



# INTRO TO TRIAL PREPARATION

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# Checklist for planning an experiment

- ✓ Define the objectives
- ✓ Identify the sources of variation
- ✓ Experimental design
- ✓ Specify the measurements
- ✓ Run a pilot experiment?
- ✓ Specify the model
- ✓ Outline the analysis
- ✓ Review/revise the above decisions



# Purpose of the trial

- Why to conduct a trial?
- Objective of the trial?
- Selection/availability of resources:
  - Germplasm
  - Machinery
  - Inputs
- Design
- Management
- Analysis
- Learning



# Trial Design

- When we design an experiment there are three components that should carefully be taken
  - Treatment design
  - Error-control design
  - Sampling/observation design



Treatment design



- Which treatment *factors* should be included?
- How many levels of each factor?
- Will there be combination of the factors?
- What will be the range of factor levels?

Error-control design



- What is the actual arrangement of the treatments in the experimental plan? (CRD, RCBD, IBD, etc)
- Depending on the **availability of experimental units**, the **structure** of those units, **precision** of estimation desired.

Sampling and observation design



- What kind of observations will be taken?
- Are the observational units the same as the experimental units?



# The three principles of experimental design

Replication



Each treatment (or some of the treatments) must be applied to several EU. Replications will allow to estimate the experimental error.

Randomization



This leads to an unbiased estimate of variance as well as unbiased estimate of treatment differences.

Local control (blocking)

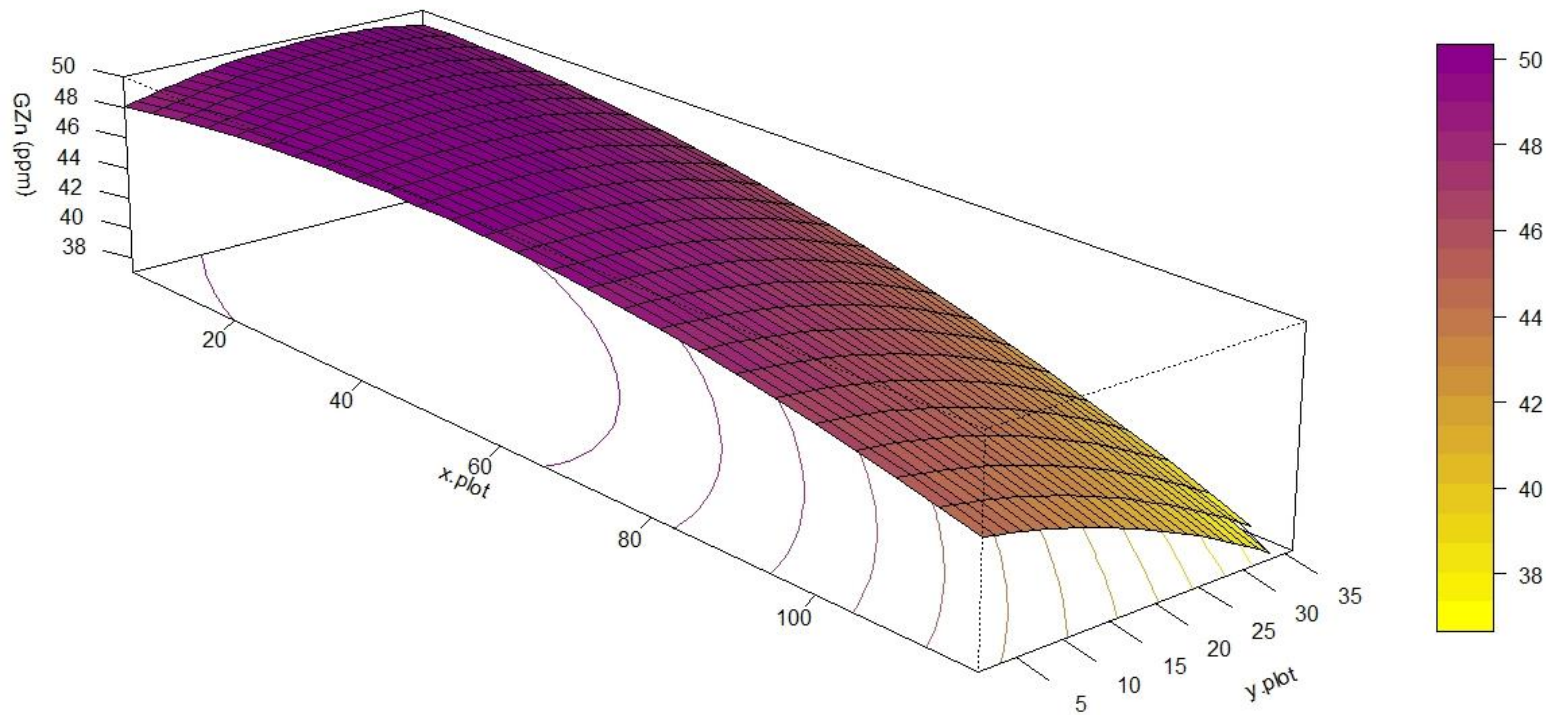


The partition of the total set of experimental units into subsets (blocks) that are as homogeneous as possible.



# Trial designs

## RCBD



Perhaps the most known & used block design

Experimental units (EUs) are blocked

Each block ( $b$ ) with  $t$  (treatments)

EU ∴ Each block is complete

Big variability between blocks makes it more efficient



# Trial designs

$\alpha$ —Lattice (Incomplete block design)

Plots/Block < # genotypes ( $t$ )

Lattices can revert to complete blocks  $\therefore$  are never less efficient than a RCBD

$t = 0, 1, \dots, t-1$

$t = ks$ ;  $k = b$  size &  $s =$  number of  $b$  in each replicate ( $r$ )

$\alpha$  array =  $k \times r$  with  $a(p,q)$  elements;  
 $p = 0, 1, 2, \dots, k-1$ ;  $q = 1, 2, \dots, r$

Develop the  $\alpha^*$  intermediate array  $k \times rs$  cyclically







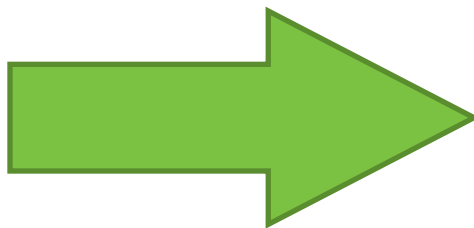
# Trial designs

$\alpha$ —Lattice (Incomplete block design)

## Construction R Example

```
library(agricolae)

trt<-1:30
t <- length(trt)
# size block k
k<-5
# Blocks s
s<-t/k
# replications r
r <- 2
outdesign<- design.alpha(trt,k,r,serie=1)
book<-outdesign$book
book
plots<-book[,1]
dim(plots)<-c(k,s,r)
for (i in 1:r) print(t(plots[,i]))
outdesign$sketch
```



```
$rep1
  [,1] [,2] [,3] [,4] [,5]
[1,] "23" "1"  "6"  "15" "2"
[2,] "28" "25" "19" "22" "30"
[3,] "13" "11" "4"  "24" "10"
[4,] "7"  "26" "14" "3"  "5"
[5,] "18" "9"  "20" "8"  "27"
[6,] "12" "29" "21" "16" "17"
```

```
$rep2
  [,1] [,2] [,3] [,4] [,5]
[1,] "2"  "28" "29" "11" "3"
[2,] "17" "22" "9"  "4"  "23"
[3,] "14" "10" "1"  "18" "12"
[4,] "21" "26" "25" "6"  "8"
[5,] "19" "24" "16" "20" "5"
[6,] "15" "27" "30" "7"  "13"
```

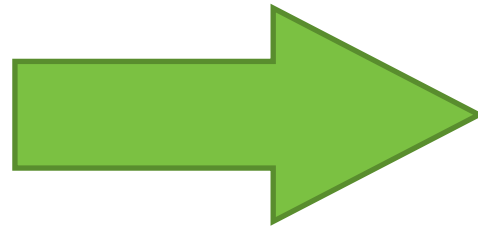


# Trial designs

$\alpha$ —Lattice (Incomplete block design)

Construction R Example

```
book<-outdesign$book  
book
```



plots	cols	block	trt	replication
11	1	1	15	1
12	2	1	29	1
13	3	1	1	1
14	4	1	2	1
15	5	1	4	1
16	1	2	17	1
17	2	2	12	1
18	3	2	30	1
19	4	2	23	1
20	5	2	8	1
21	1	3	21	1
22	2	3	3	1
23	3	3	27	1
24	4	3	16	1



# Trial designs

$\alpha$ —Lattice (Incomplete block design): The model

$$Y_{ijk} = \mu + R_j + SB_k(R_j) + G_i + \varepsilon_{ijk}$$

$\mu$  = general mean,

$G_i$  = effects of the genotypes,

$R_j$  = effects of the replicates

$SB_k$  = effects of the sub-blocks

$\varepsilon_{ijk}$  = random residual



# Trial design

## $\alpha$ —Lattice (Incomplete block design): Analysis

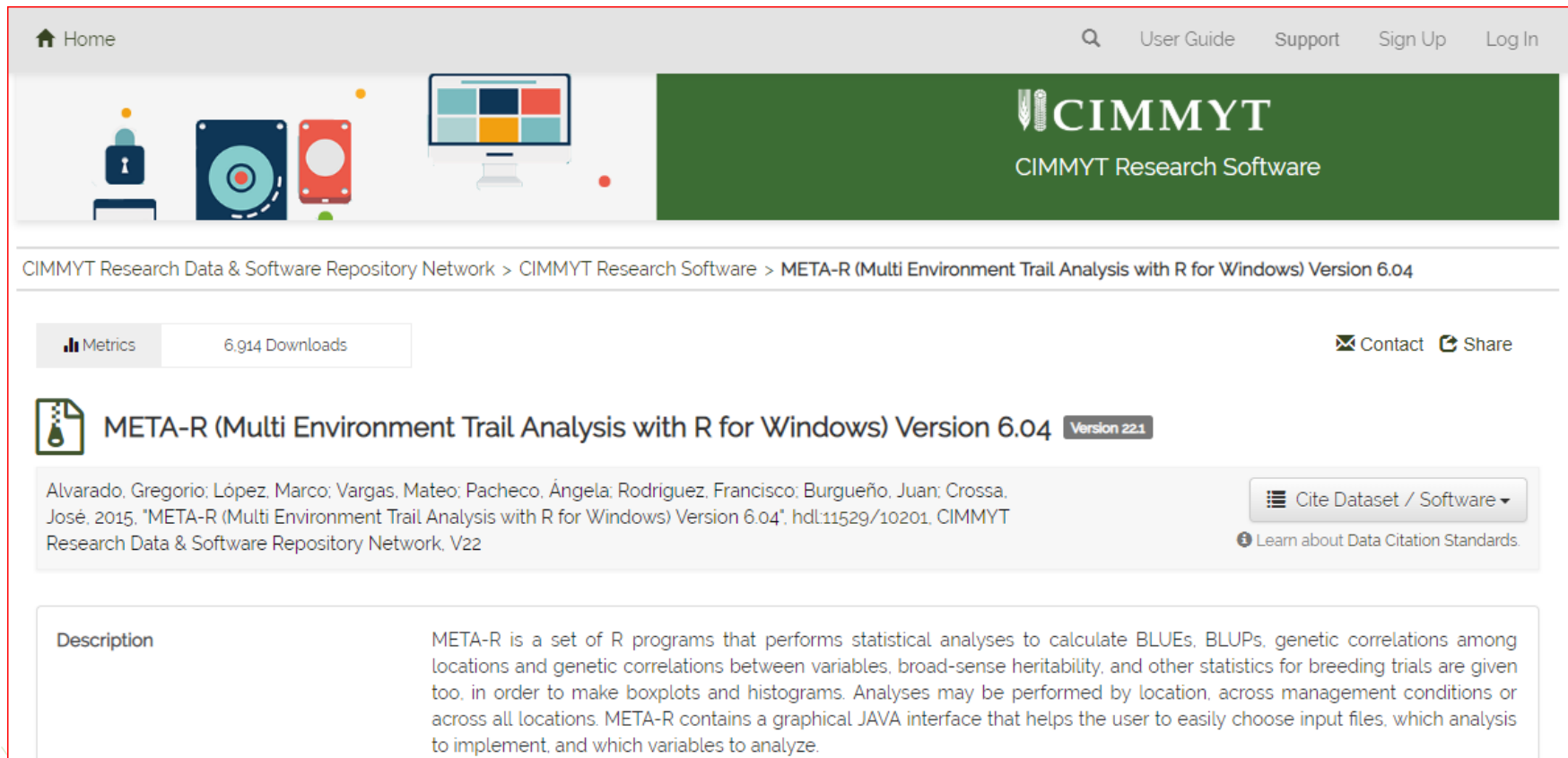
### Analysis in R

```
8 library(lmerTest)
9 library(tidyr)
10 library(reshape2)
11
12 wd = "C:/Users/LCRESPO/Documents/CIMMYT/BPAT review/TPE response"
13 setwd(wd)
14
15
16 d = read.csv("TPEs yield data ESWYT 34-38 INDIA & MEXICO.csv")
17 mv = read.csv("env-covsFinal 24-38 eswytV2.csv")
18
19 locs = unique(d$Loc_no)
20 mv.locs = mv[mv$Loc_no %in% locs,]
21 rownames(mv.locs) = mv.locs$t.occ
22 mv.locs = mv.locs[, c(4, 17:ncol(mv.locs))]|
23
24 d = d[order(d$Loc_no, d$Cycle, d$Occ),]
25 d$tons.ha = as.numeric(levels(d$tons.ha))[d$tons.ha]
26 d$Rep = as.factor(d$Rep)
27 d$Sub_block = as.factor(d$Sub_block)
28 d$GID = as.factor(d$GID)
29 d$Gen_no = as.factor(d$Gen_no)
30 d$TPE = as.factor(d$TPE)
31 d$Occ = as.factor(d$Occ)
32
33
```

```
38 m1 = lmer(tons.ha ~ t.occ + (1|t.occ:Rep:Sub_block) + (1|GID/t.occ),
39           data = d)
40 summary(m1)
41
42
43 ### split of data frame into single site/trials
44 d.list = split(x = d, f = d$t.occ)
45
46 ### Function to fit linear mixed model
47 trial.analysis.single = function(df = df){
48   lmm.s = lmer(tons.ha ~ 1 + (1|GID) + (1|Rep) + (1|Rep:Sub_block),
49               data = df)
50 }
51
52 ### Apply function over elements of trial list
53 l.s.random = lapply(d.list, trial.analysis.single)
54
55 ### Apply H2 function over list of linear models for variance components
56 stats.list = lapply(l.s.random, function(x){
57   h2.single(lm = x, df = d, varg = "GID", reps = 2)
58 })
59
60 ### Formatting the output of H2 function applied to the list of lm
61 stats.df = as.data.frame(stats.list)
62 stats.df = as.data.frame(matrix(unlist(stats.list),
63                                   nrow=length(stats.list), byrow=TRUE))
64 colnames(stats.df) = names(stats.list$`34eswyt.121`)
65 stats.df$t.occ = paste("t.occ", names(stats.list), sep = "")
66
```

# Trial design

## $\alpha$ —Lattice (Incomplete block design): Analysis



The screenshot shows the CIMMYT Research Software repository page for META-R (Multi Environment Trial Analysis with R for Windows) Version 6.04. The page features a navigation bar with links to Home, User Guide, Support, Sign Up, and Log In. Below the navigation bar is a banner with the CIMMYT logo and the text "CIMMYT Research Software". The main content area displays the software title, version, and a brief description. The description states that META-R is a set of R programs that performs statistical analyses to calculate BLUEs, BLUPs, genetic correlations among locations and genetic correlations between variables, broad-sense heritability, and other statistics for breeding trials. It also mentions that analyses may be performed by location, across management conditions or across all locations, and that META-R contains a graphical JAVA interface for choosing input files, analysis to implement, and variables to analyze.

Home


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CIMMYT Research Data & Software Repository Network > CIMMYT Research Software > META-R (Multi Environment Trial Analysis with R for Windows) Version 6.04

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 META-R (Multi Environment Trial Analysis with R for Windows) Version 6.04 Version 22.1

Alvarado, Gregorio; López, Marco; Vargas, Mateo; Pacheco, Ángela; Rodríguez, Francisco; Burgueño, Juan; Crossa, José, 2015, "META-R (Multi Environment Trial Analysis with R for Windows) Version 6.04", hdl:11529/10201, CIMMYT Research Data & Software Repository Network, V22

Cite Dataset / Software

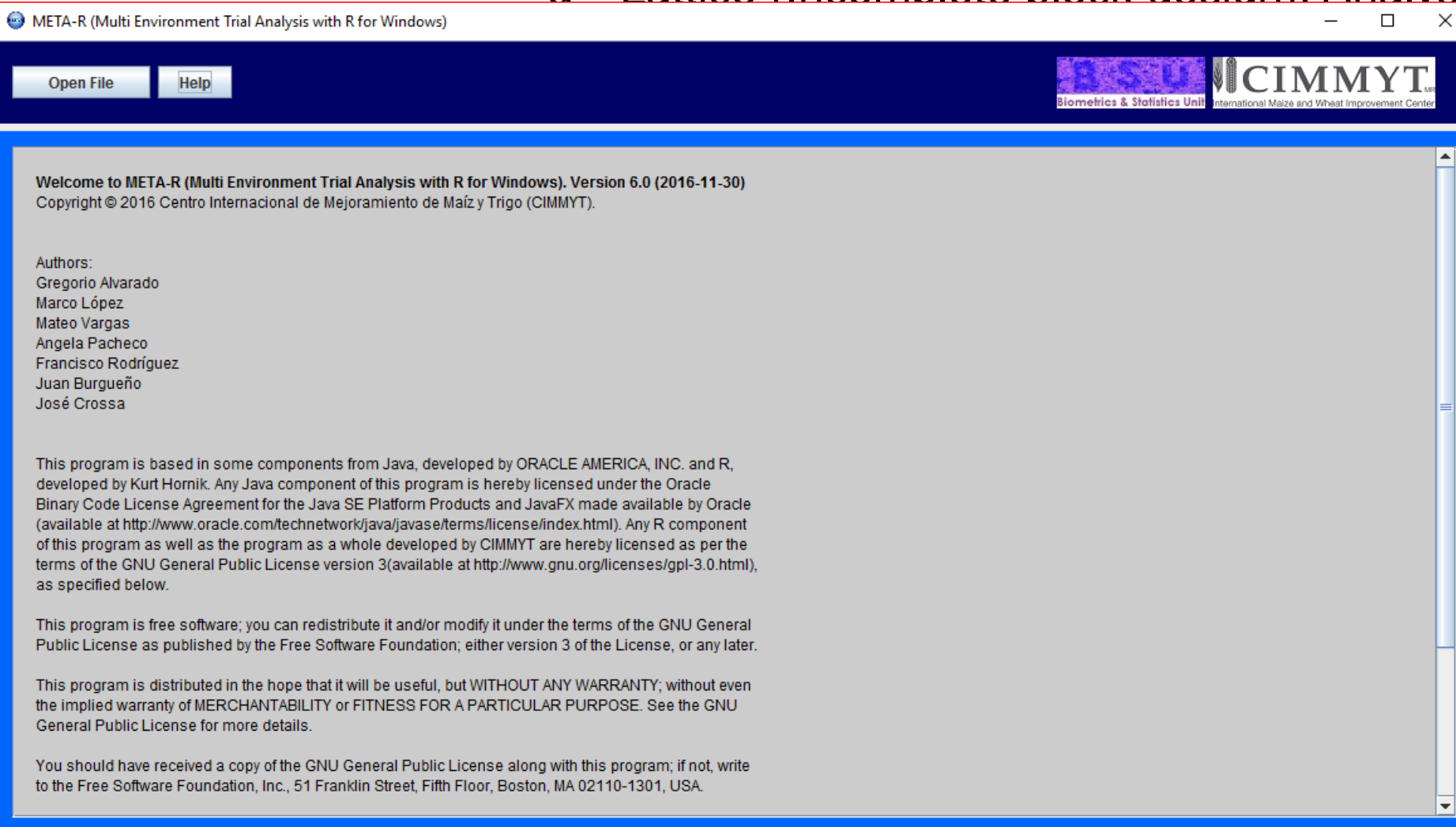
Learn about Data Citation Standards.

**Description**

META-R is a set of R programs that performs statistical analyses to calculate BLUEs, BLUPs, genetic correlations among locations and genetic correlations between variables, broad-sense heritability, and other statistics for breeding trials are given too, in order to make boxplots and histograms. Analyses may be performed by location, across management conditions or across all locations. META-R contains a graphical JAVA interface that helps the user to easily choose input files, which analysis to implement, and which variables to analyze.

# Trial design

## $\alpha$ —Lattice (Incomplete block design): Analysis



# Trial design

$\alpha$ —Lattice (Incomplete block design): Analysis

META-R (Multi Environment Trial Analysis with R for Windows)

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**Experimental Design**

Lattice

**Variable Selection**

Covariate: none

Grouping Factor: none

Environment: --select--

Replicate: --select--

Block: --select--

Genotype: --select--

Output folder: Analysis1

Welcome to META-R (Multi Environment Trial Analysis with R for Windows). Version 6.0 (2016-11-30)  
Copyright © 2016 Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT).

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Management	Site	Rep	Blk	Plot	Entry	gyf	ad	asi	ph	eh	epo
Low N	1	1	1	1	10	1.42	85	0	90	40	0.44
Low N	1	1	1	2	16	.	85	0	110	40	0.36
Low N	1	1	1	3	3	1.17	82	0	110	60	0.55



# Trial designs

## Augmented designs

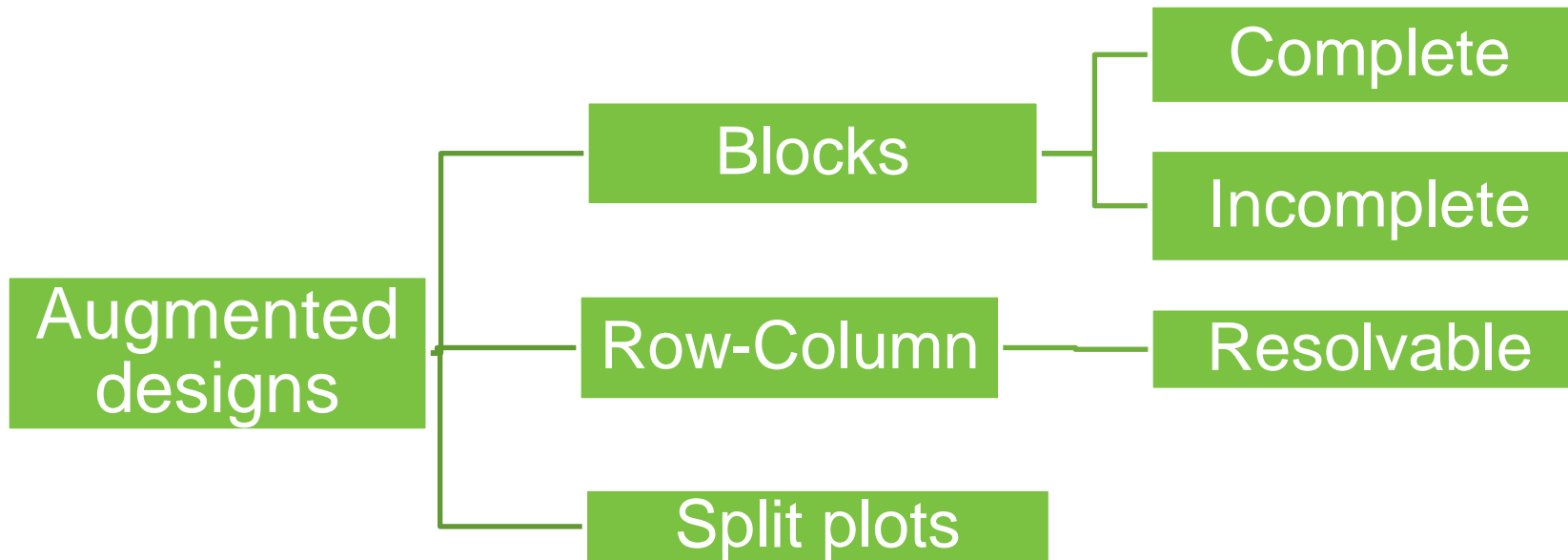
Contain  $c$  checks or standard treatments replicated  $r$  times, and  $n$  new treatments or genotypes included once (usually) in the experiment

1. The number of checks can be any kind and number  $c$ .
2. The number of new entries can be any number  $n$ .
3. The new treatments can be considered as random or as fixed effects.
5. Some of the designs in this class allow for screening when other factors are present, thereby revealing genotype-by-factor interactions.



# Trial designs

## Augmented designs



# Trial designs

## Augmented designs

Checks: A, B, C, D

New entries: 20-26

Blocks: 5

Construction in R:

```
# 4 treatments and 5 blocks
```

```
T1<-c("A","B","C","D")
```

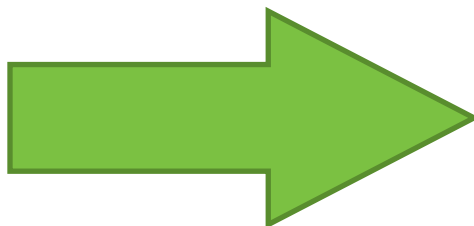
```
T2<-20:26
```

```
outdesign <-design.dau(T1,T2, r=5,serie=2)
```

```
# field book
```

```
book<-outdesign$book
```

```
by(book,book[2],function(x) paste(x[,1],"-",as.character(x[,3])))
```



block: 1

```
[1] "101 - A" "102 - 20" "103 - C" "104 - 22" "105 - B" "106 - D"
```

block: 2

```
[1] "201 - 25" "202 - C" "203 - D" "204 - 24" "205 - A" "206 - B"
```

block: 3

```
[1] "301 - B" "302 - 21" "303 - A" "304 - C" "305 - D"
```

block: 4

```
[1] "401 - D" "402 - B" "403 - 26" "404 - A" "405 - C"
```

block: 5

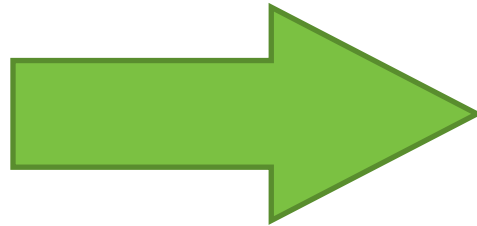
```
[1] "501 - C" "502 - B" "503 - D" "504 - 23" "505 - A"
```



# Trial designs

Augmented designs

```
book<-outdesign$book  
book
```



plots	block	trt
101	1	22
102	1	24
103	1	B
104	1	C
105	1	D
106	1	A
201	2	D
202	2	21
203	2	B
204	2	23
205	2	C
206	2	A
301	3	A
302	3	C
303	3	D
304	3	B
305	3	20
401	4	26
402	4	B
403	4	A



# Trial designs

p—Rep

In Multi-environmental (MET) testing, augmented designs can miss adjustment if checks have different error variance.

AugD allocate high number of plots to checks.

Cullist et al 2016 proposed P-rep designs: Replace replicated checks with new entries.

Williams et al 2011, applied the concept to Augmented designs in MET



# Field Maps



CIMMYT INT

File Help About

**Field Map**

**Specification of block**

Cycle : 18-19

Field : E9

Section : 1

Block : 1

Date : 24-Feb-2019

**Field Plan Layout**

Columns: 15 Rows: 6 Planting Units (PU) 90

**Select the starting point for plan.**

3) Bottom Left

☒ Serpentine

☐ Unidirectional

**Command**

<<< Back Next >>> Exit

# Field Maps



CIMMYT INT

File Help About

### Field Map

#### Specification of block

Cycle : 18-19

Field : E9

Section : 1

Block : 1

Date : 24-Feb-2019

#### Enter PU sequence numbers.

Nursery :

Number of PU/Entry : PU left : 0

Starting : PU left in row : 0

Ending :

Add + Clear

#### Commands

<<< Back

Matrix

Exit

#### Field Plan Layout

Columns: 15 Rows: 6 Planting Units (PU) 90

Select the starting point for plan.

3) Bottom Left ☒ Serpentine ☐ Unidirectional

Command Continue

#### Specifications of nurseries per block.

Nursery	Total PU	Starting	Ending	PU/Entr
EYT Flat 5IR	90	1	90	1

< ^ Up v Down Modify Del x Clear xx >

# Field Maps

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
1	BORLAUG	BORLAUG	BORLAUG	BORLAUG	BORLAUG	BORLAUG	BORLAUG	BORLAUG	BORLAUG	BORLAUG	BORLAUG	BORLAUG	BORLAUG	BORLAUG	BORLAUG	BORLAUG 100	RELLENO
2	BORLAUG	390	389	388	387	386	385	384	383	382	381	380	379	378	377	376	EYTBW-Flat-5IR 03
3	BORLAUG	361	362	363	364	365	366	367	368	369	370	371	372	373	374	375	EYTBW-Flat-5IR 03
4	BORLAUG	360	359	358	357	356	355	354	353	352	351	350	349	348	347	346	EYTBW-Flat-5IR 03
5	BORLAUG	331	332	333	334	335	336	337	338	339	340	341	342	343	344	345	EYTBW-Flat-5IR 03
6	BORLAUG	330	329	328	327	326	325	324	323	322	321	320	319	318	317	316	EYTBW-Flat-5IR 03
7	BORLAUG	301	302	303	304	305	306	307	308	309	310	311	312	313	314	315	EYTBW-Flat-5IR 03
8	BORLAUG	290	289	288	287	286	285	284	283	282	281	280	279	278	277	276	EYTBW-Flat-5IR 02
9	BORLAUG	261	262	263	264	265	266	267	268	269	270	271	272	273	274	275	EYTBW-Flat-5IR 02
10	BORLAUG	260	259	258	257	256	255	254	253	252	251	250	249	248	247	246	EYTBW-Flat-5IR 02
11	BORLAUG	231	232	233	234	235	236	237	238	239	240	241	242	243	244	245	EYTBW-Flat-5IR 02
12	BORLAUG	230	229	228	227	226	225	224	223	222	221	220	219	218	217	216	EYTBW-Flat-5IR 02
13	BORLAUG	201	202	203	204	205	206	207	208	209	210	211	212	213	214	215	EYTBW-Flat-5IR 02
14	BORLAUG	190	189	188	187	186	185	184	183	182	181	180	179	178	177	176	EYTBW-Flat-5IR 01
15	BORLAUG	161	162	163	164	165	166	167	168	169	170	171	172	173	174	175	EYTBW-Flat-5IR 01
16	BORLAUG	160	159	158	157	156	155	154	153	152	151	150	149	148	147	146	EYTBW-Flat-5IR 01
17	BORLAUG	131	132	133	134	135	136	137	138	139	140	141	142	143	144	145	EYTBW-Flat-5IR 01
18	BORLAUG	130	129	128	127	126	125	124	123	122	121	120	119	118	117	116	EYTBW-Flat-5IR 01
19	BORLAUG	101	102	103	104	105	106	107	108	109	110	111	112	113	114	115	EYTBW-Flat-5IR 01
20	BORLAUG	BORLAUG	BORLAUG	BORLAUG	BORLAUG	BORLAUG	BORLAUG	BORLAUG	BORLAUG	BORLAUG	BORLAUG	BORLAUG	BORLAUG	BORLAUG	BORLAUG	BORLAUG 100	RELLENO





# Field trial management



# Management Practices

- A key aspect in crop production
  - Sowing systems
  - Irrigation systems
  - Crop Management



# Field Preparation

## Tillage

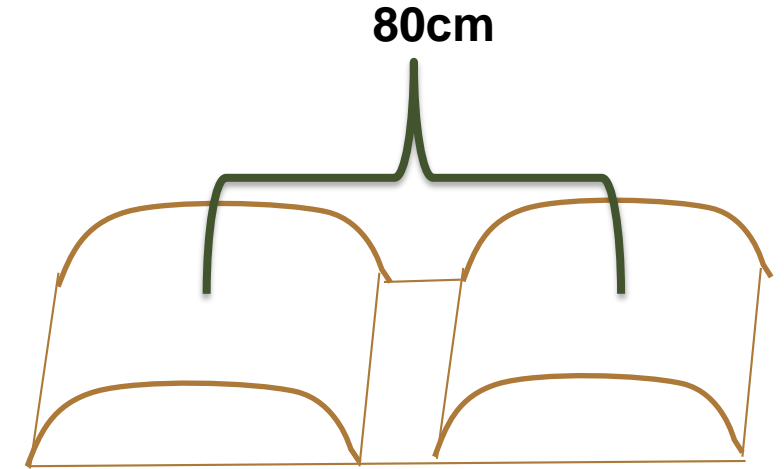
- Conventional tillage : Turning and loosening of topsoil layer using disc or plough
- Conservation tillage : Sowing on previous crop residue (30% or more) with minimum soil disruption
- Zero tillage : Direct sowing over previous crop, with no residue turnover



# Sowing Systems

- Raised Bed

- 2/3/4 rows
- Bed size varies,
  - 80cm at Obregon station
  - 3-row spacing 18cm



- Flats

- 6 or 8 rows
- 6-row spacing 18cm





# Sowing systems

- Hand planting : used for planting small plot 1m or less, head rows/plant to rows/disease evaluations
- Machine planting : planting plots of 2m or larger.
- Plot sizes for yield trials is 2m or larger (4m)
- Seeding rate: 120-180kg/ha for bread wheat & durum wheat



# Irrigation systems

- Flood furrow/basin irrigation : irrigated environment trials
- Drip irrigation : drought and irrigated yield trials
- Sprinkler irrigation : disease nursery (eg. Fusarium)





# Harvest Management



Hand harvest



Combine harvest



Arrangement



Barcode Tags



Barcode based weighing



Inventory app





**Thank you for  
your interest!**