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Statistical Designs in Agricultural Research

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ABSTRACT

Any experiment should be compatible with scientific approaches such as statistical reliability, rationality, internal and external validity, generalizability, and other specific criteria related to the subject, at the laboratory level or field level. Hence, planning a design to accomplish those approaches is critical, and selecting an appropriate model accordingly to acquire basic principles of design would make the experiment more precise. Straightforward designs of CRD, RCBD, and LSD, and complicated designs of IBD, Factorial experiments, Split-plot designs, and Lattice designs are discussed here. Consequently, the researcher can decide the appropriate experimental design.

Introduction

Experiments are conducted in every discipline in agriculture, i.e., Agricultural Biology, Agricultural Engineering, Crop Science, Animal Science, and Soil Science, in the field and in laboratories. Experimentation is an essential component before the real-world application to have better utilization of agricultural technology and final functional output, and hence, the know-how used in experimentation, as well as the experiment, should be appropriately planned to preserve the validity of the test.

Genetic factors, environmental factors, and the interaction of genetic and environmental factors trigger variability in observations, which are explainable causes. The unexplainable variability also happens, which refers to the random error variability in an experiment.

Experimental units and treatments are the main components used in an experiment. A unit or object, which is the entity to apply the treatment, is an experimental unit, while treatment is the different conditions examined under the experiment. As an example, in a fertilizer trial for testing the best Nitrogen content, fertilizer which included different concentrations of Nitrogen compounds are the treatments, and the plot in which the planting is done is the experimental unit. In an animal experiment, an animal, i.e., a cow, a chick, maybe the experimental unit.

Principles of Experimental Design

The basic principles to be necessarily considered are Replication, Randomization, and Control of unexplained variability. They must be rejected at the earliest stage if they do not apply to the experiment. When a treatment is applied to more than one experimental unit, it refers to as 'replication.' The number of replicates used for the same experiment may be similar or not similar for all treatments used. Even though the same treatment is applied in different units, variability can be observed among them, which refers to the experimental error. The number of replicates should be increased to reduce the standard error of the estimates of treatment effects, which will raise the precision. The experimental error needs to be reduced by controlling unexplained variability to facilitate the increased precision; thereby, a statistical test becomes more sensitive. Methods of decreasing unexplained variability will be discussed later in the design models. When assigning treatments into different experimental units, all units should have an equal chance of being assigned in a treatment. Such allocation is mentioned as 'randomization,' which directs to unbiased estimates.

The analyzing method of variability should be declared before discussing different types of experimental designs. The observations gained from the experiment with varying needs of an application must be evaluated in terms of variability incurred and its significance in the sense of statistics. Here, the total variability is partitioned into particular causes, including error. This procedure is called the Analysis of Variance (ANOVA). Each component added to the experiment is presented in ANOVA as a source of the variable. The hypothesis must be developed here, as usual. The next step is to design the trial in an appropriate manner after considering the researchers' requirements.

Planning an Experiment

As an example, an experiment is designed to select the best Nitrogen fertilizer, using three treatments and four replicates.

Randomized Design

For this purpose, the simplest design to use is Completely Randomized Design **(CRD).** In this method, treatments are allocated to all experiment units in a completely random manner. Thus, it should be noted that other conditions must be controlled for all experiment units, which refers to the homogeneous condition. Achieving homogeneity is difficult, and a laboratory is a good environment for CRD because it helps to achieve controlled conditions. Accordingly, there should be 12 plots within, and

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each three treatment has four replicates. Equation 1 provides the statistical model for the ANOVA.

$$y_{ij} = \mu + \tau_i + \varepsilon_{ij} \quad \text{..... Eq. 1}$$
$$i = 1, 2, \dots, t$$
$$j = 1, 2, \dots, r$$

Where

y_{ij} = **the jth observation on the ith treatment**

μ = **grand mean**

τ_i = **effect of ith treatment**

ε_{ij} = **random error**

Suppose the above experiment is to be conducted in the field, and more replicates are necessary. Thus, field conditions such as moisture content (MC) may not be the same for a particular direction, which refers to the non-homogeneous condition. Then the experimental units become non-homogeneous. If non-homogeneity is achieved in one known direction, the possible design is Randomized Complete Block Design (RCBD). Hence, experimental units can be grouped into perpendicular to the direction, which is named as 'blocking.' The arranged blocks should be homogeneous.

Minimum variability should be assured within the blocks while high variability between the blocks. When planning the experiment, the number of blocks to be formed could be considered as per the non-homogeneity in the field. However, each treatment should be allocated into each block, i.e., each block must contain every treatment once. At the same time, randomization also should be considered without affecting the block combination explained above. Thus, randomization is restricted. Correspondingly, if there are four blocks, there should be 12 plots. Equation 2 provides the statistical model for ANOVA in this case.

$$y_{ij} = \mu + \tau_i + \rho_j + \varepsilon_{ij} \quad \text{..... 2}$$
$$i = 1, 2, \dots, t$$
$$j = 1, 2, \dots, r$$

Where y_{ij} = **the ith treatment of jth block**

μ = **grand mean**

τ_i = **effect of ith treatment**

ρ_j = **effect of jth block**

ε_{ij} = **random error**

As there is additional variability, i.e., block, compared to CRD, the error is reduced since some part of residual is explained by the variability of blocks. Thus, precision is increased compared to CRD. In addition, the efficiency of blocking (E) can be assessed through MS error of CRD and RCBD. E = MS error (CRD)/ MS error (RCBD).

Incomplete Block Design

However, when the number of treatments in our experiment is large, achieving homogeneity in one block becomes a failure in practice. Any circumstance can occur when the block size is smaller than the number of treatments. Thus, all treatments cannot be positioned in the same block, the number of replicates may not be the same for all treatments, and the size of the block may vary within an experiment. This type of design is referred to as Incomplete Block Design **(IBD).** The statistical model for the ANOVA is the same as RCBD.

Using IBD alone will not permit higher precision, and hence we can use Balanced Incomplete Block Design **(BIBD).** The same size of blocks, the same number of replicates for all treatments, and one associate class should be accomplished to attain BIBD.

When planning an experiment, another possibility is having a lesser number of blocks than the number of treatments where BIBD cannot be used. Hence, the design can be planned in a form where the number of times a pair of treatment occurs together in the blocks would be dissimilar for different pairs, i.e., λ parameter. Therefore, Partially Balanced Incomplete Block Design **(PBIBD)** can be constructed having two λ values maximum. In special cases, three λ values are allowed.

Latin Square Design

In addition to the variability of MC, there are different salinity levels perpendicular to the direction of moisture difference (east and south). There, non-homogeneity has occurred in two known directions, and RCBD is not valid in such situations. Here, Latin Square Design **(LSD)** can be used instead of the above experimental design. It allows blocking into both row-wise and column-wise while each treatment is allocated in a way that it appears once in each row and once in each column. Randomization should also be considered in this situation.

First, a possible arrangement is built as the above designs, and then the randomization of rows and columns needs to be done in two separate steps. At the end of designing LSD, there will be 3^2 experimental units, as there are three treatments (t). It should be noted that it must have a t^2 number of experimental units, and thus, higher the "t," higher the experimental units to be used, which is not possible in practice. In contrast, lower the "t" lower the error degrees of freedom (DF), which leads to lower precision. Therefore, lower t value, i.e., 4-6, is recommended for the LSD. Equation 3 gives the statistical model for the ANOVA.

$$y_{ijk} = \mu + \tau_i + \rho_j + \gamma_k + \varepsilon_{ijk} \quad \text{..... Eq. 3}$$
$$i, j, k = 1, 2, \dots, t$$

Where

y_{ijk} = **the observation on ith treatment of jth row and kth column**

μ = **grand mean**

τ_i = **effect of ith treatment**

ρ_j = **effect of jth row**

γ_k = **effect of kth column**

ε_{ij} = **random error**

There are ways to overcome the mentioned limitations of LSD. DF can be increased by replicating basic LSD, which refers to the Replicated Latin Square Design **(RLSD).** It means that more than one square can be used for the experiments. Four kinds of settings are available in practical situations. The differences occurred in squares of columns and rows are mentioned as nested within squares. To increase replicates, we required to establish two squares in the field. Then it is identified that the row effect (MC) and the column effect (salinity) are not different in squares. Thus, there is no nesting effect. Therefore, we can use the design of RLSD with both rows and columns not nested. The same squares can be replicated within randomization again. Equation 4 presents the model.

$$y_{ijkl} = \mu + \tau_i + \rho_j + \gamma_k + \delta_l + \varepsilon_{ijk} \quad \text{..... Eq. 4}$$
$$i, j, k = 1, 2, \dots, t$$
$$l = 1, 2, \dots, s$$

Where

y_{ijkl} = **the observation on ith tratment of jth row, kth column and lth square**

μ = **grand mean**

τ_i = **effect of ith treatment**

ρ_j = **effect of jth row**
γ_k = **effect of kth column**
γ_l = **effect of lth square**
ε_{ij} = **random error**

The above design is not valid when conditions of only rows or columns or both rows and columns are different from square to square, where we hope to establish an experiment. Assume that the difference has occurred only in conditions of rows of square to square, then the design to be used is RLSD with rows only nested while columns are kept as basic LSD. The design of RLSD with columns only nested is used when the condition is the opposite direction. Then the model for "RLSD with rows only nested" appears as in equation 5.

$$y_{ijkl} = \mu + \tau_i + \rho_{j(l)} + \gamma_k + \delta_l + \varepsilon_{ijk} \quad \text{……Eq. 5}$$
$$l = 1, 2, \dots, s$$

Where

y_{ijkl} = **the observation on ith treatment of jth row, kth column and lth square**
μ = **grand mean**
τ_i = **effect of ith treatment**
$\rho_{j(l)}$ = **effect of jth row in lth square**
γ_k = **effect of kth column**
γ_l = **effect of lth square**
ε_{ij} = **random error**

If variations are observed in conditions of both rows and columns, the design we need to consider is RLSD with both rows and columns nested. Equation 6 provides the model.

$$y_{ijkl} = \mu + \tau_i + \rho_{j(l)} + \gamma_{k(l)} + \delta_l + \varepsilon_{ijk} \quad \text{……Eq. 6}$$
$$i, j(l), k(l) = 1, 2, \dots, t$$
$$l = 1, 2, \dots, s$$

Where

y_{ijkl} = **the observation on ith treatment of jth row, kth column and lth square**
μ = **grand mean**
τ_i = **effect of ith treatment**
$\rho_{j(l)}$ = **effect of jth row in lth square**
$\gamma_{k(l)}$ = **effect of kth column in lth square**
γ_l = **effect of lth square**
ε_{ij} = **random error**

The error DF of RLSD is higher than basic LSD, which leads to high precision with RLSD.

Mean Separation

Making conclusions is possible by considering the difference between means of the treatment; however, it is limited as it gives conclusions such that "all means are different" or "at least two means are different" by ANOVA. Such conclusions are not adequate to make recommendations on treatments. Thus, the mean separation procedure can be utilized according to our objectives, to make exact conclusions on treatments. To test the mean difference between two treatments **t-test** (equation 7) is used.

$$t = \frac{\text{mean difference between two treatments}}{\sqrt{2RMS/_2}}$$

……..Eq. 7

Where RMS is residual mean square,

$$H_0 : T_1 - T_2 = 0; \; H_A : T_1 - T_2 \neq 0$$

If we are dealing with more than two sample cases, and we are planning to compare particular combinations, and hence the number of comparisons is small, the t-test can be generalized to evaluate the mean difference. The test used here is the Least Square Difference **(LSD)** (equation 8) test. Equation 8 gives the LSD test.

$$LSD = t_{\frac{\alpha}{2},dferror} \times \sqrt{2RMS/_r} \quad \text{……Eq. 8}$$

where 'r' is the number of replicates.

If we need to make all possible comparisons in an experiment, Duncan's Multiple Range Test **(DMRT)** (Equation 9) can also be used in addition to the particular comparisons as above.

$$DT = q_{\alpha,p,dferror} \times \sqrt{RMS/_r} \quad \text{……. Eq. 9}$$

where $q_{\alpha,p,dferror}$ is from the DMRT table.

Thus, if our target is the evaluation of pair-wise comparisons for all possible treatment combinations, the test used is Tukey's Studentized Range test **(TSRT)**. Equation (10) is,

$$H = m_{\alpha,k,dferror} \times \sqrt{RMS/_r}$$

where $m_{\alpha,k,dferror}$ is from the TSRT table.

Suppose we aim to compare total means against the control, then the possible test is Dunnett's Test. Equation (11) is given by

$$D = d_{\alpha,k,dferror} \times \sqrt{2RMS/_r}$$

where $d_{\alpha,k,dferror}$ is from the Dunnett's table.

If we intend to compare more than two means, i.e., group comparisons, Orthogonal Contrast is used: for instance, testing of T_1 & T_2 vs. T_1, T_2, & T_3 and testing of control vs. rest.

Factorial Experiments

Suppose we assess the effect of fertilizer and MC for plant growth. As we discussed up to this point, we evaluated only the individual effect of factors, which are fertilizer and MC, separately. It is sufficient as long as the effects of fertilizer and MC for plant growth are checked independently, i.e., fertilizer and MC are not interacting with each other.

Imagine there are three levels of fertilizers (a_1, a_2, and a_3) and two levels of MC (b_1 and b_2). If the effect of one factor (fertilizer) is varying when one level of the other factor (MC) is changed, there is an interaction between these two factors. In such situations, not only the individual factors alone, but the interaction between the two factors also effect on yield or the particular response, which should also be investigated. The Factorial Experiment will resolve this situation.

The effects of treatment combination, i.e., interaction effects in addition to the individual effects (main effects) is another factor to be considered. For the above example, there are six factorial combinations, which refer to the treatments, i.e., a_1b_1, a_1b_2, a_2b_1, a_2b_2, a_3b_1, and a_3b_2. Besides, this is a 2*3 factorial experiment. When the experiment is planned, randomization should be achieved by allocating replicates of factorial combinations into the experimental units. The design may be CRD or RCBD, as usual. Equation 12 presents the statistical model for ANOVA (CRD).

$$y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk} \quad \text{…….. Eq. 12}$$
$$i = 1, 2, \dots, a$$
$$j = 1, 2, \dots, b$$
$$k = 1, 2, \dots, r$$

Where

y_{ijk} = **the kth observation on the ith level of N and jth level of MC**
μ = **grand mean**

α_i = **effect of ith level of N**

β_i = **effect of j level of MC**

$(\alpha\beta)_{ij}$ = ***interaction*** **effect of ith level of factor N and jth level of MC**

ε_{ij} = **random error**

Equation 13 gives the statistical model for ANOVA (RCBD).

$$y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \rho_k + \varepsilon_{ijk} \quad \text{. Eq. 13}$$

$$i = 1, 2, \dots, a$$
$$j = 1, 2, \dots, b$$
$$k = 1, 2, \dots, r$$

Where

y_{ijk} = **the observation on the ith level of N and jth level of MC in kth block**

μ = **grand mean**

α_i = **effect of ith level of N**

β_i = **effect of j level of MC**

$(\alpha\beta)_{ij}$ = ***interaction*** **effect of ith level of factor N ad jth level of MC**

ρ_k = **effect of kth block**

ε_{ij} = **random error**

Further, the factorial experiment can be extended to more than two-factor cases. If there are three factors, i.e., fertilizer, MC, and salinity, there are three main effects, three two-way interactions, and one three-way interaction. Here, priority should be given to the main effect, while less priority for lower-order interactions.

Only if we can use an equal size of small experimental units, above designs discussed up to this point are valid. If we are to evaluate the effects of irrigation, ploughing, shading, and so forth, the use of simple small plots are not practical; hence, lager plots are necessary. Plots should be able to evaluate all kinds of effects, which need different sizes of plots. As an example, our experiment is extended to evaluate the effect of fertilizer and irrigation on plant growth. Treatments of irrigation need larger plots, whereas fertilizer requires smaller plots. Even though there would be an interaction between these effects, the standard factorial experiment cannot be held practically. As replication is essential, blocks have to be constructed. Randomization is performed in a way that first randomizes the levels of irrigation, i.e., large plot factor (main plot) within each block separately, and then randomized levels of fertilizer, i.e., small plot factor (subplot) within each plot of irrigation, separately. This design is named as Split-plot Design. Even if this includes block, it is not the same as standard RCBD as an interaction between blocks and main plots is possible since conditions of main plots within each block are not equal. Further, it should be noted that the effect of the main plot factor has less precision than the effect of the sub-plot factor. Received information on the main plot factor is also lesser than the sub-plot factor.

We cannot be satisfied by analyzing the above two factors, as we want to investigate the effects of irrigation, fertilizer, as well as salinity on plant growth. Then fertilizer and salinity treatments which can be used same size of plots are combined to make sub-plots to randomize into the main plot of irrigation. A split-plot can be used by forming into a two-factor setting, irrespective of the number of factors required to be investigated.

Suppose we aim to investigate the effect of irrigation, pest control, and fertilizer, which needs three sizes of plots for three factors, i.e., large, medium, and small. Randomization is done, as we discussed in the Spilt-plot design. The difference here is three steps are involved in the procedure. The first randomization is done for irrigation, which needs large plots; thereafter, treatments of pest control, having medium plots, are randomized into each large plot separately. Finally, fertilizer treatments are randomized into each medium plot separately. This design is called the Spit-Split Plot Design.

Another circumstance has occurred to evaluate irrigation with two levels and ploughing with three levels in the same experiment together, where both factors need large plots. Then, Strip-Plot design is used. Accordingly, plots of two factors are positioned perpendicular to each other in a block. First, the block is divided into two to allocate two levels of irrigation, followed by each plot of irrigation is divided into three to allocate three levels of pest control. Here, the precision is low when testing the main effects, whereas higher precision is obtained when interaction effects are evaluated.

When we extend our experiment to measure the effects of more factors, and there are more and more factorial combinations, i.e., 2^n; two levels with n factors (2^3=8, 2^4=16, 2^5=32), 3^n; three levels with n factors (3^3=27, 3^4=81, 3^5=243). The issue created in this setting is also the inability to obtain homogeneity in one block since the number of treatments is large. Therefore, block size has to be reduced, as it is smaller than the number of treatments. Consequently, one or more effects become inestimable, as it cannot be distinguished from the block effect. This phenomenon is called *confounding*. When it can be confounded highest order interaction, severity can be diminished. In this settlement, all factorial combinations are preserved in the experiment itself. Assume, we must undertake more than five factors, i.e., 2^7=128, 2^8=256 treatment combinations. Capturing all treatment combinations into one single experiment is not possible. Therefore, only fractions of treatment combinations, i.e., ½ of factorial combinations are utilized to experiment. This design is named as the Fractional Factorial experiment.

Lattice Design

Another design introduced to overcome the issue of non-homogeneity of larger blocks is referred to as the Lattice Design, utilized with many number of treatments. When we require to examine more than 50 varieties, e.g., plant breeding experiments, the lattice design is suggested. Most of them are equivalent to BIBD or PBIBD. Correspondence between treatment and treatment combination of the factorial experiment is the base of the construction of lattice design. In each replicate, one effect is confounded to obtain the composition of the block. This is also called as Quasi-factorial design. The relationship between the number of treatments (t) and block size (k) is,

$$t = k^2 \text{ or } t = k^3 \text{ or } t = k(k+1)\cdot$$

Conclusion

The design should be planned before implementing an experiment, according to the research objectives, availability of resources, laboratory conditions, and field, and the number of factors to be tested. Among the straightforward designs of CRD, RCBD, and LSD, and complicated designs of IBD, Factorial experiments, Split-plot designs, and Lattice designs, the researcher should decide the most appropriate experimental design for the planned study.

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