DESIGN OF EXPERIMENTS

K.Vijaya Kumar

The variability in experimental material is common in any field of research. It may vary in size from experiment to experiment but its presence cannot be disputed. When the variability is small as compared to the group or class differences and if it is not expensive to take more observations, a detailed experimental set up is not needed. But when there is lot of variation from observation to observation and if it is not economical to take large number of observations it is necessary to adopt an experimental design which allows to estimate true treatment differences in an unbiased way with a specified degree of precision.

While conducting an experiment, a researcher would be having different objects of comparison called 'treatments'. For example, In an agricultural field trial there may be different varieties, cultivation practices or different doses of fertilizer to be compared. In Post Harvest Technology (PHT) there may be different doses of a chemical to preserve a product for longer shelf life. Through a simple trial it may be possible to see a particular treatment doing better than others. But, the better/poor performance of the particular treatment may be due to other reasons rather than the real superiority of a particular treatment. In a field trial it may be because the plots allotted for the particular treatment might have been more fertile or might have been located under better environmental conditions. In the PHT problem mentioned earlier, some of the treatments might have poorly performed because the samples used for the study might have been contaminated earlier. So, it is necessary that all treatments are tried under identical conditions and without any bias. Statistical designs for an experiment will help us to determine the intrinsic worth of a treatment in an unbiased manner.

The basic requirement of a statistical design consists of three principals viz., randomisation, replication and local control.

Randomisation: This consists of allocation of treatments to various plots without any bias of the experimenter. By randomisation, we can ensure that various treatments in long run, by repetition of the experiment, will be subjected to equal environmental effects.

Replication: By replication we mean that the repetition of the treatment under study. Since the inherent variation in the experimental material cannot be allowed directly because of unpredictable nature, the experimenter wishes to average out its influence over the different treatments by replication. If we repeat a treatment 'r' times, the mean of these repetitions will be subject to a standard error (SE) of $\sigma/\sqrt{r}$, where σ is the standard deviation (SD) of individual plots which is estimated from the experiment. The percentage SE to which this mean will be subject equal to $C/\sqrt{r}$ where C is coefficient of Variation (%). We will be able to estimate the number of replications required for inferring the difference between two treatments (given as % of general mean say d%) as significant at a given level of significance (say at 5%) when observed difference exceeds a given per cent of mean. Therefore,

$$\frac{\text{difference}}{\text{S.E of difference}} \geq 1.96.$$

Solving 'r' from this gives the minimum number of replications required. However, since the CV is an estimated value only and not true value, this will give only an approximate idea of the minimum number of replications required for the study.

Local Control: The variation in the experimental material existing which cannot be controlled by the experimenter is termed as experimental error. Therefore, the observed differences between two treatments may be due to i) Real treatment differences ii) Experimental error which influences the yield irrespective of the real treatment differences.

Thus it is required to find out the magnitude of experimental error and compare it with the treatment variations to see if there is any real difference between treatments. It is possible to reduce the experimental error by suitable arrangement of experimental units within homogeneous blocks. This is known as the principle of **Local control.**

Different ways of arrangements of experimental plots to investigate the treatments is known as **Experimental designs. Completely Randomized Block Design(CRD):** This is the simplest of all the designs. Here, all the experimental units available for the experiment are assumed to be homogeneous. The treatments are allotted at random to different units(plots). CRD is a flexible design as there is no restriction of equal number of replication for each treatment. However, the principle of 'local control' is overlooked since randomization is carried out throughout the field. This has its own disadvantages and advantages. If the experimental area/material is homogeneous as it is assumed, the design is most efficient as compared to other designs. If it is not, this design is to be avoided. The following example illustrates the layout of a CRD.

Example 1: A trial is to be conducted to find out a treatment schedule for preserving coconut wood for timber purpose. Six treatments(coded as A,B,C,D, E and F) are to be compared. Forty six logs of equal size and which are homogeneous with regard to their initial physical property are available for the study. For treatment F chemical which is enough for only 6 logs is available. Design an experiment which will help to decide the best treatment schedule.

The experiment.can be laid out in a CRD as the experimental material is given to be homogeneous. Since chemicals for maximum of 6 logs only is available, for treatment F we can have 6 replications and 8 replications each for treatments A to E. For this purpose, first all the 46 logs are serially numbered from 1 to 46. With the use of random number tables the numbers 1 to 46 are arranged randomly. The first 8 numbers are allotted to treatment A and the next 8 to treatment B and so on till treatment E. The remaining 6 numbers are allotted to treatment F. The analysis of variance consists of finding out sum of squares due to the only source of variation viz., treatments. The sum of square for unknown causes(error) is found out by subtracting treatment sum of square from total sum of square. When the ratio of treatment mean square to error mean square exceeds table value of F for corresponding degrees of freedom, the null hypothesis of equality of treatment means is rejected at 5% or 1% level.

Randomized Block Design:

This is the most commonly used design. Since the principle of local control is utilized

for reducing the experimental error by restricting the treatments within homogeneous blocks, this is an improvement over CRD.

In the case of a field trial a block can be contiguous area of land and in industrial experiments, the block can be materials prepared in one single batch so that within a block homogeneity is maintained. In a RBD, each block is a complete replication and there is a reduction in error variance as replication differences is eliminated from the error variation thereby making treatment comparisons more precise. The following example will illustrate the procedure of layout of an RBD.

Example 2: In the example 1 given above it is known that the 46 logs of wood belong to palms of 6 different age groups viz.., below 40,41-50,51-60,61-70,71-80 and above 80 years and in each group a minimum of 7 logs of wood are available. Design an experiment to find out the best treatment schedule.

Since it is known that the logs belong to 6 age groups, it is better to allot treatments within each group separately as logs within a particular age group are expected to be more homogeneous than logs belonging to different age groups. So, each treatment is allotted randomly to a log within each age group so that each age group forms a complete replication. Since the variation due to age group is removed from the error variation, there will be reduction in error. In RBD there are two known sources of variation viz., replication and treatments. The variation (Sum of squares) due to experimental error is found out again by subtraction of variation (Sum of squares) due to known sources of error from the total sum of squares and test for treatments is carried out like in CRD.

Latin Square Design(LSD): In this design the heterogeneity is controlled in two directions so that variation from two sources are eliminated from error variation with a result that the treatment comparisons are generally more precise than RBD. So, in case of a Field trial, if it is known that the fertility gradient is in two directions perpendicular to each other,there can be two sets of blocks made one set called Rows horizontally and other set of blocks called Columns vertically over the previous set. The area formed by the intersection of these sets of blocks is a plot. In LSD , each treatment occurs exactly once in each Row and Column. This design lacks the flexibility of RBD or CRD as there should be equal number of replication as that of treatments. This is a major limitation of this design. The sum of square due to error is found out by subtraction of sum of squares due to rows, columns and treatments from the total sum of squares.

The following example illustrates the layout of an LSD

Example 3: In the examples 1 and 2 given above, it is further known that the 46 logs belong to 6 varieties of coconut such that a minimum of 1 log is available in each variety in each age group. Lay out the experiment in a suitable design to find out the best treatment schedule.

It is now known that the experimental materials have two known sources of variation viz., age differences and varietal differences which if not eliminated, will increase the error variation. The logs are first arranged age wise in 6 groups. Within each age group, the logs are arranged again variety wise. A 6X6 Latin square arrangement is then chosen after usual randomization procedure for the LSD. Since there is atleast one log

satisfying both the classifications, different treatments are imposed on each log as per Latin square arrangement.

The Latin square arrangement for the experiment may look as below after randomization of rows, columns and treatments.

F	D	B	E	A	C
E	B	F	C	D	A
C	A	E	D	F	B
A	F	D	B	C	E
D	E	C	A	B	F
B	C	A	F	E	D

Here 6 rows represent 6 age groups and 6 columns represent 6 varieties so that each treatment is tested on one log belonging to a particular age group and the variety.

Factorial Experiment: During some occasions, the experimenter may have to investigate simultaneous variation in more than one factor, For example, he may be interested in finding out the optimum dose combination of Irrigation and fertilizer for a crop. One way of doing is to experiment first with Irrigation and arrive at an optimum dose and then conducting another experiment using the optimum dose of irrigation already found with different doses fertilizer. But this method is not only time consuming but also erroneous; because if the two factors are interacting, the dose of irrigation which is found optimum earlier may not be really optimum at lower/higher dose of fertilizer. So, in such situations, only way is to take all possible combinations of the two or more factors in one and the same experiment and try to find the optimum combination. The factorial experiment can be laid out in a CRD or RBD depending on the experimental material.

Confounding: When there are more than two factors and more levels for each factor, there will be too many treatment combinations. In a field trial it is difficult to get large uniform area to accommodate all these combinations. Since a smaller area is expected to be more homogeneous than a larger area, a portion of the treatment combinations can be tried in these homogeneous blocks; thus a replication wherein all the treatment combination appear once consists of more than one block. This procedure of dividing the replication into two or more homogeneous compact blocks is called as **confounding.** Such a subdivision of a replication disturbs the precision of treatment comparisons and some effects will be mixed up with block differences. However, with judicious allocation of treatment combinations in different blocks, it is possible to retain all the important comparisons intact.

The following example of a 2^4 experiment will illustrate method of confounding. In a 2^4 factorial experiment 4 factors(say a,b,c,d) each at two levels (say absence and presence) are tried so that there will be a total of 16 treatment combinations. These can be listed as below.

(1),a,b,ab,c,ac,bc,abc,d,ad,bd,abd,cd,acd,bcd,abcd

Here, (1) represents the absence of all the factors and 'a' the presence of factor 'a' and the absence of all other factors.

The following blocking system with treatment combinations in the respective blocks in one replication is an arrangement which confounds the highest order interaction ABCD. Here the effect ABCD is mixed up with block differences.

Block 1 a, b, c, d, abc, abd, acd, bcd
Block 2 (1), ab, ac, bc, ad, bd, cd, abcd

If there are 2 or more replications in the experiment,and same treatment combinations as above are tried in the two blocks of the other replications also,then ABCD cannot be estimated at all and it is the case of **Complete confounding** the effect ABCD. **Partial confounding** is an arrangement so that the effect confounded in one replication can be estimated from other replication(s).

Split Plot design:

In Factorial experiments, when we consider two dissimilar factors (ex: Irrigation and Spacing) first factor(Irrigation) usually shows larger differences than the second(Spacing). With a factorial layout, both the above factors will be tested with equal precision. So it is possible that the differences between Spacings may go unnoticed as the other factor produces larger differences and both are tested with the same error which will generally be large because of the first factor. One way of reducing the error is to restrict the randomization of the factors producing smaller differences within compact blocks instead of scattering them throughout the replication. So, a replication is divided into as many blocks as there are levels of first factor and the levels of first factor are allotted randomly to these blocks(Main Plots). Within each of these Main Plots, the levels of the 2nd factor are allotted at random. Similar procedure is followed in other replications also. The resulting layout is called **Split Plot Design.** Since two types of plots are involved, one larger plots for the main plot treatments and the other smaller plots for sub plot treatments, two error variations are involved one for the main plots and the other for sub plots and the interaction. The comparison of subplots thus becomes more precise as the main plot comparisons are done with the separate error.

Split Plot Design has several practical advantages and is found to be very useful in agricultural field experiments. In this design greater precision is obtained for the Sub plot treatments and Interaction - of course at the cost of Main plot treatments. Therefore we have to be careful in choosing the factors for the Main plot treatment.

Strip Plot Design:

This is analogous to Split Plot design. When two factors can be tried on larger plots, one set of factors is superposed over the other at right angles. So, a replication is first divided into as many strips as there are levels of first factor horizontally and then into as many strips as there are levels of 2nd factor vertically. Both the sets of treatments are allotted at random within a replication. Such an arrangement is called Strip Plot design. Since there will be three types of plots in this layout, three different error mean squares are involved. The test of significance for interaction is more sensitive by virtue of large number of error degrees of freedom available for the corresponding error.

Incomplete Block Designs:

When there are 10 - 15 treatments for comparison, the experiment can be laid in a simple RBD. But when there are a large number of treatments especially in Plant Breeding where number of varieties or strains to be compared, it will not be possible to accommodate all of them in a block because of soil heterogeneity. The situation here is somewhat similar to that of factorial experiments where replicates are divided into homogeneous blocks. So, the same solution

of spreading replication to more than one homogeneous block is applied here also. Designs of this type are called Incomplete Block designs. In the case of confounded factorial designs the idea is to estimate the main effects and first order interactions at the cost of estimating higher order interaction. In Incomplete block design, there is nothing like interaction and we are interested in having the comparison between all the treatments. Therefore something like a partial confounding is adopted so that the treatment comparison which are confounded in one replication can be estimated from other replications. Thus comparison between all the pairs of varieties are available. So, though some information is lost by this type of confounding, ultimately because of smaller size of the blocks, the comparisons are generally more precise than what we would have obtained by adopting a simple RBD by taking all the treatments in one single block.

Simple lattice or Double lattice designs: This is the simplest case of an Incomplete Block design. In this case, the number of varieties is 'V' where V is a perfect square(q^2). The designs are available for 16,25,36,49 etc. This layout requires a minimum of 2 replications in blocks of q plots each. So,if there are 36 varieties, first the varieties/ treatments are numbered 1-36 serially and the numbers are arranged in a square array of 6X6. First replication which may be called the X group is formed by taking the row sections as blocks; so, 6 blocks are formed this way in a replication. Second replication called the Y group will have 6 more blocks consisting in each block the varieties/treatments occurring in the columns of the array. The two groups together constitute the simple or double lattice design.

The following 6X6 array shows the layout of a simple/Double lattice design.

1	2	3	4	5	6
7	8	9	10	11	12
13	14	15	16	17	18
19	20	21	22	23	24
25	26	27	28	29	30
31	32	33	34	35	36

Here, the first replication(X group) consists of 6 blocks, each consisting of treatments mentioned in the 6 rows of the array. The second replication(Y group) consists of another set of 6 blocks consisting of treatments mentioned in the 6 columns. These two sets of blocks constitute the simple lattice design for 36 treatments.

Factorial experiment in Fractional replication: In a factorial experiment, if the factors are in large number a single replication itself will have unmanageable number of treatment combinations. For example, even in comparatively small experiment like 2^6 factorial experiment. there will be 64 combinations. For estimation of main effects or two factor interactions even half this number of combinations will be enough without much loss of precision. So, it is a great saving if we can reduce the number of treatment combinations to a manageable level and still get all the main effects and important interactions. Such factorial experiments where only a fraction of the treatment combinations are tried in a replication is known as factorial experiments in fractional replications. These types of experiments are very useful in industrial experiments where sometimes the combinations are in thousands for a single replication and it will be physically impossible to carry out the complete trial. However, by using half or one fourth of a replication, we will be mixing up certain

effects with some other effects called 'aliases'. So, for example while estimating the effect of say A, the alias of the component A (which is normally a higher order interaction) also is included in that. Mostly these 'aliases' are so chosen in the layout such that effects of them can be neglected. This means that it is necessary that we know few basic things about the experimental material before laying out the experiment so that an effect which is an alias of another effect will not be simply ignored. The following example illustrates laying out a 2^5 factorial experiment in fractional replication by using a 'half' replicate.

In the above example there are 32 treatment combinations. The highest interaction is ABCDE. So, the treatment combinations can be divided into two sets such that from one of these all the main effects and 2 factor interactions can be estimated. The 3 factor and 4 factor interactions which can be assumed as negligible go as 'aliases' of these 2 and 3 factor interactions. The 5 factor interaction ABCDE is taken as Defining contrast which cannot be estimated. The combinations which come under the 2 sets with ABCDE as the defining contrast are:

1st set a, b, c, d, e, abcde, abc, abd, abe - acd, ace, ade, bcd, bce, bde, cde

2nd set abcd, abce, abde, acde, bcde, ab, ac, ad, ae, bc, bd, be, cd, ce, de, (1) +

Taking the first set only in the experiment i.e half replication, we can estimate the following main effects and interactions and the corresponding alices are also given.

Main effect	Alices	Interaction	Alices
A	BCDE	AB	CDE
B	ACDE	AC	BDE
C	ABDE	AD	BCE
D	ABCE	AE	BCD
E	ABCD	BC	ADE
		BD	ACE
		BE	ACD
		CD	ABE
		CE	ABD
		DE	ABC