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Design of experiments: a powerful tool for agriculture analysis

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ABSTRACT

This Article introduces the basic concepts, terminology, goals and procedures underlying the proper statistical design of experiment. This section describes in detail the process of choosing an experimental design. The choice of design in agricultural field experiments depends on the objectives of the experiment and the number of factors to be investigated.

Introduction

All fields of research have at least one feature in common i.e. the variability of experimental material. When this variability (Known as experimental error) is small relative to the observed treatment differences and measurements are not costly, an elaborate experimental design is not required. But when the results of experiments are affected by this inherent variability in addition to action of the treatments, we need to design our experiments.

In agricultural research, the key questions to be answered are generally expressed in terms of hypothesis that has to be verified or disapproved through experimentation. These hypotheses are usually suggested by past experiences, observations, and by theoretical considerations too. Once the hypothesis is framed, the next step is to design a procedure for its verification. These procedures usually consist of four phases.

- Selecting the appropriate material to test.
- Specifying the characters to measure.
- Selecting the procedure to measure those characters.
- Specifying the procedure to determine whether the measurements made support the hypothesis framed.

These procedures used to test the hypothesis constitute much of what is generally termed design of experiment which has three essential components.

- Estimation of error
- Control of error, and
- Proper interpretation of results

Designing of Experiment

Designing is a process or set of rules by which treatments are assigned to experimental units to verify or discard the hypothesis made by the experimenter. The purpose of designing of experiment is to increase the precision of the experiment i.e. to get more and more information per observation. In order to increases the precision, the experimental error needs to be reduces.

Basic principles of experimental designs

The techniques used to reduce experimental error to increase the precession of experiment form the basic principles of experimental designs. viz; replication, randomization and local control.

Replication

The repeated application of the treatments under investigation is known as replication. The main advantages of replication are:

- To provide an estimate of experimental error.
- To reduce the experimental error.

Randomization

It is process of allocating treatments to various experimental units randomly so that each treatment gets an equal opportunity to show its worth. The purpose of randomization is to assure that sources of variation due to extraneous factors operate randomly so that the average effect on any group of units is zero. Thus randomization technique ensures unbiasness of the estimate of experimental error. It is a prerequisite for the validity of the statistical tests of significance. e.g. T-test, F-test etc.

Local Control

In field experiments, grouping of homogenous experimental units into blocks is known as local control of errors. The commonly used techniques for controlling experimental error in agricultural research are:

- Selection of experimental material
- Proper plot technique (Shape and size of plots)
- Data analysis (competition effect)

Statistical control of error

In case blocking alone may not be able to achieve adequate control of experimental error, proper choice of data analysis (analysis of co-variance) can help to a great extent. In this type of data analysis, the uncontrolled source of variance can be measured by taking additional observations on some character called ancillary variable (x) e.g. plant population, straw yield which is highly correlated with the character under study (y). The analysis of covariance controls the experimental error by taking into consideration the dependence of y on x. This form of error control is known as statistical control of the error.

Analysis of variance (ANOVA)

ANOVA is a technique by which the total variation in the responses observed in an experiment may be split into several

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physically assignable components. This total variation is expressed as sum of these non-negative components. Each of these components is a measure of variation due to some specific independent source or factor or cause. Structure of these component parts is determined by the design of experiments. ANOVA consists in estimation of the amount of variation due to each of these independent factors separately and then comparing these estimates with the estimate due to experimental error. Thus ANOVA is a powerful tool for test of significance of treatments (F-test).

By ANOVA, when the treatments show significant effect then we would be interested to find out which pair of treatments differ significantly. Two most commonly used test procedures for pair wise comparisons in agricultural experiments are the least significant difference (LSD) test and Duncan's multiple range (DMRT) test.

Assumptions of ANOVA models

For validity of F-test in ANOVA, the following assumptions are made:

- Observations are independent.
- The parent population from which observations are taken is normal i.e. observations should be normally distributed.
- Various treatment and extraneous effects are additive in nature.
- The experimental error is independently and normally distributed with mean zero and a constant variance.
- All groups should have homogeneous variances.
- Violation of these assumptions may lead to misleading of results.

ANOVA Model

$$Y_{jk} = \mu + T_i + e_{ijk}$$

where Yj_k = the k^{th} value of response variable for i^{th} treatment

μ = is overall population mean of response variable

T_i = the difference between the population mean of the i^{th} treatment and the overall mean, μ. This is referred to as the effect of treatment *i*.

e_{ijk} = the difference between the observed value of the k^{th} observation in the i^{th} group and the mean of i^{th} group. This is called the error term.

The above model is called linear additive model. Depending on the design the ANOVA model will take different forms. The predicted value in ANOVA is the group mean. A residual is the difference between the observed value of the response and predicted value of the response variable.

Steps for ANOVA Summary

- Null Hypothesis: All treatment means are equal
- Alternative Hypothesis: At least one treatment is different.
- Produce descriptive statistics
- Verify assumptions
- Examine the *p*-values on the ANOVA table. If the *p*-value is less than *alpha*, rejects null hypothesis.

Fixed, Random and Mixed affect Models

Usually the treatments in an experiment are fixed by the experimenter as per his convenience. They are not selected randomly from all possible treatments. When the treatments are fixed (like four varieties of crop) the ANOVA model is called fixed effect model.

In random effect model, instead of fixed varieties we take a random sample of varieties from set of all varieties and see their effects. Such ANOVA models are called random effect models. The model that contains both fixed and random effects is called mixed model.

Types of Experimental Designs

Single Factor Experiments

Experiments in which only a single factor varies while all other factors are kept constant are called single-factor experiments. In such experiments, the treatments consist solely of the different levels of a single variable factor. All other factors are applied uniformly to all plots at a single prescribed level. Examples of single factor experiments are:

- Varietals trials where several varieties of a crop are tested.
- Fertilizer trials where several rates of a single fertilizer are tested.
- Insecticide trials where several insecticides are tested.

There are two groups of experimental designs that are applicable to a single factor experiment. One group consists of complete block designs, which is suited for experiments with a small number of treatments and is characterized by blocks, each of which contains at least one complete set of treatments. CRD, RBD and LSD are examples of complete block designs

The other group is the family of incomplete block designs which are suited for experiments with a large number of treatments and are characterized by blocks, each of which contains only a fraction of the treatments to be tested. Lattice, group balanced block design are examples of incomplete block designs.

Factorial Experiments

In practice, the response of biological organism to the factor of interest is expected to differ under different levels of other factors. For example the yield of wheat varieties may differ under different rates of fertilizers application, spacing and irrigation schedules Thus when the effect of several factors are investigated simultaneously in a single experiment, such experiment is known as factorial experiment. In factorial experiments, the treatments consist of all possible combinations of the selected levels of two or more factors. Factorial experiments are advantageous over single factor experiments in following ways.

In factorial experiments we can evaluate combined effect of two or more factors when they are used simultaneously, i.e. we can study the individual effects of each factors as well as their interactions in one experiment.

The factorial approach results in a considerable saving of experimental resources.

Simple and Main Effects

The simple effect of factors is the difference between its responses for a fixed level of other factors. The mean of the simple effects of a factor is called the main effect of the factor.

Interaction Effects

The effect of one factor changes as the level of other factor changes i.e. the factors are not independent, the dependence of factors in their responses is known as interaction the interaction is measured as the mean of the differences between simple effects of the factors.

Split Plot Design

The Split plot design is specifically suited for a two factor experiment that has more treatments than can be accommodated by a complete block design. In this design, one of the factors is assigned to the main plot (main plot factor). The main plot is divided into subplots to which the second factor, called the subplot factor, is assigned. With a split plot design, the precision for the measurement of the effects of main-plot factor is sacrificed to improve that of the subplot treatment. Measurement of the main effect of the subplot factor and its interaction with the main plot factor is more precise than that obtainable with RCBD. The following considerations are required for assigning main plot and subplot factors-

- Degree of precision.
- Relative size of main effects.
- Management practices.

Strip Plot Design

Strip plot design is specifically suited for a two-factor experiment in which the desired precision for measuring the interaction effect between two factors is higher than that for measuring the main effect of either one of the two factors. This is accomplished with the use of three plot sizes-

- Vertical-strip plot for first factor-a vertical factor.
- Horizontal-strip plot for second factor- the horizontal factor.
- Intersection plot for the interaction between the two factors.

The vertical-strip plot and the horizontal-strip plot are always perpendicular to each other. In strip-plot design, the degree of precision associated with the main effects of both factors is sacrificed in order to improve the precision of the interaction effect.

Two-Factor Experiments

When response to factor of interest is expected to differ under different levels of the other factors, we consider the factorial experimental design to handle simultaneously two or more variable factors. Two factor factorial design. Split--plot design and Strip- plot design come under this category of experiments.

Three or More - Factor Experiments

A two factor experiment can be expanded to include a third factor, a third factor experiment to include a fourth factor and so on. With the increase in factors we come across with two important consequences.

There is a rapid increase in the treatments to be tested, and

There is an increase in the number and type of interaction effects, e.g. a four factor experiment has 10 interaction effects.

Choice of Experimental Designs

It depends on the objective of the experiment and number and nature of the treatments under study. Another important consideration for the choice of any experimental design is the availability of resources.

Some considerations under which the different designs are appropriate are as under:

- CRD is appropriate when the experimental material is limited and homogeneous, such as the soil in the pot experiments.
- RBD is appropriate when the fertility gradient of the field is in one direction.
- LSD can replace RBD when the fertility gradient is in two directions instead of one.
- When there are several factors with different levels to be studied simultaneously with the same precision, a factorial scheme may be adopted.
- When the factors are such that some of them require large plots, like irrigation, sowing dates etc. and may be studied with different precision, split plot design may be used.
- An incomplete block design may be used when treatments are more than plots in a block and small block size can be maintained even if the number of treatments is very large.

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