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INCOMPLETE BLOCK DESIGN IN DUS TRIALS

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INCOMPLETE BLOCK DESIGN IN DUS TRIALS

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Introduction

1. In many crops, the number of varieties to be tested is large and is increasing with time. As the influence of soil variability within a block usually increases with increasing block size, the increasing number of varieties to be tested means that, if complete block designs are used, there is more variability and it is more difficult to discriminate between candidate varieties and reference varieties. Several approaches exist for decreasing block sizes.

2. Firstly, the total number of varieties may be decreased by using grouping characteristics to separate the varieties and carry out an independent experiment for each group. This requires that the groups are non-overlapping at that all varieties can be assigned to the correct group with high probability. If those two requirements are not fulfilled, it may be risky to carry out an experiment for each group because this means that varieties in different groups cannot be compared using standard approach. Depending on the design, varieties in different groups either cannot be compared or they can be compared only within so-called inter block stratum, which usually leads to higher variance of comparisons. This is because the variance of blocks is usually much higher than the variance of plots. The result is that only very big differences can be effectively detected, see e.g. Caliński and Kageyama(2002). See also the discussion.

3. Another approach is to split the total number of plants per variety into more complete blocks, i.e. 6 blocks with 10 plants per plot in each instead of 3 blocks with 20 plants per plot, or may be even 10 blocks with 6 plants per plot or 12 blocks with 5 plants per block. However, the use of many blocks instead of just a few increases the work needed for sowing/planting and other tasks needed to maintain the trial. Also, the total area needed for the trial increases as the number of blocks are increased because more area is needed for guard areas and for gangways etc.. In the extreme case, the block sizes will only be decreased very little as most of the area will be used for guard areas and gangways etc.

4. The last approach to be mentioned here is to use designs where each block does not contain all varieties (called incomplete block designs). For instance there may be 80 varieties, which are to be compared using 3 plots with 20 plants per variety. Instead of having 3 blocks each with 80 plots (one with each variety) it could be arranged in 30 blocks with 8 plots each. This of course means that each block only contains a subset of the varieties. If this assignment of varieties to blocks is done randomly, the design may turn out to be a disaster where it is impossible to compare some pairs of varieties and other varieties may only be compared with very low precision (or high variability). However, if the assignment of varieties to blocks is carried out in a sensible way (based on statistical methods), it is possible to construct a design where all pairs of varieties can be compared with almost the same precision. Also, this precision may be considerably better than the precision which can be

obtained using complete blocks. This is because the variance between plots in small blocks (e.g. 8 plots) is smaller than the variance between plots in large blocks (e.g. 80 plots).

5. Incomplete block designs have been used in trials for many years. The ideas originate back to Yates (1939). Such designs have also long been used for comparing the performance of a large number of varieties or lines (see, e.g. Le Clerg, 1966 or Patterson and Hunter, 1983). During the last few years Incomplete block designs have also been used in DUS trials (e.g. Watson, 2002, Pilarczyk, 2001 and Kristensen, 2000). Most of this paper will focus on such designs, and the principles, the availability and the benefits/draw backs of incomplete block designs.

6. Another situation where incomplete blocks may be useful is when the comparison of certain pairs of varieties are to be optimised. However, the type of designs used for this purpose may be different from those used to compare many varieties.

Incomplete block designs for comparing many varieties

7. Several types of incomplete block designs exist, but here we will only mention one type, the type called α -designs (or generalised lattice designs). The α -designs were proposed by Patterson and Williams (1976) as a generalisation of lattice designs (Yates, 1939). The α -designs are very flexible as they are available for any number of varieties, for any number of replicates (at least two replicates) and for a wide range of block sizes. Another feature of these designs is that the incomplete blocks may be collected so that they form complete replicates. This means that the designs may be laid out in the field such that from a practical point of view they look like randomised complete block designs.

8. The construction of optimal designs is most easily done using a computer program. Two such programs are mentioned here: the first is Alpha+, which is written by CSIRO, Australia and Biomathematics & Statistics Scotland and is available from any of those institutes. The second is CycDesign, which is written by D. Whitaker, E.R. Williams and J.A. John. Near optimal designs can also be constructed manually from the generating arrays given by Patterson and Williams (1976). Briefly, this design type gives designs where: a) the number of times pairs of varieties comes together in an incomplete block is as equal as possible; and b) all pairs of varieties can be compared indirectly through as many other varieties as possible. The randomisation of the plan is performed in 4 steps:

- (1) the order of the replicates is randomised;
- (2) the order of the incomplete blocks within each replicate is randomised;
- (3) the order of the plots within each block is randomised; and
- (4) the varieties are allocated to the numbers 1 to v , the total number of varieties.

9. The analysis of the data can be done using the same statistical model as for designs with complete blocks, i.e. assuming additive variety and block effects (model 1 below). However, because the incomplete blocks can be, and usually are, grouped to form complete replicates, the block effects are usually subdivided into a replicate effect and an effect of the incomplete blocks within replicates (model 2). The effect of incomplete (and complete) blocks can be either fixed or random. When it is reasonable to assume that the effect of incomplete blocks are random (model 3) the generalised lattice will give a precision that is at least as good as if a randomised complete block design were used.

10. Models to analyse α -designs:

- (1) [response]=[mean]+[effect of variety]+[effect of block]+[random effect of plot]
- (2) [response]=[mean]+[effect of variety]+[effect of replicate]+[effect of block]+[random effect of plot]
- (3) [response]=[mean]+[effect of variety]+[effect of replicate]+[random effect of block]+[random effect of plot]

or more mathematically

- (1) $Y_{vb} = \mu + \alpha_v + \gamma_b + E_{vb}$ Effect of blocks are assumed systematic
 - (2) $Y_{vrb} = \mu + \alpha_v + \beta_r + \gamma_{rb} + E_{vrb}$ Effect of blocks within reps. are assumed fixed
 - (3) $Y_{vrb} = \mu + \alpha_v + \beta_r + C_{rb} + E_{vrb}$ Effect of blocks within reps. are assumed random
- E_{vb} , E_{vrb} and C_{rb} asumed independent and normal distributed with constant variance, σ_E^2 and σ_C^2

11. The models result in the following analyses of variance tables (showing only the column with the degrees of freedom):

Source	Model (1)	Model (2)	Model (3)
Varieties	V-1	V-1	V-1
Replicates		R-1	R-1
Blocks	RB-1	R(B-1)	R(B-1)
Residual	VR-V-RB+1	VR-V-RB+1	VR-V-RB+1
Total	VR-1	VR-1	VR-1

Note: V, R and B are the number of varieties, replicates and blocks within replicates

12. Note that, even though the degrees of freedom are identical for model (2) and model (3), the Mean Squares will usually not be identical

13. When using incomplete blocks, the user first has to choose an appropriate block size and then later decide how to place the blocks in the field. In the following we try to formulate the authors' experience on these matters. The block size is usually chosen to be approximately the square root of the number of varieties - e.g. with 80 varieties we would usually chose a block size of around 9. If the field to be used is heterogeneous, we would decrease the block size and use a design with 8, 7 or may be even 6 plots per block. In the field we would first subdivide the area into complete replicates such that the area covered by each replicate is as homogeneous as possible. We then subdivide each of the complete replicates into incomplete blocks such that the plots within each incomplete block are as homogeneous as possible. In addition to these principles, we try also to fulfil the following: an incomplete block should cover only one row of plots in the field and should be compact. In large trials (with many varieties) we also try to ensure that field operations such as sowing, planting etc. can be stopped at a border between complete replicates.

Efficiency factors and effectiveness of block designs

14. Every block design is characterised by a so-called efficiency factor (often denoted by ϵ) that denotes the amount of information contained in the within-block stratum (it is a theoretical characterisation of the design). Two extreme cases are:

(a) complete blocks – all information is contained in the within-block stratum (efficiency factor $\varepsilon=1$), and

(b) design with all blocks of one plots (efficiency factor $\varepsilon=0$), all information is contained in the between block stratum.

15. Generally bigger blocks provide higher efficiency factors. So, under the assumption that the variance of error is not dependent on the block size, the randomised complete block design is the best as it has the highest efficiency factor. However, in practice in plot experiments, we observe that the variance of error increases when the block size increases. So, finding the best design in practice means balancing the loss in the efficiency factor and the gain through reducing the variance of error - both caused by the reduction of block size. So, in practice, every design can be better characterised by so-called effectiveness. It is the ratio of the average variance of treatment comparisons provided by randomised complete block design to the same variance provided by the design under consideration. The best design is the one with the highest effectiveness. Let $MS_e(k)$ stand for mean square for error (it is the estimate of the error variance) in a design with blocks of k plots and let ε_k be its efficiency factor. The effectiveness of block design with blocks of k plots can be calculated as:

$$(a) \quad MS_e(v)/(MS_e(k)/\varepsilon_k) = \gamma \varepsilon_k \quad \text{under model 2 given above,}$$

$$(b) \quad \gamma \left\{ \varepsilon_k + \frac{(1 - \varepsilon_k)(s - 1)}{\gamma(v - 1) - (v - s)} \right\} \quad \text{under model 3 given above, see Patterson and Hunter (1983).}$$

Here $\gamma = MS_e(v)/MS_e(k)$, v is the total number of varieties and s denotes the number of incomplete blocks contained within one complete replicate.

16. The variety estimates obtained from the analyses are different from those obtained from the analysis of data from a complete block design. In complete block designs the variety estimates are simply the means of the variety performance in each complete block. This is still the case even if the block effects are very large, because all varieties occur once in each block and so they will all be favoured to the same extent by a very high yielding block. However, this is not the case for incomplete block designs as only some varieties are present in each block. So the varieties present in e.g. the highest yielding block will gain a false benefit from this when compared with the other varieties. Therefore they have to be adjusted downwards. Similarly varieties in blocks with low yield have to be adjusted upwards. In this adjustment it also has to be taken into account that a block may also be low yielding just because it happens to consist of low-yielding varieties, so the adjustment has to be done in an optimal way. The variety estimates obtained by $\hat{Y}_v = \hat{\mu} + \hat{\alpha}_v$ can be regarded to be such an optimal adjustment of the simple means.

Examples of designs

17. An example of an α -design with 71 varieties is shown in appendix 1. Each column of plots forms a complete replicate, which is then subdivided into 9 incomplete blocks of which one contains 7 plots and the rest contain 8 plots each (two block sizes are used in order to let the number of plots per replicate add to 71). So in replicate 1 (first column) the first

incomplete block consists of the varieties 5, 60, 57, 9, 26, 47, 61 and 33. The next block consists of the varieties 48, 53, ... and 68 and so on. In this example the varieties that occur together in the same incomplete block in replicate 1 do not occur together in replicate 2 and replicate 3. This is a feature of all α -designs where the size of the blocks are less than or equal to the number of blocks in each replicate and the number of replicates is small. If the size of the blocks becomes larger than the number of blocks per replicate or if the number of replicates becomes large, then some pairs of varieties may occur together in an incomplete block more than once.

18. This design was used for Yellow Mustard in Denmark in 1999, using Test Guidelines for Rape Seed (document TG/36/6), and the records for UPOV characteristic numbers 6, 8, 9, 10, 13, 14, 16, 17, 18, 19 and 20 were analysed using a model where the effects of incomplete blocks within replicates are assumed random (model 3 above). This model reduced the LSD-values for these characters by 0 to 24%. In other years the reduction may be different. In Table 1, the reductions in LSD-values are shown for each characteristic in each of the years 1997-1999 (based on Kristensen, 1999 and Kristensen, 2000). In these years there were 55, 66 and 71 varieties and the block sizes were 11, 11 and 8(7), respectively. In the last column the reduction in COYD LSD-values are shown. The reductions in COYD LSD-values are calculated by comparing the COYD LSD values when the COYD is based on simple variety by year means and when it is based on yearly estimates using the model with random incomplete-block effects. In most cases the reduction for the COYD LSD is smaller than the average of the reduction in the individual trials (years). This is also to be expected, as the variety by trial interaction must be expected to be unaffected by the used design. The largest reductions were found for characteristic numbers 16 and 17.

Table 1: Reduction of LSD-values, %, for some characters of Yellow Mustard (using Test Guidelines for Rape Seed, document TG/36/6) when α -designs are used instead of randomised complete block designs. The last column shows the reduction in COYD LSD values.

No.	Character name (TG/36/6)	1997	1998	1999	COY-D
06	Leaf: Number of lobes (fully developed leaf)	4.4	0.1	0.2	1.2
08	Leaf: Length (blade and petiole)	0.5	0.0	3.0	1.0
09	Leaf: Width (widest point)	1.7	0.0	1.0	2.0
10	<u>Varieties with long leaves only</u> : Leaf: Length of petiole	7.7	1.0	0.5	2.5
13	Flower: Length of petals	0.3	0.2	0.1	0.0
14	Flower: Width of petals	0.1	0.5	0.1	0.2
16	Plant: Height (at full flowering)	9.0	8.1	23.8	8.2
17	Plant: Total length incl. side branches	25.6	22.9	9.7	14.9
18	Siliqua: Length (between peduncle and beak)	4.8	3.4	3.9	4.2
19	Siliqua: Length of beak	3.3	3.5	3.3	2.6
20	Siliqua: Length of peduncle	0.6	0.0	0.6	0.0

19. A similar investigation was performed in Poland. In 1995-1996 three experiments on maize were conducted. The numbers of varieties involved were 200, 212 and 98 respectively. The four characteristics for which calculations were performed were:

- c₁ – plant length,
- c₂ – height of insertion of upper ear,
- c₃ – leaf width,
- c₄ – length of peduncle.

20. The effectiveness of incomplete blocks in these three trials is reported in Table 2.

Table 2: Reduction of LSD-values, %, for some characteristics of maize when α -designs are used instead of randomised complete block designs.

Year	Place	V	k	ε	Reduction of LSD-values			
					c ₁	c ₂	c ₃	c ₄
1995	Słupia W.	200	13(12)	0.870	18.4	20.8	2.6	1.7
1996	Słupia W.	212	15(14)	0.889	32.8	18.1	5.0	6.1
1996	Przeclaw	98	10(9)	0.846	12.7	10.1	15.3	14.1

As one can see, the effectiveness is dependent on the year and the characteristic.

21. Similar calculations performed for a trial on French Bean conducted in 1998 at Słupia Wielka showed the effectiveness of the applied incomplete block design ($v=40$, $k=10$, $\varepsilon=0.8540$) for plant height. For three characteristics concerning pod, incomplete blocks had no advantage over complete ones.

22. A post-blocking analysis of a DUS experiment on Onion (with $v=114$ varieties and $r=2$ replicates) performed in 2003 at Experimental Station Słupia Wielka, Poland, showed no advantage of incomplete blocks over complete ones for all nine analysed characteristics and for different incomplete block sizes (Pilarczyk, 2004)

Incomplete block designs for optimising comparison of variety pairs

23. The theory used in a split-plot design may be used for setting up a design where the comparisons between certain pairs of varieties are to be optimised. When setting up the design, the pairs of varieties are treated as the whole-plot factor and the comparisons between varieties within each pair is the sub-plot factor. As each whole-plot consist of only two sub-plots, the variance used to compare varieties within pairs will be relative small and the comparisons within pairs will be (much) more precise than if a randomised block design was used.

24. If 4 pairs of varieties are to be compared very efficiently, then this may be done using the following design.

Pair 1 variety A	Pair 3 variety E	Pair 4 variety H
Pair 1 variety B	Pair 3 variety F	Pair 4 variety G
Pair 3 variety F	Pair 2 variety D	Pair 1 variety A
Pair 3 variety E	Pair 2 variety C	Pair 1 variety B
Pair 4 variety G	Pair 1 variety B	Pair 2 variety C
Pair 4 variety H	Pair 1 variety A	Pair 2 variety D
Pair 2 variety D	Pair 4 variety H	Pair 3 variety E
Pair 2 variety C	Pair 4 variety G	Pair 3 variety F

25. In this design each column represents a replicate. Each of these are then divided into 4 incomplete blocks each consisting of two plots. The four pairs of varieties are randomised to the incomplete blocks within each replicate and the order of varieties is randomised within

each incomplete block. The model to analyse data from such a design may be written in the following way:

$$[\text{response}] = [\text{mean}] + [\text{effect of replicate}] + [\text{effect of variety pairs}] + [\text{effect of varieties within pairs}] + [\text{random effect of block}] + [\text{random effect of plot}]$$

or mathematically

$$Y_{rpv} = \mu + a_r + \beta_p + \gamma_{pv} + D_{rp} + E_{rpv}$$

D_{rp} and E_{rpv} assumed independent and normal distributed with constant variance, σ_D^2 and σ_E^2

26. This will lead to the following analyses of variance table (showing only the column with the degrees of freedom and mean squares).

Source	DF	MS
Replicates	R-1	MS_R
Pairs of varieties	P-1	MS_P
Varieties within pairs	P	MS_V
Blocks within replicates	(R-1)(P-1)	MS_B
Residual	(R-1)P	MS_{Res}
Total	2RP-1	

27. When comparing varieties belonging to the same pair the mean square for the residual, MS_{Res} , has to be used whereas a weighted mean of MS_B and MS_{Res} , has to be used if two varieties in different pairs have to be compared.

Discussion and conclusion

28. The benefit of using incomplete block designs includes the possibility of comparing many varieties in one design with a precision that is about as good as if only few varieties were to be compared in a design with complete blocks. Compared to randomised complete block designs with many varieties the incomplete block designs is superior for characteristics that are sensitive to soil fertility. For characteristics which are less sensitive to soil fertility the gain in precision will be smaller or zero (but only in few cases marginally negative). As the gain from using incomplete block designs instead of randomised complete block designs is usually largest in trials where the randomised complete block design would have yielded large LSD-values, the LSD-values from trial to trial are expected to be more equal when using incomplete block designs.

29. The construction, layout in the field, and analysis of incomplete block designs are slightly more complicated than for randomised complete block designs. However, efficient programs for PC's are available that can ease these processes.

30. The use of incomplete block designs in principle prevents the user from imposing "restricted randomisations" in order to let groups of varieties be located close to each other. Different methods may be used to combine α -designs and grouping:

- The simplest method is to place some restriction on the allocation of varieties to the variety numbers 1 to v in one of the replicates, say replicate 1, such that the order of the

varieties in this replicate follows the grouping characteristics. From a statistical point of view this is not completely correct, so some investigations should be performed in order to ensure that the assumptions are not violated too heavily.

- Grouping may be imposed by using designs similar to how α -designs may be used to construct incomplete split plots (Kristensen, 2003). Similar designs may also be constructed using some of the methods described in Caliński and Kageyama (2002). When it is possible to use this method, it allows varieties in the same group to be compared with a high precision whereas varieties in different groups will be compared with a lower precision. This method still needs some practical investigations in order to find out where and how the method should be used.

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APPENDIX 1

1 1 05	2 1 16	3 1 29
1 1 60	2 1 60	3 1 53
1 1 57	2 1 20	3 1 10
1 1 09	2 1 64	3 1 64
1 1 26	2 1 55	3 1 65
1 1 47	2 1 25	3 1 04
1 1 61	2 1 28	3 1 14
1 1 33	2 1 62	3 1 23
1 2 48	2 2 48	3 2 08
1 2 53	2 2 42	3 2 68
1 2 63	2 2 56	3 2 46
1 2 15	2 2 57	3 2 47
1 2 31	2 2 02	3 2 34
1 2 37	2 2 04	3 2 07
1 2 25	2 2 46	3 2 03
1 2 68	2 2 44	3 2 66
1 3 42	2 3 14	3 3 20
1 3 01	2 3 24	3 3 15
1 3 65	2 3 33	3 3 36
1 3 07	2 3 35	3 3 38
1 3 17	2 3 15	3 3 27
1 3 32	2 3 19	3 3 56
1 3 16	2 3 01	3 3 69
1 3 27	2 3 34	3 3 26
1 4 28	2 4 52	3 4 62
1 4 36	2 4 67	3 4 11
1 4 10	2 4 07	3 4 17
1 4 59	2 4 49	3 4 24
1 4 03	2 4 10	3 4 44
1 4 19	2 4 05	3 4 54
1 4 54	2 4 63	3 4 61
1 4 12	2 5 13	3 4 49
1 5 04	2 5 39	3 5 21
1 5 66	2 5 68	3 5 39
1 5 18	2 5 23	3 5 51
1 5 55	2 5 58	3 5 28
1 5 35	2 5 61	3 5 45
1 5 58	2 5 43	3 5 63
1 5 21	2 5 36	3 5 42
1 5 11	2 6 41	3 5 33
1 6 70	2 6 38	3 6 41
1 6 51	2 6 03	3 6 67
1 6 43	2 6 11	3 6 58
1 6 52	2 6 09	3 6 60
1 6 56	2 6 70	3 6 30
1 6 08	2 6 31	3 6 48
1 6 29	2 6 65	3 6 32
1 6 62	2 7 59	3 6 12
1 7 50	2 7 17	3 7 02
1 7 64	2 7 30	3 7 43
1 7 69	2 7 66	3 7 40
1 7 39	2 7 22	3 7 05
1 7 44	2 7 51	3 7 01
1 7 34	2 7 69	3 7 31
1 7 41	2 7 37	3 7 59
1 8 46	2 8 45	3 7 55
1 8 20	2 8 18	3 8 18
1 8 40	2 8 53	3 8 19
1 8 22	2 8 54	3 8 25
1 8 23	2 8 08	3 8 06
1 8 67	2 8 32	3 8 22
1 8 71	2 8 71	3 8 57
1 8 24	2 8 26	3 8 70
1 9 45	2 9 40	3 9 52
1 9 06	2 9 29	3 9 09
1 9 14	2 9 50	3 9 37
1 9 13	2 9 47	3 9 50
1 9 02	2 9 21	3 9 71
1 9 38	2 9 06	3 9 35
1 9 30	2 9 27	3 9 13
1 9 49	2 9 12	3 9 16

Layout of a DUS trial in Denmark with 71 varieties of Yellow Mustard.

The trial has 3 complete replicates that are subdivided into 9 blocks of 8 (7) plots each.

Each cell is a plot. The first digit in each cell gives the number of the whole replicate; the next one gives the incomplete block number (numbered from 1 to 9 in each whole replicate) and the last two digits show the variety number (numbered from 01 to 71).

