	1
.	.	A	C	.	2
B	.	.	.	C	3

Based on

$$\begin{aligned} \text{Aditivity: } B-C &= (B-C)_3 \\ &= (B-A)_1 - (C-A)_2 \end{aligned}$$

Experimental error independent of treatment

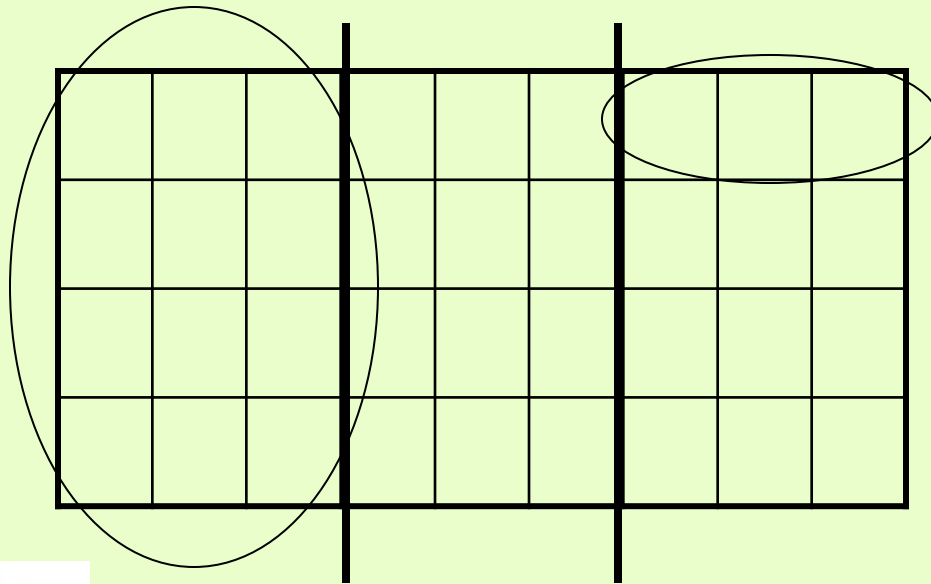
Incomplete Blocks

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

Resolvable designs

i.e.: $g=k \cdot b_i$

α -lattice, latinized, row-columns,...



Complex
specific Software

interblock info

I.B. design Efficiency

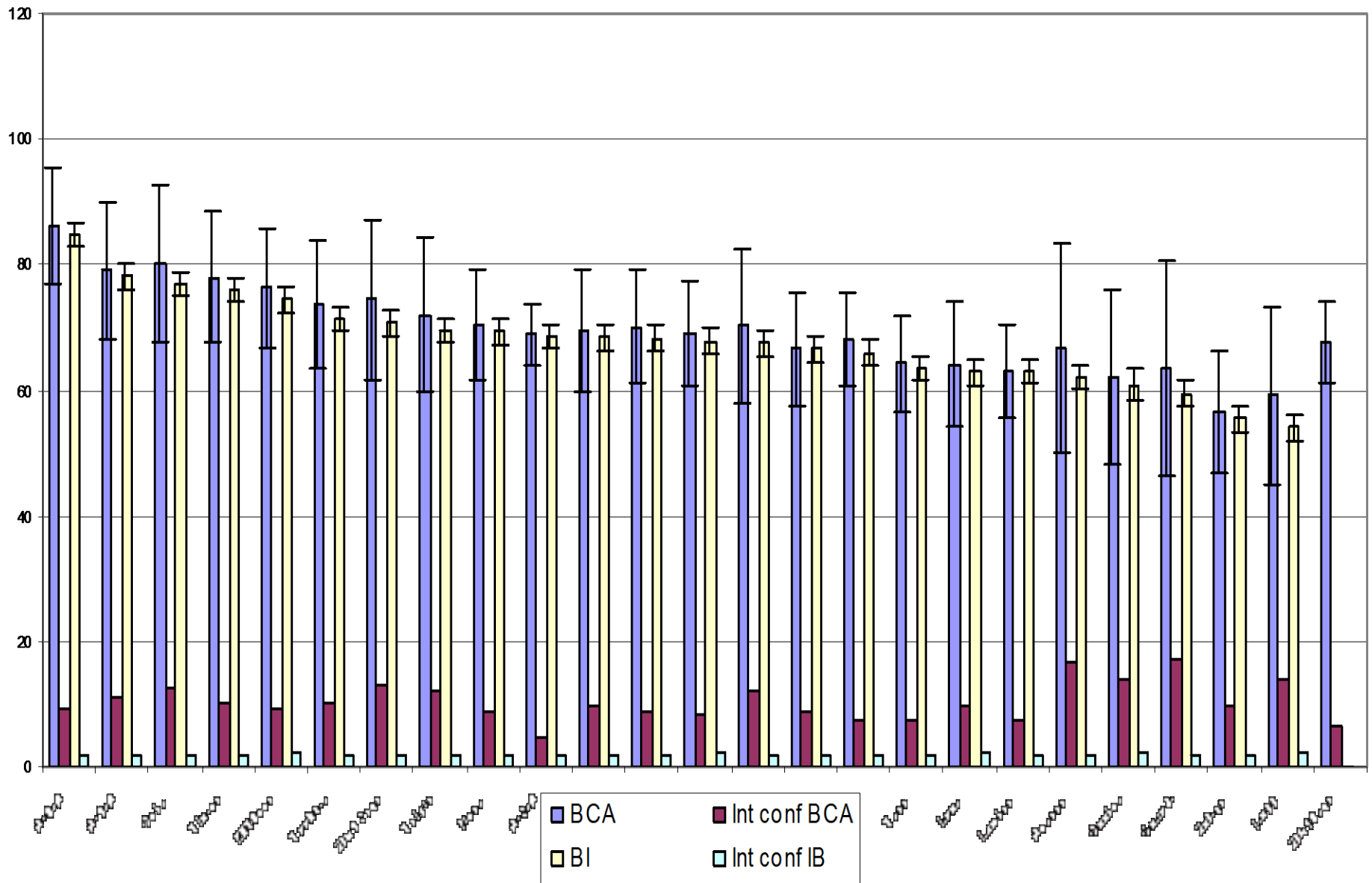
Objetive: To compare genotypes con la mayor precisión

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

$$\left(\frac{\sqrt{\left(\frac{2\sigma^2}{r} \right)}}{\sqrt{\left(\frac{2\sigma^2}{rxE} \right)}} \right)^2 = E$$

A IB with 4 reps y $E=1.5$ equals to a RCB with $4 \times 1.5 = 6$ CB

“Efficiency” ~ costs

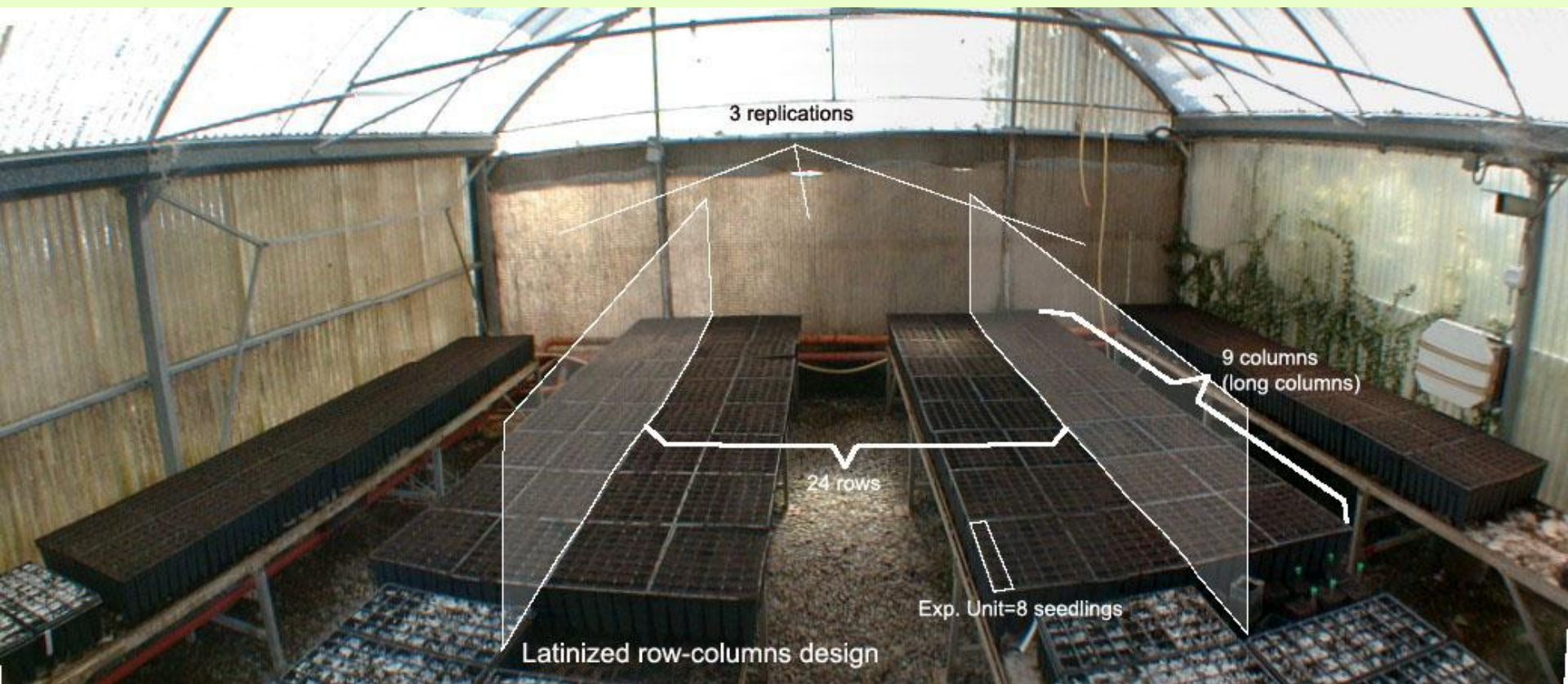


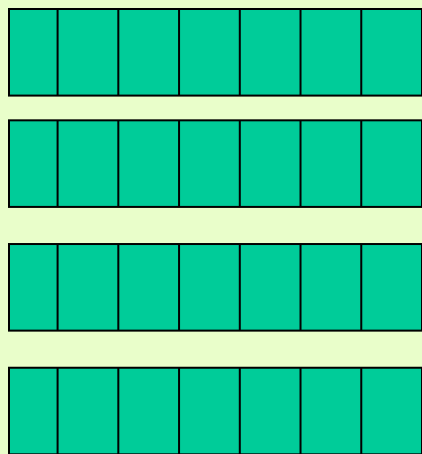


$$x_{ijklmn} = \mu + repl_i + block_j(repl_i) + plot_k + pop_l + seedlot_m(pop_l) + \varepsilon_n$$

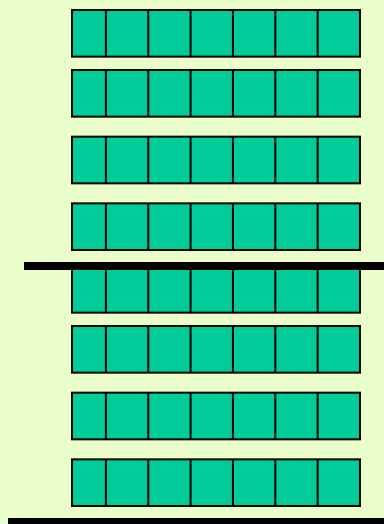
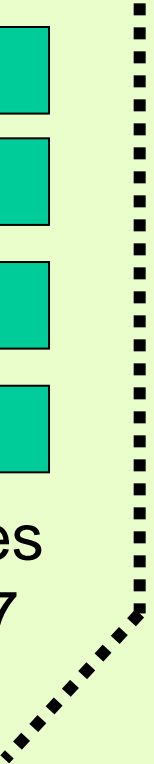
Mixed model REML BLUP & BLUE

Layout software: CyCDesignN

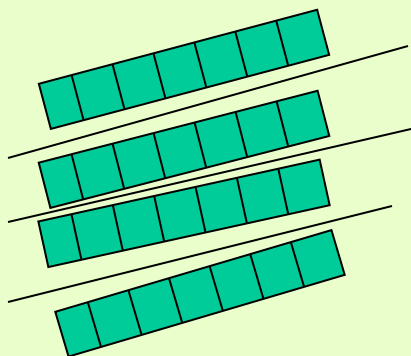




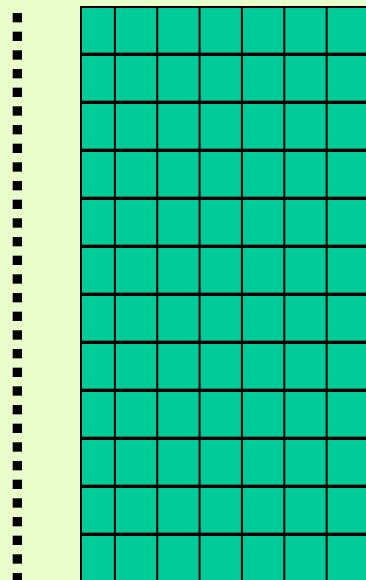
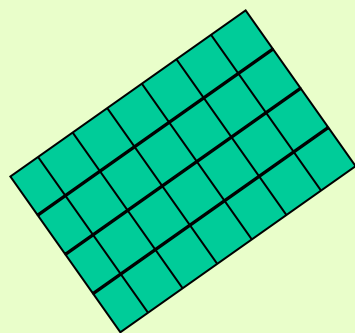
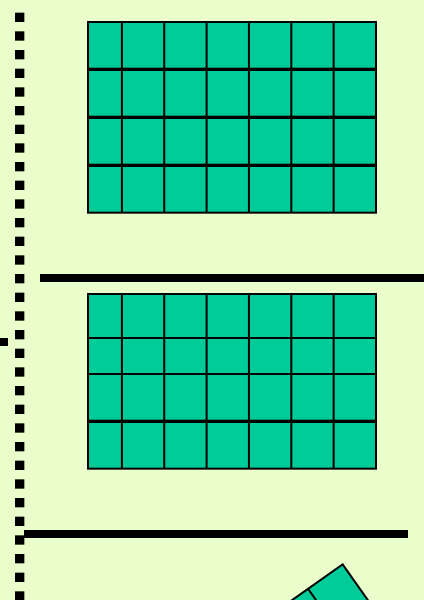
28 genotypes
4 blocks of 7



3 rep α -lattice



Row-Column



Latinized
Row-column



CycDesign 4.0

Home

What's New

CycDesign

CycXOver

CycAnalysis

F.A.Q.

CycDesign 4.0 is a computer package for the generation of optimal or near-optimal experimental designs. It comprises three modules:

CycDesign provides a comprehensive design generation module for experimenters; particularly those involved in plant breeding, horticulture, agriculture, forestry and market research and for all field, glasshouse and laboratory trials.

CycXover provides a wide choice of designs for experiments that involve sequences of treatments (such as stimuli, diets or drugs) applied to subjects over successive time periods. Known as crossover (or changeover) experiments, they are used in such areas as clinical trials, sensory perception experiments, psychological testing and dietary experiments.

CycAnalysis allows you to tailor your output from a **CycDesign** or **CycXover** session into a form ready for analysis. It also gives you the option to generate either **GenStat** or **SAS** code for the analysis of the design you have chosen.

CycDesign 4.0 is written in Visual C++ and runs under Windows 95, 98, NT, 2000, XP, Vista and Windows 7.

CSIRO Forestry and Forest Products
Canberra
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Hamilton
New Zealand

CycDesign

Version 2.0



CycDesignN

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

Enter your new working directory

C:\Archivos de programa\DataPlus\pr Browse

Cancel OK

CycDesignN Version 2.0

Setup View Help

W L D I ?

CycDesignN

Block design

Resolvable

Design parameters [24,4,4]

Single factor.. [1]

Alpha design

Not latinized

Not spatial

Seed: clock

< Back Next >

Kind of design: Incomp. Block or row-column

Resolvable, if $v=ks$ v treatments= s blocks of k treatments

Parameters: n^0 reps, n^0 i.b., n^0 treatments

Factorial or nested

Latinization and spatial restrictions

Generation of algorithms (starting point)

Choose your design

Working directory: C:\Archivos de programa\DataPlus\pruebas NUM

Block design

Block design

Resolvable

Design parameters [24,4,4]

Single factor.. [1]

Alpha design

Not latinized

Not spatial

Seed: clock

< Back

Next >

Row-column design

Row-column design

Resolvable

Design parameters [30,6,5,3]

Single factor.. [1]

Alpha design

Not latinized

Spatial: Integer

Seed: clock

Two stage

< Back

Next >

No operative differences for user

Design parameters [120,10,3]

Block design

Row-column design

Design parameters

Number of treatments: 120

Number of units/block: 10

Number of replicates: 3

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Design parameters

Number of treatments: 120

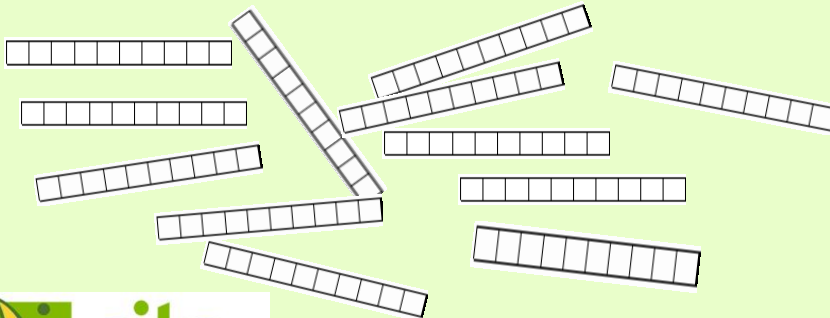
Number of rows: 10

Number of columns: 12

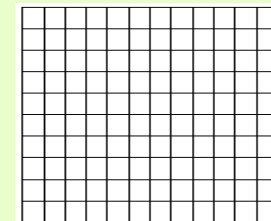
Number of replicates: 3

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12 I.B. of 10 treat.



1 REP



Single factor.. [5]

NESTED

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

Treatment structure

Single factor

Number of treatment groups 5

Factorial

< 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
 $10 \times 12 = 120$ treat.

Treatment structure

Single factor

Factorial

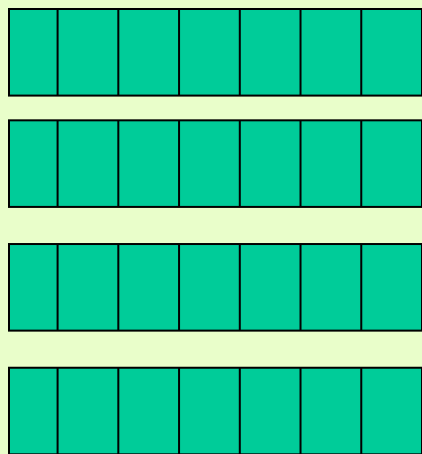
Number of factors 2

< Back Next >

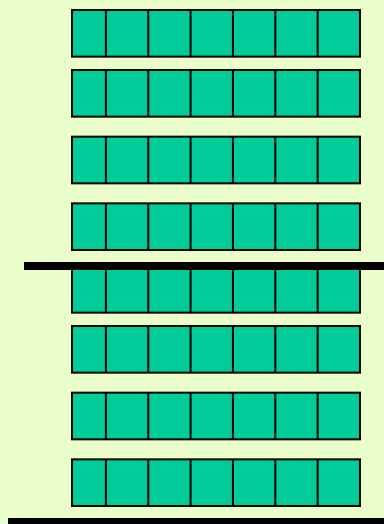
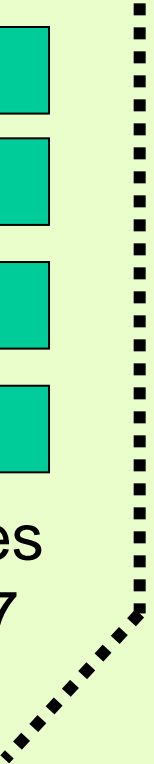
Factor levels

1	10	2	12
---	----	---	----

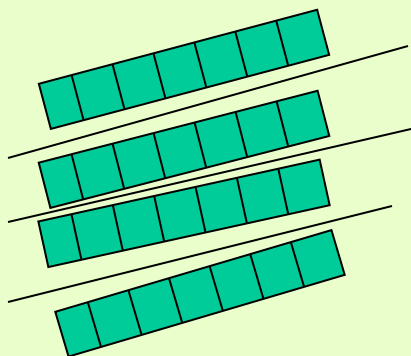
< Back Next >



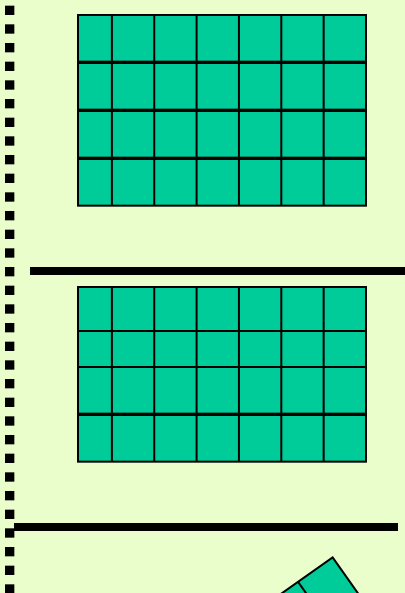
28 genotypes
4 blocks of 7



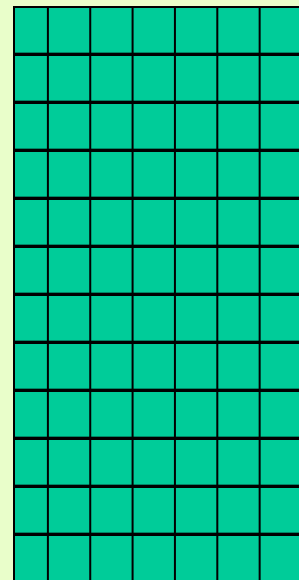
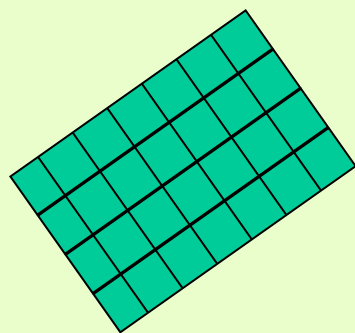
3 rep α -lattice



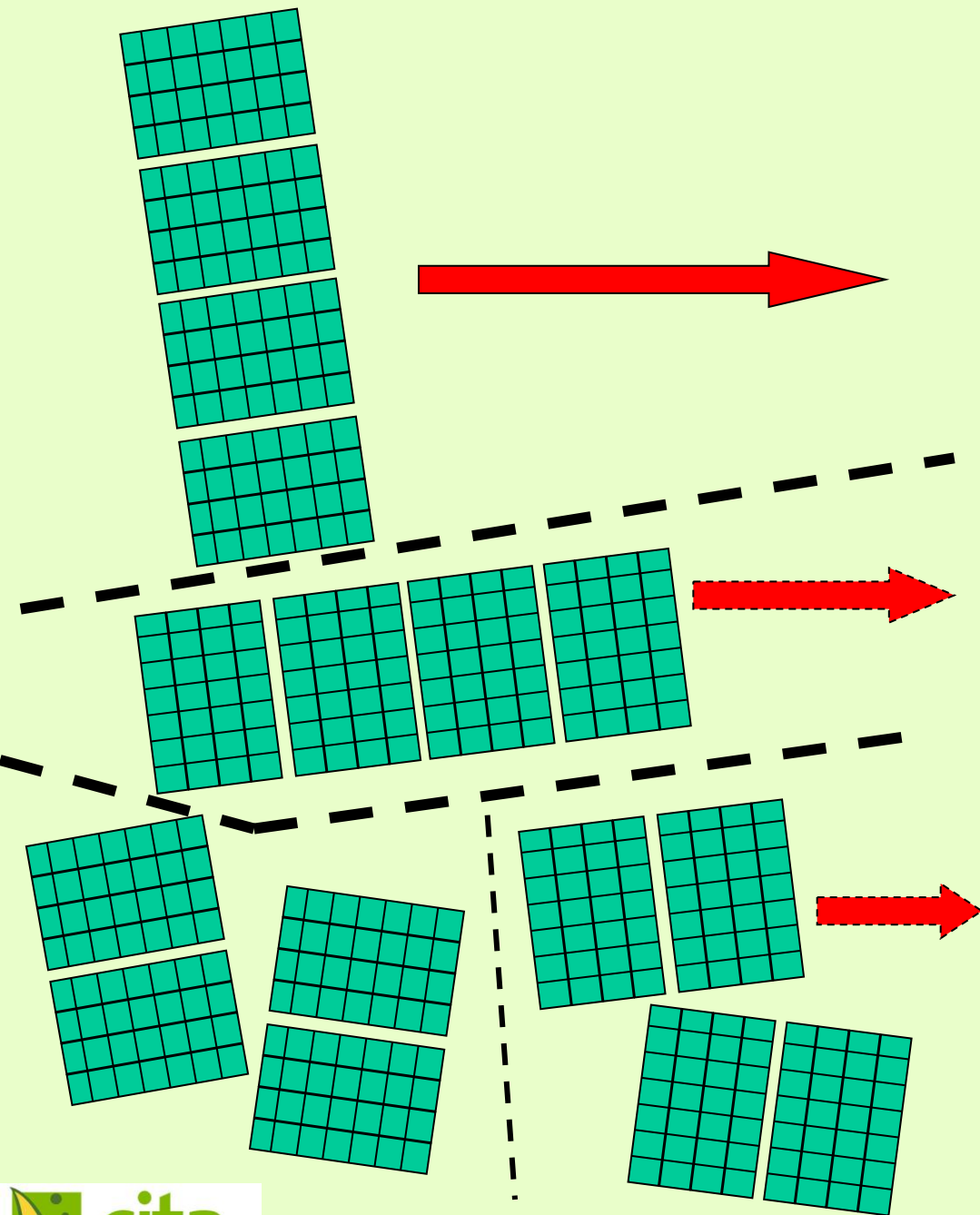
Row-Column



Row-Column



Latinized
Row-column



Latinized design

Columns

Not latinized

Latinized

Number of contiguous columns:

Rows

Not latinized

Latinized

Number of contiguous rows:

Replicate layout

Number of groups:

< Back Next >

CycDesignN Version 2.0

Setup View Help

W L D I ?

CycDesignN

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

Enter your new working directory

C:\Archivos de programa\DataPlus\pr Browse

Cancel OK

Randomization/Output

Randomize

Number of randomizations 1

Seed: clock

Default levels

Rows by columns

Index file: design.ind

Design file: design.out

Log file: design.log

< Back Next >

Choose your design

Working directory: C:\Archivos de programa\DataPlus\pruebas NUM

Possibility of recoding our levels (treats o genotypes)

Row-column design

Resolvable

Design parameters

Number of treatments = 120
 Number of rows = 10
 Number of columns = 12
 Number of replicates = 3

Latinized by columns

Random number seed for design generation = 46

Two stage

Average efficiency factors (Upper bounds)

Row 0.895737 (0.896446)
 Column 0.875536 (0.875563)
 Row-Column 0.789671 (0.795142)

Concurrence

	Row	Column
0	5229	5520
1	1842	1620
2	69	0

Randomization 1

Random number seed for randomization = 203

Treatment randomization:

Group 1:

98	100	102	97	103	36	105	108	110	112	115	70	39	25	67
63	77	74	10	72	78	116	88	90	93	19	57	114	28	117
30	26	8	118	119	21	40	24	64	91	104	5	23	81	47
82	60	9	79	14	94	6	80	34	84	86	59	4	95	41
20	46	101	38	37	111	65	52	107	17	32	56	76	92	12
55	11	106	2	69	29	7	15	53	87	43	71	99	66	109
113	62	120	18	31	42	49	3	16	54	27	1	61	48	75
83	45	50	44	51	85	89	33	22	35	68	73	96	13	58

Replicate randomization:

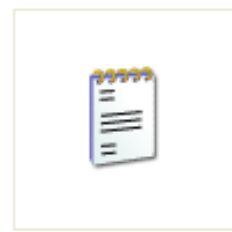
3 1 2

Column randomization:

11 4 8 12 1 10 2 3 5 6 7 9

Row randomization:

2	9	8	10	3	4	5	6	1	7
6	4	2	8	5	3	1	9	7	10
10	6	7	9	8	2	5	4	1	3



design.log

rep 1	1												
column	1	2	3	4	5	6	7	8	9	10	11	12	
row	+	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	
1		86	70	92	54	107	96	6	106	101	20	13	114
2		113	12	4	9	115	93	82	14	94	18	103	60
3		2	59	102	53	15	71	116	76	78	77	75	1
4		49	72	73	89	50	43	5	51	87	22	8	79
5		67	28	85	68	84	21	46	80	11	64	105	23
6		110	104	37	40	3	98	100	33	117	111	57	16
7		58	48	47	119	52	108	10	99	27	26	30	95
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
10		24	7	34	63	74	39	25	41	65	69	56	35

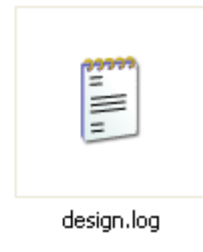
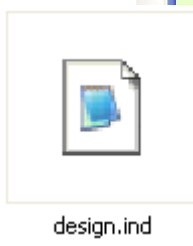
rep 2	1												
column	1	2	3	4	5	6	7	8	9	10	11	12	
row	+	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	
1		17	58	18	70	102	63	67	61	116	80	117	50
2		9	15	81	85	54	19	26	24	106	43	12	100
3		92	97	33	75	49	111	53	28	74	30	82	118
4		71	36	35	21	94	90	57	89	32	52	51	20
5		3	40	86	27	23	76	115	47	8	25	29	93
6		72	14	59	16	120	107	65	6	108	110	68	31
7		56	11	119	45	104	83	79	64	37	60	96	2
8		77	46	87	66	41	103	112	98	114	55	34	109
9		62	13	84	5	39	95	91	78	22	4	38	48
10		99	69	88	113	10	73	7	44	42	101	1	105

rep 3	1												
column	1	2	3	4	5	6	7	8	9	10	11	12	
row	+	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	
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
3		84	117	41	59	42	20	113	56	43	47	97	83
4		103	89	108	92	118	25	1	67	15	104	5	29
5		69	62	75	94	28	79	106	107	23	112	119	110
6		70	120	40	91	71	37	52	82	63	105	24	87
7		53	2	12	8	16	99	39	36	6	19	80	21
8		60	33	90	44	26	68	72	27	34	116	93	13
9		51	114	64	58	4	81	111	88	48	3	65	102
10		31	115	101	95	46	49	61	22	45	35	77	9

```

design.ind - Bloc d
Archivo Edición Formato Ver Ayuda
Rnd rep row col trt
1 1 1 1 86
1 1 1 2 70
1 1 1 3 92
1 1 1 4 54
1 1 1 5 107
1 1 1 6 96
1 1 1 7 6
1 1 1 8 106
1 1 1 9 101
1 1 1 10 20
1 1 1 11 13
1 1 1 12 114
1 1 1 1 113
1 1 1 2 12
1 1 1 2 3 4
1 1 1 2 4 9
1 1 1 2 5 115
1 1 1 2 6 93
1 1 1 2 7 82
1 1 1 2 8 14
1 1 1 2 9 94
1 1 1 2 10 18
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1 1 1 2 12 60
1 1 1 3 1 2
1 1 1 3 2 59
1 1 1 3 3 102
1 1 1 3 4 53
1 1 1 3 5 15
1 1 1 3 6 71
1 1 1 3 7 116
1 1 1 3 8 76
1 1 1 3 9 78
1 1 1 3 10 77
1 1 1 3 11 75
1 1 1 3 12 1
1 1 1 4 1 49
1 1 1 4 2 72
1 1 1 4 3 73
1 1 1 4 4 89
1 1 1 4 5 50
1 1 1 4 6 43
1 1 1 4 7 5
1 1 1 4 8 51
1 1 1 4 9 87
1 1 1 4 10 22
1 1 1 4 11 8
1 1 1 4 12 79
1 1 1 5 1 67
1 1 1 5 2 28
1 1 1 5 3 85
1 1 1 5 4 68
1 1 1 5 5 84
1 1 1 5 6 21
1 1 1 5 7 46
1 1 1 5 8 80
1 1 1 5 9 11
1 1 1 5 10 64
1 1 1 5 11 105
1 1 1 5 12 23

```



```

design.out - Bloc de notas
Archivo Edición Formato Ver Ayuda
Row-column design
Resolvable
Design parameters
  Number of treatments = 120
  Number of rows      = 10
  Number of columns   = 12
  Number of replicates = 3
Latinized by columns
Randomization 1

```

rep	1	-----											
column	1	2	3	4	5	6	7	8	9	10	11	12	
row	1	86	70	92	54	107	96	6	106	101	64	105	23
2	113	12	4	9	115	93	82	14	94	78	57	14	94
3	2	59	102	53	15	71	116	76	78	78	57	14	94
4	49	72	73	89	50	43	5	51	87	78	57	14	94
5	67	28	85	68	84	21	46	80	11	64	105	23	
6	110	104	37	40	3	98	100	33	117	111	57	16	
7	58	48	47	119	52	108	10	99	27	26	30	95	
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	
10	24	7	34	63	74	39	25	41	65	69	56	35	


```

rep 2
column
row
1 17 58 18 70 102 63 67 61 116 80 117 50
2 9 15 81 85 54 19 26 24 106 43 12 100
3 92 97 33 75 49 111 53 28 74 30 82 118
4 71 36 35 21 94 90 57 89 32 52 51 20
5 3 40 86 27 23 76 115 47 8 25 29 93
6 72 14 59 16 120 107 65 6 108 110 68 31
7 56 11 119 45 104 83 79 64 37 60 96 2
8 77 46 87 66 41 103 112 98 114 55 34 109
9 62 13 84 5 39 95 91 78 22 4 38 48
10 99 69 88 113 10 73 7 44 42 101 1 105

```



```

rep 3
column
row
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
3 84 117 41 59 42 20 113 56 43 47 97 83
4 103 89 108 92 118 25 1 67 15 104 5 29
5 69 62 75 94 28 79 106 107 23 112 119 110
6 70 120 40 91 71 37 52 82 63 105 24 87
7 53 2 12 8 16 99 39 36 6 19 80 21
8 60 33 90 44 26 68 72 27 34 116 93 13
9 51 114 64 58 4 81 111 88 48 3 65 102
10 31 115 101 95 46 49 61 22 45 35 77 9

```