Effective statistical evaluation of grower group on-farm trials

Session 2 ... Concerning analysis/interpretation of results

Bev Gogel[1] Dini Ganesalingam[2]

[1]University of Adelaide beverley.gogel@adelaide.edu.au

[2]University of Western Australia aanandini.ganesalingam@uwa.edu.au

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Five fundamentals of on farm experimentation

trial design

1. *decide on the aim of the research... the theory to be addressed/research question to be answered*
2. *generate a statistically valid experimental design to address this aim ...*

trial management

3. conduct a successful on-farm trial...
   - *choose an appropriate trial site*
   - *establish and manage trial efficiently and effectively*
   - *collect data as accurately as possible* including *covariates if appropriate*
   - *prepare the data for analysis*

analysis and inference

4. do a valid statistical analysis...
5. make sound statistical inference/ draw some valid conclusions...

Outline

In this session

- a selection of standard trials
- a textbook analysis of these trials
  - ANOVA
- the danger of fitting a text book analysis without a model fitting process
  - including covariates and/or other extraneous terms
- improving the analysis by accounting for spatial gradients/trends
  - spatial methods of analysis
- analysis of a data example(s)

Completely randomised design

simple on farm trial:

- dimension 3 rows $\times$ 6 ranges
- 3 reps of 6 treatments completely randomly allocated to plots
  - no structure *ie. no blocking of plots into groups*

completely randomised design

- yield measured on each plot

aim:

- form predicted treatment means
- compare yield performance for the treatments relative to one another

	range					
	1	2	3	4	5	6
row 1	v3	v5	v4	v4	v6	v3
row 2	v6	v5	v5	v6	v1	v2
row 3	v1	v2	v1	v3	v2	v4

Completely randomised design

in the completely randomised design (CRD)

- all reps of a given variety can fall together in the randomisation
- ok for an even patch of ground
- assumed for this example

	range					
	1	2	3	4	5	6
row 1	v3	v5	v4	v4	v6	v3
row 2	v6	v5	v5	v6	v1	v2
row 3	v1	v2	v1	v3	v2	v4

Completely randomised design

simple set of yield trial data for a CRD sown on an *even patch of ground*

plot data:

- same order of magnitude across plots but vary between plots
- even scatter about an average level

but...

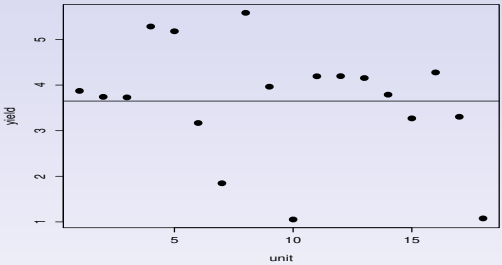


Figure: Scatterplot of yield data for CRD

Completely randomised design

- treatment 5 high trait values: 4, 5, 8
- treatment 4 lower trait values: 7, 10, 18

$\longrightarrow$ **treatment** effects

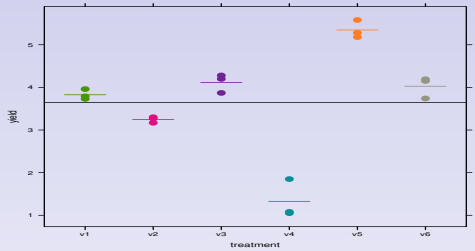


Figure: Scatterplot of yield data grouped by treatment

baseline linear model

$$\textit{yield} \quad = \quad \textit{treatment} + \texttt{residual error}$$

- *treatment* = mean yield for each treatment
- `residual error` = deviations from *treatment* mean due to measurement error/unexplained variation

Completely randomised design: the model

baseline linear model

$$\textit{yield} \quad = \quad \textit{treatment} + \texttt{residual error}$$

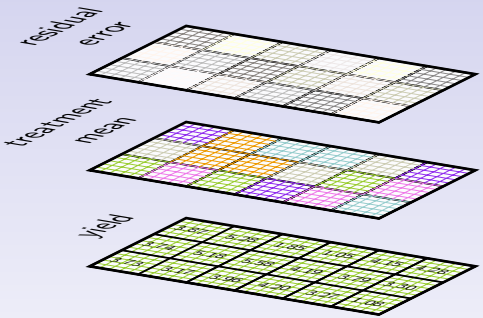


Figure: Layers diagram for the CRD

Randomised complete blocks

for the CRD:

- 3 reps of 6 treatments
- 3 reps completely randomly allocated to plots

for the RCB:

- 3 reps of same 6 treatments
- 3 reps randomly allocated to the 3 replicate blocks

$$b1 = 1\&2,\ b2 = 3\&4,\ b3 = 5\&6$$

	range					
	1	2	3	4	5	6
row 1	v6 b1	v3 b1	v5 b2	v4 b2	v2 b3	v1 b3
row 2	v4 b1	v5 b1	v2 b2	v6 b2	v3 b3	v6 b3
row 3	v1 b1	v2 b1	v1 b2	v3 b2	v4 b3	v5 b3

Randomised complete blocks

blocking plots into groups such that plots within groups are more alike than plots from different groups is:

- to control for *broadscale/block-style variation* in the field
- reasonably effective in controlling broadscale/block-style variation

but

- typically need to account for *local* or *plot-to-plot* variation as well... *in a while*

Randomised complete blocks

a simple set of yield trial data for an RCB sown on an *even patch of ground*:

as for CRD:

- treatment 5 high trait values
- treatment 4 lower trait values

$\longrightarrow$ **treatment** effects

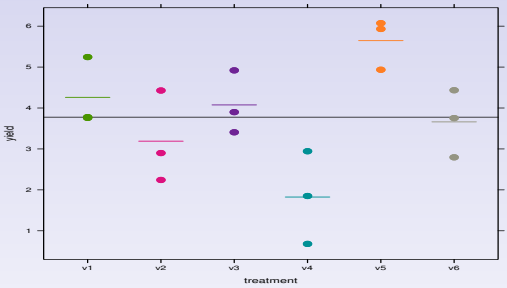


Figure: Scatterplot of yield data for RCB

Randomised complete blocks: the model

same plot but data grouped by replicate block

- yield data in **b2** consistently higher
- yield data in **b1** consistently lowest

$\longrightarrow$ **block** effects

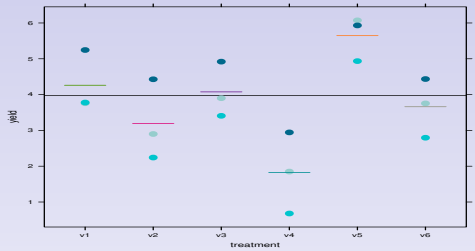


Figure: Scatterplot of yield data by block

baseline linear model

$$\textit{yield} \quad = \quad \textit{treatment} + \texttt{block} + \texttt{residual error}$$

- *treatment* = mean yield for each treatment
- `block` = model term containing 3 random block effects
- `residual error` = deviations from *sum of treatment mean and the block effect* due to measurement error/unexplained variation

Randomised complete blocks: the model

baseline linear model

$$\textit{yield} \quad = \quad \textit{treatment} + \texttt{block} + \texttt{residual error}$$

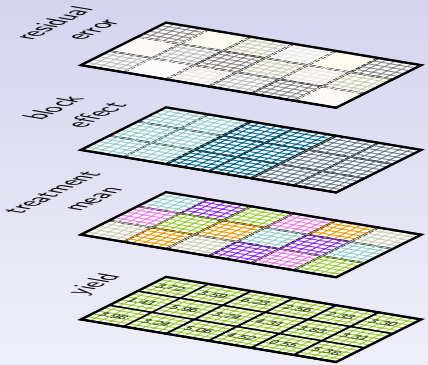


Figure: Layers diagram for the RCB

Split-plot trial

split-plot trial:

- 12 rows $\times$ 6 ranges
- 3 replicate blocks in pairs of ranges
- 24 plots in each replicate block divided 6 main plots
  - mainplots = sets of 4 adjacent plots in ranges
  - 6 varieties randomly allocated to mainplots in each replicate block
- 4 treatments randomly applied to the 4 subplots in each mainplot

	range 1	2	3	4	5	6
row 1	v6	v3	v5	v4	v2	v1
row 2	v4	v5	v2	v6	v3	v6
row 3	v1	v2	v1	v3	v4	v5

main aim:

- assess the treatments within each variety (eg. herbicide tolerance trial)

Split-plot trial: the model

for the spPLOT we have the baseline model:

$$\textit{yield} \quad = \quad \textit{variety*treatment} + \texttt{block/mplot} + \texttt{residual error}$$

which expands to:

$$\begin{aligned} \textit{yield} \quad = \quad & \textit{variety} + \textit{treatment} + \textit{variety:treatment} \\ & + \texttt{block} + \texttt{block:mplot} + \texttt{residual error} \end{aligned}$$

- *variety* and *treatment* are model terms for the varieties and treatments being tested
- `block` = model term containing 3 random block effects
- `mplot` = model term with 6 random mainplot effects
- `residual error` = deviations due to measurement error/unexplained variation

Split-plot trial: the model

baseline linear model

$$\textit{yield} = \textit{variety} + \textit{treatment} + \textit{variety:treatment} + \texttt{block} + \texttt{block:mplot} + \texttt{residual error}$$

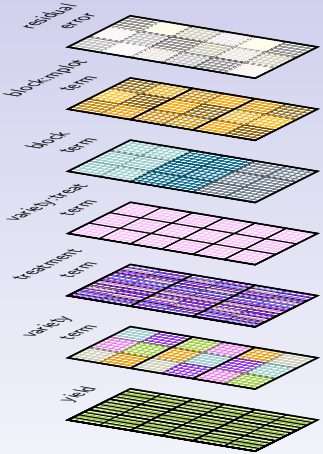


Figure: Layers diagram for the spPLOT

Fitting the model

So how do we *fit* the model?

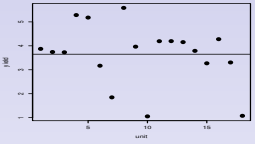
Analysis of Variance (ANOVA) probably most familiar method

ANOVA

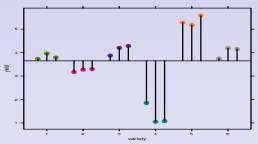
- partitions the total variation in the data into variation due to each term in the model and leftover or residual variation
- allows us to test how important each term is in determining the response measured
- gives an estimate of the trial error variance

All of this information is summarized in an ANOVA table

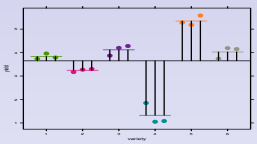
ANOVA estimation: the CRD



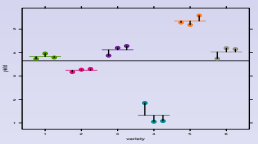
simple scatterplot



TotSS



TrtSS



ResSS

Table: one-way ANOVA

source	sum of squares squares	degrees of freedom	mean square	variance ratio	probability
treatment *between treatments*	TrtSS $= \sum_{i=1}^{n} \left(\bar{y}_j - \bar{\bar{y}}\right)^2$	$t-1$	TrtMS = TrtSS/$(t-1)$	*F*= TrtMS/ResMS	$P = Pr(F > \mathrm{F}_{t-1,n-t})$
residual *within treatments*	ResSS $= \sum_{i=1}^{n} \left(y_{i_{jk}} - \bar{y}_j\right)^2$	$n-t$	ResMS = ResSS/$(n-t)$		
total	TotSS $= \sum_{i=1}^{n} \left(y_{i_{jk}} - \bar{\bar{y}}\right)^2$	$n-1$			

ANOVA vs REML estimation

Most on-farm trials use standard designs therefore highly structured... RCB, spPLOT, ...

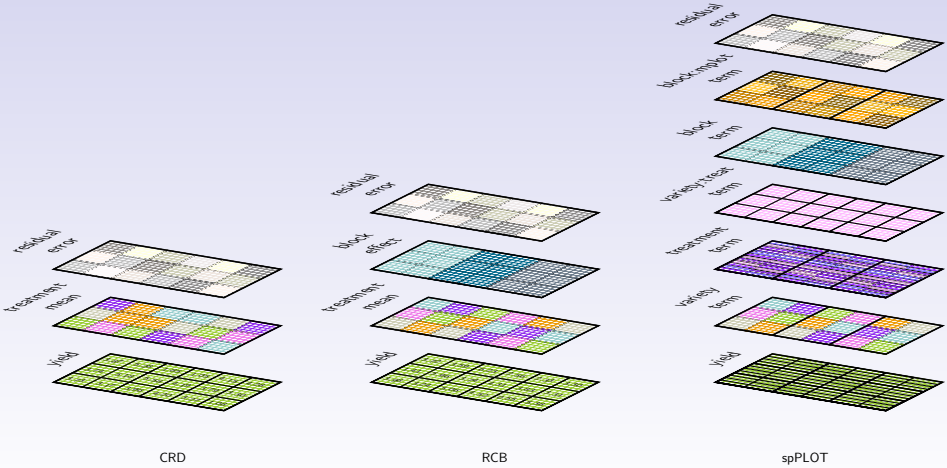
ANOVA estimation relies on

- the structure in the data
- a very basic assumption of independence in the residual errors (IID assumption)

The data we see is typically has some degree of imbalance so we use REML estimation *(Patterson and Thompson, 1971)*) which

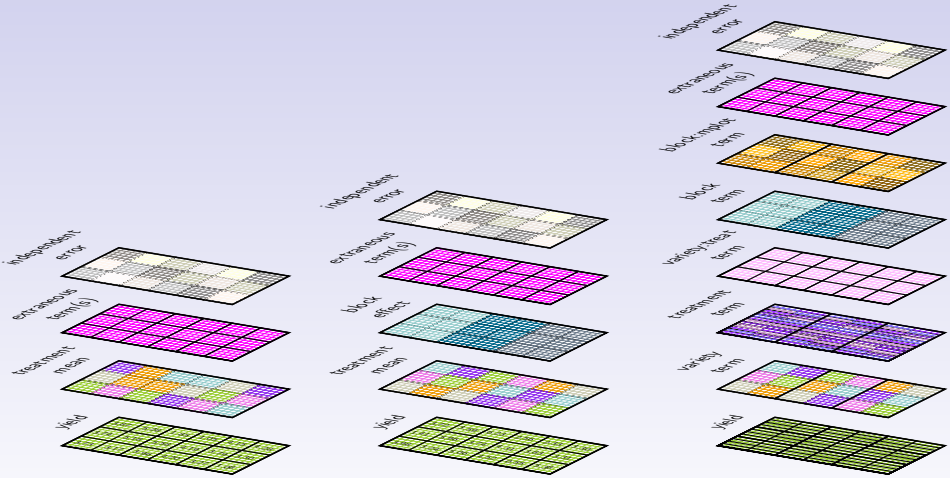
- does not rely on balance and IID errors so accommodates imbalance and other variance/covariance structures
- makes it a lot more powerful and flexible than ANOVA
- gives the same results as ANOVA for generally balanced data and simple assumptions about the residual errors

Baseline models: CRD, RCB, spPLOT



CRD RCB spPLOT

More general models: covariates/extraneous effects



CRD RCB spPLOT

More general models: covariates

What is a covariate?

- *a source of variation that is not accounted for in the initial design of the experiment, but can impact on the dependent variable* (Lomax & Hahs-Vaughn, 2012)

- accounts for a source of *uncontrolled* or *nuisance* variation in the dependent variable

- includes effects such as water logging, weeds, herbicide damage, ...

More general models: covariates

Key condition:

- a covariate *cannot be driven by/must be independent of* the treatment (Elashoff, 1969)
- eg. in NVT yield trials the treatment is the variety so the covariate cannot be a genetic characteristic (trait) of a variety

What if assumption fails ie. covariate is genetically driven?

- covariate could *remove part of* the treatment effect, or
- covariate could result in *an inflated* treatment effect (Lomax & Hahs-Vaughn, 2012)

... so what can be classified as a covariate then?

Covariate or not?

- agronomy type measurements *are not* covariates
  - early growth score
  - establishment
  - plant counts
  - patchiness
- in these examples the trait measured is a genetic characteristic of a variety, ie. early plant vigour

Covariate or not?

- trial management type measurements *are* covariates
  - fungicide damage
  - herbicide damage
  - weed contamination
- in these examples the trait measured is not a genetic characteristic of a variety but a source of uncontrolled/nuisance variation

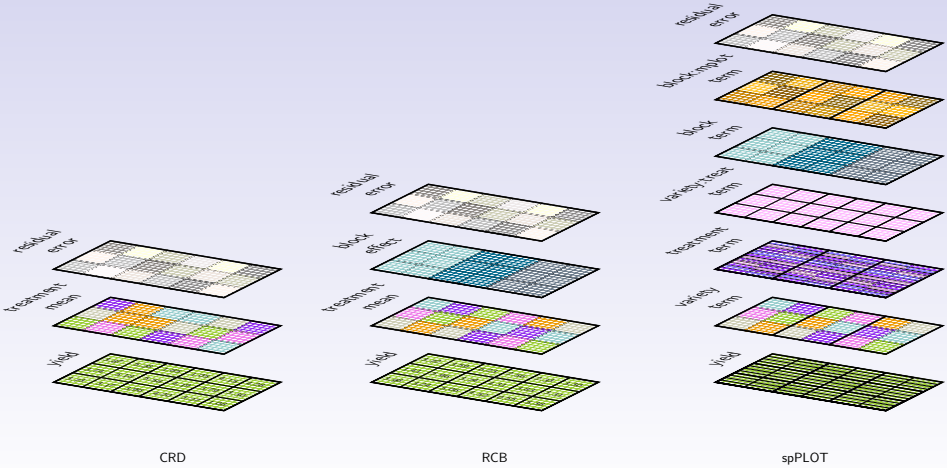
Covariate or not?

- for environment based measurements it's *not clearcut*
  - non-wetting soils
  - water logging
  - drought severity
  - wind damage
- bird and animal damage *are* covariates
- diseases in general *are not* covariates but Rhizoctonia damage *is* a covariate

More general models: extraneous effects

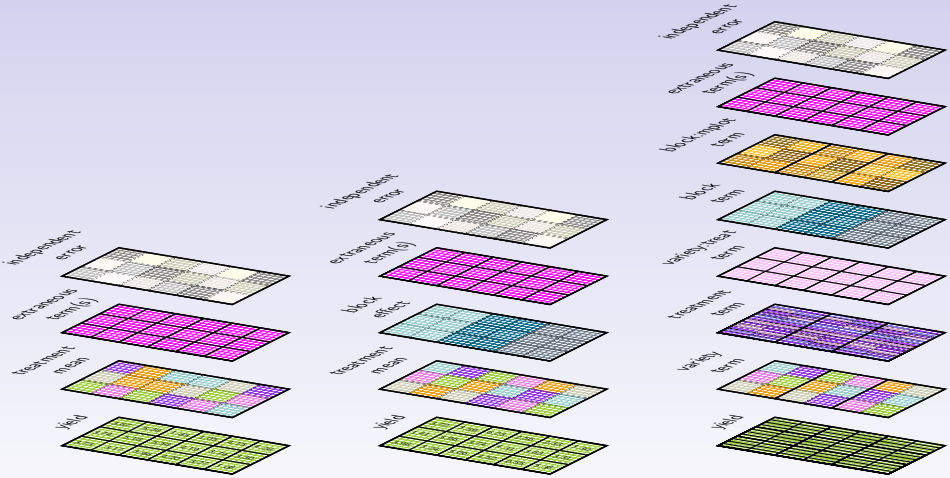
- covariates are sources of variation that are not accounted for in the initial design of the experiment, but can impact on the dependent variable
- often see other effects induced by management practices which are also are not accounted for in the initial design of the experiment, but are believed can impact on the dependent variable, eg. row effects due to serpentine harvesting, ... *extraneous effects*
- can add these to the model as a part of the model fitting process as well...

Baseline models: CRD, RCB, spPLOT



CRD RCB spPLOT

More general models: covariates/extraneous effects



CRD RCB spPLOT

More general models: spatial methods

the models we've seen so far:

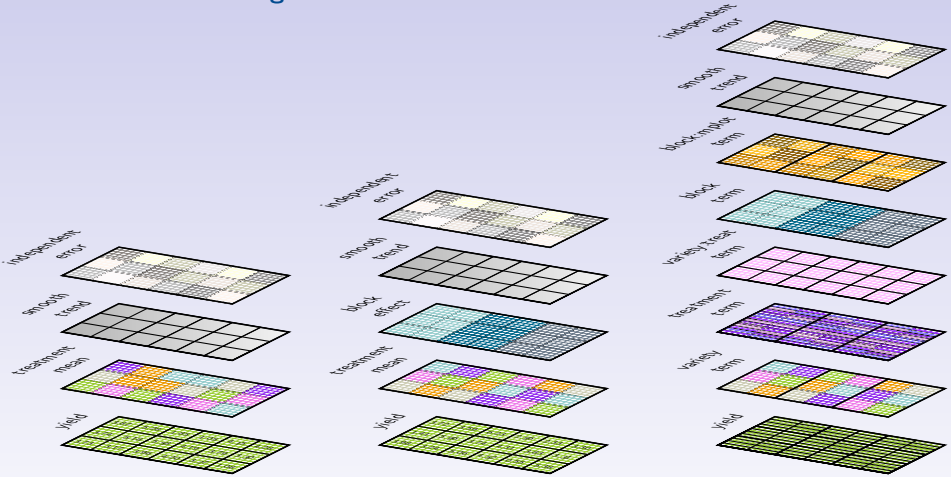
- model *broadscale variation* (variation between blocks of plots)
- don't capture *local/plot-to-plot/spatial variation*
  - due to moisture gradients/fertility trends
  - induce higher correlation between plots that are closer together in the field

we routinely use *spatial methods of analysis*:

- in spatial analysis
  - broadscale variation accommodated with blocking terms ...`block` in RCB, `Block` and `Block:Mplot` in spPLOT
  - spatial variation accommodated by more sophisticated assumptions about residual errors

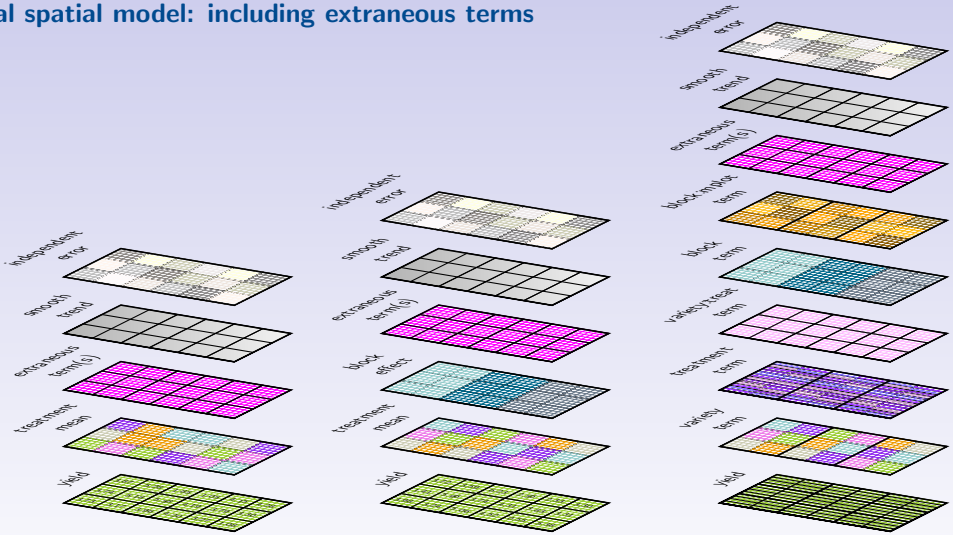
spatial methods $\longrightarrow$ estimated treatment effects that are *more more accurate and precise* than estimates using straight blocking methods

Our baseline model: including smooth trend



CRD RCB spPLOT

General spatial model: including extraneous terms



CRD RCB spPLOT

Analysis of Brome Grass control trial

standard RCB trial:

- 4 reps of 9 treatments
- 4 row $\times$ 9 range array of plots
- replicate blocks = rows
- treatments randomly allocated to 4 blocks

aim:

- compare grass control treatments

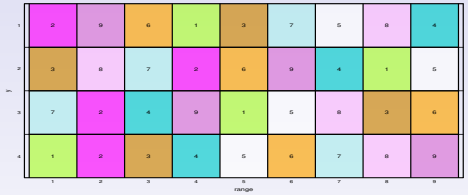


Figure: Allocation of varieties to plots

Example 5.1 Simple spatial analysis

late stage barley data

- 3 reps of 42 varieties
- 21 row $\times$ 6 range array of plots
- blocking in 2 directions
  - one complete replicate in each pair of ranges
  - second complete replicate blocks of 7 consecutive rows

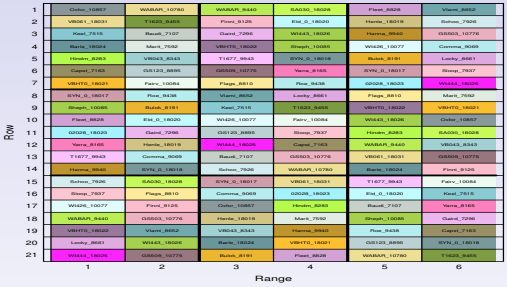


Figure: Variety allocation in the NVT barley trial