

ADDIS ABABA UNIVERSITY
OFFICE OF GRADUATE STUDIES
COLLEGE OF NATURAL SCIENCES
DEPARTMENT OF STATISTICS



CONSTRUCTION OF PARTIAL TRIALLEL CROSS DESIGNS
AND THEIR ANALYSIS

A thesis submitted to the Department of Statistics in partial fulfillment of the requirements for the Degree of Master of Science in Statistics

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ABSTRACT

Construction of Partial triallel cross designs and their analysis.

Addisu Jember.

Two new methods of construction of mating designs for partial triallel cross for $p > 3$ lines are proposed. In the first method we have used a Latin square of order p , where p a prime or power of a prime is, then bordered rows and columns of the Latin square by the directrix of the Latin square. Each cell together with border elements gives a design for Partial triallel cross after eliminating the elements of directrix. In the second method, first we obtain initial blocks and then develop cyclically mod (p) these initial blocks, we get block designs for partial triallel cross. These designs are found to be optimal in the sense of Das and Gupta (1997). Diallel mating designs are useful in estimating the additive and dominance genetic variances only under the absence of epistatic effects. If epistatic effects are present the variance component due to interaction between additive and additive (σ_{AA}^2), additive and dominance (σ_{AD}^2) and dominance and dominance (σ_{DD}^2) can be estimated by raising only Triallel Crosses. However, the number of all possible three way crosses would be unmanageable even for a moderate number of lines p . To handle this situation, we are giving here two new methods of constructing mating design for partial triallel cross. A method of analysis of these mating designs is also shown by numerical example.

Keywords: *directrix of Latin squares, mating designs, triallel cross, partial triallel cross, general effects of first kind, general effects of second kind.*

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Acronyms

ANOVA	Analysis of variance
BIBD	Balanced incomplete block design
CRD	completely randomized designs
CTC	Complete triallel Cross
IBD	Incomplete block designs
LS	Latin Square
MD	Mating designs
MOLS	mutually orthogonal Latin square
PTC	Partial triallel Cross
RCBD	Randomized complete block design

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1 INTRODUCTION

1.1 BACKGROUND OF THE STUDY

The subject of statistics deals with random phenomena and uncertainty. Its foundation is based on mathematics and probability, and that part of the subject has acquired the name of “mathematical statistics”. As an applied science, however, it has grown into a body of knowledge which deals with measuring uncertainty surrounding the data generating in a given discipline like Biology, Agriculture and Engineering etc. In fact, it is at the interface of statistical methods and the discipline of application that statistics really exists in the form of a science of the meaning and use of data. This makes statistical science as inter and cross disciplinary research activity.

The foundation of statistical approach to experimentation was laid by R.A.Fisher in the early 1930s. The subject evolved in agriculture but is now applicable to almost all sciences, to genetics, to engineering and to other areas or fields.

Research in genetics is based almost exclusively on experimental investigation, different facets of in genetics using different techniques. The interpretation of and conclusions drawn from these experiment is often based on statistical analyses of some sort. This implies in turn that the design of such experiments must be based, implicitly or explicitly, on statistical considerations in order to obtain data that are relevant to experimenter’s questions in the sense that if analyze appropriately they will provide answers to these questions (Hinkelmann, 1975).

One of the main aims of genetical investigation involving crops is to develop improved crop varieties using genetic architecture. When there are a numbers of strains of a crop of one type of investigation consisting of crossing between such strains to evolve new varieties. Through these types of investigation the combining capabilities of the strains can be studied. Normally highly inbred parental lines are considered for such investigation.

Agriculture is the deliberate planting and harvesting of plants and herding animals. This human invention has, and continues to, impact on society and the environment. Plant breeding is a branch of agriculture that focuses on manipulating plant heredity to develop new and improved variety for use by society. People in society are aware and appreciative of the enormous diversity in plants and plant products. They have preferences for certain varieties of flowers and food crops. They are aware that whereas some of this variation is natural, humans with special expertise (plant breeders) create some of it. Generally, also, there is a perception that such creations derive from crossing different plants

It is obviously impossible to cover and summarize all aspects of design for genetical experiments in one single paper. A selection therefore has to be made based on personal preference and on the theme of study. A natural choice then is to discuss experiments in quantitatively genetics or, more precisely, certain types of such experiments (Hinkelmann, 1975).

1.1.1. Purpose of Genetical Experiments

Within the framework of this paper, following Hinkelmann (1975), we shall classify experiments in quantitative genetics into two groups: comparative experiments and exploratory experiments.

1.1.1.1. Comparative Experiments

Comparative experiments in quantitative genetics have the same purpose that comparative experiments have in general: to compare treatments and draw conclusions from the comparisons

for practical applications or further theoretical investigations. The “treatments” in a genetical experiment are genetic entities, such as individuals (plant, animal), lines, clones, strains, populations. The main objective is to compare these entities in some form on their genetic merits and predict their performance in or ability to transmit this performance to future generations.

The genetic components of a comparative model are considered to be fixed effects, the p treatments could have been specifically chosen by the experimenter. The goal here is to test hypotheses about the treatment means and estimate the model parameters. Conclusions reached here only apply to the treatments considered and cannot be extended to other treatments that were not in the study.

1.1.1.2. Exploratory Experiments

The main difference between a comparative experiment and an exploratory experiment can be best explained in terms of underlying linear models: the genetic components of a comparative model are considered to be fixed effects, whereas those for an exploratory experiment are considered to be random effects, the p treatments could be a random sample from a larger population of treatments. Conclusions here extend to all the treatments in the population; we are not interested in the particular ones in the model. In other words: the inferences to be drawn from these two types of experiments are quite different and aimed at answering different questions. An explanatory experiment is designed to answer questions such as: what is the genetic structure of a population; i.e., what types of gene action are present in the population;

The objectives of comparative and exploratory experiments are that: how can these objectives be achieved? This brings us to the actual design aspects for genetical experiments, which means finding ways to generate data that are appropriate for answering relevant questions of a comparative or exploratory nature.

Here are a few examples taken from *Peterson: Design and Analysis of Experiments*:

1. *Fixed*: A scientist develops three new fungicides. His interest is in these fungicides only.
Random: A scientist is interested in the way a fungicide works. He selects, at random, three fungicides from a group of similar fungicides to study the action.
2. *Fixed*: Measure the rate of production of five particular machines.
Random: Choose five machines to represent machines as a class.
3. *Fixed*: Conduct an experiment to obtain information about four specific soil types.
Random: Select, at random, four soil types to represent all soil types.

1.1.2 Trialallel or Three - Way Crosses

Trialallel cross system or three –way crosses is an extension of Diallel crossing. The analysis of crosses can provide additional information regarding epistatic components of variances and the effects of order in which the parental lines are involved in crosses.

Trialallel crosses are a type of mating design (a systematic method of developing offspring of parents or lines which are subjected to analysis in order to draw inferences about parents) used to study the genetic properties of a set inbred (pure) line in plant breeding¹. Suppose there are p inbred lines, the number of differences crosses for a complete trialallel experiments is $n_c = 3\binom{p}{3} = p(p-1)(p-2)/2$ of the type $(i \times j)k$, $i < j < k$, $i, j, k = 0, 1, 2, \dots, p-1$ (Das and Gupta, 1997). To understand this, the three –way crosses in the above the two distinct lines i and j , and let $(i \times j)$ be their F1 hybrid and k be another or unrelated line, then a cross between $(i \times j)$ and k is a three way hybrid or crosses, denoted by $(i \times j)k$. Here line i and j are grandparents or half parents, and k is the parent of the three-way cross. For p lines in the experiment, then in the

¹ *Plant breeding is as an art, a science, and technology of improving the genetic makeup of plants in relation to their economic use for the mankind.*

absence of reciprocal effects and maternal-paternal interactions (i.e. $(i \times j)k$, $(j \times i)k$, $k(i \times j)$ and $k(j \times i)$) are the same and represent one. Most of the common commercial hybrids in corn are either three-way hybrids or double cross-hybrids. The experience gained in corn and maize breeding has helped the cause of diallel in other plants and animal breeding such as swine breeding, silk worm breeding and chicken breeding. Diallel has helped the breeders to improve the quantitative traits of economic and nutritional importance in crops and animals. It has been established that the three-way hybrids are more stable than the pure lines and single cross hybrids and exhibit individual as well as population buffering mechanisms because of the broad genetic base.

Rawlings and Cockerham (1962) were the first to introduce mating design for diallel crosses, that is useful for the estimation of σ^2_A and σ^2_D and first order epistatic component σ^2_{AA} , σ^2_{AD} , σ^2_{DD} . Where genotypic variance is further chiefly partitioned into Additive variance (σ^2_A), Dominance variance (σ^2_D).

Additive variance is the most important component of genotypic variance as it determines the observable genetic properties. It is also the chief cause of resemblance between relatives and known as variance of breeding values under random mating conditions (Falconer and Mackay, 1996). Additive variance is the fixable component of the genetic variance and is generally the largest portion of the total genetic variance. Additive variance is also most easy to estimate in comparison to other components. Non-additive variance consists of dominance and interaction variance. Dominance deviation arises due to dominance action among alleles and also known as deviation due to within locus interaction, while the deviation due to the interaction among genes at different loci, i.e. inter-locus interaction is known as epistatic deviation. Fisher (1918) was first to construct a gene model to study the inheritance of quantitative traits with dominance at

single locus and further stated about the deviation from simple additive effects among loci in case of more than one locus and gave it a term called “epistacy”. In statistical terms the epistatic² deviation is defined as the deviation of multilocus genotypic values from the additive combination of their single-locus components (Lynch and Walsh, 1998).

The mating designs for triallel crosses introduced by Rawlings and Cockerham (1962a) are generally conducted in completely randomized designs (CRD), or in randomized complete block (RCB)³ designs as environmental designs involving n_c crosses. As p increases, the number of triallel crosses, n_c increases manifold. As a consequence more resources are required for conducting experiments. Furthermore, accommodation of large number of crosses in a RCB design usually results into large intra block variances. A sample of the complete triallel crosses, i.e., partial triallel crosses (P.T.C.) introduced by Hinkelmann (1965) and subsequently studied by Arora and Aggarwal [(1984), (1989)], Ceranka *et al.* (1990) and Ponnuswamy and Srinivasan (1991) and others may be used in such situations.

1.2 STATEMENT OF THE PROBLEM

Triallel crosses as mating designs are used to study the genetic properties of inbred lines in plant and animal breeding experiments, and triallel analysis had clearly shown its advantage over diallel analysis by giving additional information on magnitude of all types of epistatic components and order in which parents to be crossed in three way crosses for obtaining superior transgressive segregants. Triallel cross experiments are generally conducted using a completely randomized design (CRD), or a randomized complete block (RCB) design as environmental design

² *The suppression of a gene by the effect of an unrelated gene*

³ *Randomized Block Design (RBD) is an improvement of Completely Randomized Design (CRD). It provides error control measures for elimination of block variations. i.e., in RBD, the sources of variation are eliminated in only one direction namely blocks wise.*

involving in total number of crosses. Suppose there are p inbred lines and it is desired to perform a trialallel crossing experiment involving $p(p - 1)(p - 2)/2$ crosses. The number of crosses in such a mating design increases rapidly with increase in the number of lines, p ; for $p = 5$, there are only 30 crosses while, with $p = 10$ lines, the number of crosses is 360. If there is heterogeneity in the experimental material in one direction, adoption of a randomized complete block design with crosses as treatments would result in a large error variance, even with a moderate number of inbred lines.

To handle this situation, Hinkelmann (1965) proposed Partial trialallel cross (PTC). In this paper we are presenting two new methods of construction of partial trialallel crosses in incomplete block design.

1.3 OBJECTIVE OF THE STUDY

1.3.1 General objective

The main objective of this study is to construct some new methods for partial trialallel cross designs in the existing literature.

1.3.2 Specific objective

- ❖ To develop environmental designs for the crosses obtained through mating designs.
- ❖ To analyze data using the proposed PTC designs, to estimate the general effects of the first and second kind for PTC.
- ❖ The optimality of the proposed PTC design.

1.4 APPLICATION OF THE RESULTS

- ❖ A systematic method of developing or raising the offspring of parents or lines, which are subjected to analysis in order to draw valid inferences about parents, is called a Mating Design (MD). A Mating Design represents only a part of a genetic experiment. A judicious choice of a Mating Design is essential to attain the Breeder's goal.
- ❖ The result of this study provides information to researchers and further investigation on triallel crosses.
- ❖ The results help as a basis for further study in this area.

1.5 LIMITATIONS OF THE STUDY

The study has the following limitations.

- ❖ The mating designs for triallel crosses are not available for every value of inbred line p .
- ❖ In the analysis, the data (illustrative) had been taken from abroad, because in Ethiopia it seems that the experiments in triallel cross design are not conducted.

1.6 ORGANIZATION OF THE DOCUMENT

This chapter provided an overview of the research undertaken in this thesis. The remainder of the document is organized as follows. Chapter 2 provided some concepts on mating and environmental design and the importance why we used, also there is some characteristics of triallel cross designs and also it summarizes research in the area of environmental and mating designs. Chapter 3 describes two new methods of construction for partial triallel cross designs in blocked and unblocked cases. In chapter 4 we discuss about the optimality and analysis of proposed PTC designs; in chapter 5 we give illustrative example that summarizes the above

analysis of PTC, discussions over there and concludes the discussion by highlighting the salient contribution of the research making recommendation for future research.

2 LITERATURE REVIEW

2.1 Why use experimental designs?

Experimental designs are used so that the treatments may be assigned in an organized manner to allow valid statistical analysis to be carried out on the resulting data. Different designs identify different known or suspected sources of variation so that the treatments effects can be evaluated free of extraneous environmental or other influences. Statistical theory also requires that certain conditions be met during the execution of the experiment to make valid probability statements to about the experiment (Sharma, 2000).

A major objective of plant and animal breeding programmes is to improve the genetic potential of plants and animals. The breeding experiment comprise two types of design namely, mating and environmental design.

2.1.1 Mating design

Mating design is a procedure of producing the progenies. This, however, is only one component of the actual experiment since the offspring of these crosses have to be reared subjecting these progenies to environmental conditions in a systematic manner by using an environmental design.

The “treatments” in genetical experiments are genetic entities or, more precisely, crosses among genetic entities. Inferences about these entities are made by evaluating the offspring from these crosses (matings). More generally, offspring of various degrees are used in genetical experiments. The offspring are obtained by following a certain propagation scheme, which shall

be referred to as mating design. “Simple” mating designs usually allow one to investigate a limited number of questions or hypotheses, relying furthermore on a number of assumptions. There is, therefore, a need for more elaborate and specific mating designs. The choice of the mating is sometimes severely limited by the genetic material one is working with and by practical considerations. Whereas complicated mating designs can be handled for many plants and small animals, they become impractical or even impossible for large animals. The limitations and other difficulties, usually beyond the control of the experimenter, may not always enable us to use the mating design that fully achieves the objectives of the experiments: to estimate the desired comparisons or to estimate genetic variance components such that they are unconfounded with other variance components (Hinkelmann, 1975). Artificial crossing or mating is a common activity in plant breeding programs for generating various levels of relatedness among the progenies that are produced. Mating in breeding has two primary purposes: To generate information for the breeder to understand the genetic control or behavior of the trait of interest. To generate a base population to initiate a breeding program. The breeder influences the outcome of a mating by the choice of parents, the control over the frequency with which each parent is involved in mating, and the number of offspring per mating, among other ways.

2.1.2 The environmental designs

Just as a factorial experiment has to be embedded in an experimental design, a mating design represents only a part of a genetical experiment. The genetic crosses, as specified by mating design, can be observed only under certain environmental conditions. That is to say one has to superimpose on the mating design an environmental design. To assure that proper inferences can be made the environmental design must have certain properties and must be feasible from an experimental points of view. It must assure that genetic parameters can be estimated free from

environmental effects. Within the framework of the combined underlying linear model and unbiased estimation, this means that the expected value of the estimator for genetic parameters must be free from parameters representing the environmental design. In many cases the environmental designs are familiar experimental designs, such as completely randomized designs, complete and incomplete block designs, split plot designs, etc. In some cases the mating designs lead quite naturally to certain known environmental designs. In other cases specific environmental design form an integral part and can hardly be separated. In other words, the mating design is drawn up with the specific environmental conditions in mind (Hinkelmann, 1975).

2.1.3 Three- factor mating design

The concept of Diallel or two factor mating design can be extended easily to three factor mating design. The number of all possible three way crosses would be unmanageable even for moderate number of line p .

The triallel mating can also be defined as set of all possible three way hybrids based on a given set of lines constituting the triallel cross. If p is the number lines in the triallel there would be $n_c = p(p - 1)(p - 2)/2$ distinct three way crosses. Each line will occur in $r_H = (p - 1)(p - 2)$ three way crosses as a grandparent and in $r_F = (p - 1)(p - 2)/2$ three way crosses as a parent. Similarly each pair of lines occur in $r_d = (n - 2)$ three way crosses both of them as grandparents and in $r_s = (n - 2)$ three way crosses with one of them as grandparent and others as a parent.

2.1.4 Review of past research

Hinkelmann (1965) introduced for the first time the concept of Partial Triallel Crosses (PTC) and provided a method of construction of PTC based on Generalized Partially Balanced Incomplete Block Designs (GPBIBD). Ponnuswamy *et.al.* (1974) gave combining ability type analysis of triallel cross in maize. Arora and Aggarwal (1984,1989) discussed about confounded triallel experiments in block design. Ceranka *et.al* (1990) estimated the parameters for triallel for triallel crosses in block design. Ponnuswamy and Srinivasan (1991) constructed partial triallel crosses in doubly balanced incomplete block designs. Ponnuswamy and Subbarayan (1991) gave the analysis of Partial triallel crosses. Panda *et al.* (2001) investigated the robustness of optimal block designs for triallel cross experiments against exchange of a cross. Dharmalingam (2002) also constructed PTC using Trojan squares. Actually Trojan squares are mutually orthogonal Latin squares (MOLS). Sharma and Fanta (2010) constructed optimal partial triallel cross in the sense of Das and Gupta (1997). Sharma.*et.al* (2011) developed partial triallel cross (PTC) by taking any two mutually orthogonal Latin squares and superimpose any one over the other and border the columns of composite Latin squares. Remove first row in composite Latin square, and then attach border elements in each cell corresponding to their column and also discussed their analysis.

Optimality aspects of partial triallel cross were discussed by Das and Gupta (1997). Das and Gupta (1997) have constructed block designs for triallel crosses that start with p lines rather than n_c crosses in the experiment. This approach yields designs which are universally optimal in $D(p, b, k)$, the class of connected block designs for triallel crosses in p lines with b blocks each of size k such that the total number of experimental units are $n < n_c$, where here $n_c=3pC_3$. Das and Gupta (1997) used nested balanced block designs with sub-block size 3 in the construction of optimal

block designs for trialallel crosses. These optimal designs perform well under ideal conditions. However, disturbances may occur due to some reasons at different phases from planning to execution of an experiment. Exchange of a cross in one such aberration or discrepancy that is said to have occurred, if one of the crosses gets substituted by any of the remaining crosses during experimentation due to the following reasons

- (i) In environmental design, a different cross has been applied in a block instead of what should have been, due to human mistake or due to erroneous tagging and labeling.
- (ii) In environmental design, suppose one cross did not germinate due to incompatibility of the cross or for any other reason and was substituted by an already existing surplus cross.
- (iii) If resource is not available for a particular cross, then exchange of that cross with any other surplus cross is necessitated.

Batra *et al.* (1997) studied the robustness of block designs against interchange of a pair of treatments. Recently, Panda *et al.* (2001) investigated the robustness of optimal block designs for trialallel cross experiments against exchange of a cross. Here, an attempt has been made to investigate the robustness of optimal block designs for trialallel crosses against interchange of a pair of crosses.

In the present study we are attempting to give some new methods of construction of partial trialallel cross designs for blocked and unblocked situations. Our blocked designs are optimal in the sense of Das and Gupta (1997). Following we have also presented an analysis of unblocked partial trialallel crosses in randomized block design.

3 METHODS OF CONSTRUCTION

3.1 CONSTRUCTION OF PARTIAL CROSS DESIGNS

3.1.1 Definition of a PTC

Suppose we have p lines, a three way cross is then represented by a triplet $(i \times j) \times k$, where $i \neq j \neq k = 0, 1, 2, \dots, p - 1$, as explained above $(i \times j)$ stands for an offspring of the single cross $(i \times j)$. We shall call i and j half parents and k full parents and we always assume that no two of the lines in the three way cross are the same, i.e. we assume $i \neq j \neq k$. Also we do not distinguish between reciprocal crosses (Hinkelmann, 1965).

Definition 3.1.1.1: Following Hinkelmann (1965), A set of mating or crosses

is said to be a PTC if it satisfies the following conditions:

- (i) Each line occurs exactly r_H times as half parents and r_F times as full parents.
- (ii) Each cross $(i \times j) \times k$ occurs either once or not at all.

Condition (ii) does not exclude the simultaneous occurrence of $(i \times j) \times k$, $(i \times k) \times j$ and $(j \times k) \times i$.

The total number of crosses is pr_F . Since each line is equally often represented as half parents it follows immediately that $r_H = 2r_F$. Let $r_F = r$, thus which follows $r_H = 2r$. If $r = (p - 1)(p - 2)/2$ we obtain the complete triallel crosses. The degree of fractionation for a PTC

is then given by $2r/(p-1)(p-2)$, where r is a replication of a line, r_H, r_F is a replication of half parents and full parents respectively.

Definition 3.1.1.2: A Latin square is an arrangement of p treatments (lines) in p rows and p columns in such a way that each symbol occurs once in a row and once in a column.

3.2 METHODS OF CONSTRUCTION

3.2.1 METHOD ONE

The first method developed in this paper is based on directrix of Latin squares to construct Partial Triallel Cross designs which is different from any other methods.

Start with a Latin square of order p , where p is a prime or a power of a prime and border the columns and the rows of square with elements of the transversal or directrix (where transversal or directrix of a Latin square of order p in a set of p cells each row, column and symbol is represented exactly once in the set; see Hedayal and Seiden, 1974). Each cell together with the border elements give a triplet of the type $(i \times j) \times k$, where $i \neq j \neq k = 0, 1, \dots, p-1$ in the upper and lower triangular and triplet of the type $(i \times j) \times k$ where $i = j = k = 0, 1, \dots, p-1$ in the transversal.

Now deleting the triplet in transversal we get a mating design for Partial triallel cross experiment which satisfy the condition of partial triallel cross given in definition 3.1.1. The method is illustrative below with example.

Example 1 Consider the following Latin square of order 5 transversal bracketed.

[0]	1	2	3	4
1	[2]	3	4	0
2	3	[4]	0	1
3	4	0	[1]	2
4	0	1	2	[3]

Now we border the columns and rows of the above Latin square by the bracketed elements of the transversal. We get the following arrangements.

Table 3.2.1 the transversal of Ls in the row and column

	0	2	4	1	3
0	0	1	2	3	4
2	1	2	3	4	0
4	2	3	4	0	1
1	3	4	0	1	2
3	4	0	1	2	3

Now we attach border elements with each cell. Then we get the following design

$[(0 \times 0) \times 0]$	$(0 \times 1) \times 2$	$(0 \times 2) \times 4$	$(0 \times 3) \times 1$	$(0 \times 4) \times 3$
$(2 \times 1) \times 0$	$[(2 \times 2) \times 2]$	$(2 \times 3) \times 4$	$(2 \times 4) \times 1$	$(2 \times 0) \times 3$

$$\begin{array}{ccccc}
(4 \times 2) \times 0 & (4 \times 3) \times 2 & [(4 \times 4) \times 4] & (4 \times 0) \times 1 & (4 \times 1) \times 3 \\
(1 \times 3) \times 0 & (1 \times 4) \times 2 & (1 \times 0) \times 4 & [(1 \times 1) \times 1] & (1 \times 2) \times 3 \\
(3 \times 4) \times 0 & (3 \times 0) \times 2 & (3 \times 1) \times 4 & (3 \times 2) \times 1 & [(3 \times 3) \times 3]
\end{array}$$

Now deleting the bracket cells, we get mating design for partial triallel cross in which

- (i) Each line occurs $r_H (= 8)$ lines as half parent and $r_F (= 4)$ lines as full parent.
- (ii) Each triple cross $(i \times j) \times k$ either once or not at all in d_1 (mating design for PTC).

$$\begin{array}{ccccc}
(2 \times 1) \times 0 & (0 \times 1) \times 2 & (0 \times 2) \times 4 & (0 \times 3) \times 1 & (0 \times 4) \times 3 \\
(4 \times 2) \times 0 & (4 \times 3) \times 2 & (2 \times 3) \times 4 & (2 \times 4) \times 1 & (2 \times 0) \times 3 \\
(1 \times 3) \times 0 & (1 \times 4) \times 2 & (1 \times 0) \times 4 & (4 \times 0) \times 1 & (4 \times 1) \times 3 \\
(3 \times 4) \times 0 & (3 \times 0) \times 2 & (3 \times 1) \times 4 & (3 \times 2) \times 1 & (1 \times 2) \times 3
\end{array}$$

We denote this design by d_1 .

Note: To construct PTC the Latin square directrix must be different.

For example for $p = 6$ the directrix of the Latin Square is repeated so we cannot construct PTC in case.

$$\begin{array}{cccccc}
[1] & 2 & 3 & 4 & 5 & 6 \\
2 & [3] & 4 & 5 & 6 & 1 \\
3 & 4 & [5] & 6 & 1 & 2 \\
4 & 5 & 6 & [1] & 2 & 3
\end{array}$$

5 6 1 2 [3] 4

6 1 2 3 4 [5]

3.2.2 METHOD TWO

From the above mating design, we may get another blocked mating design. Consider any column of the above mating designs as initial blocks and then developing these blocks cyclically in row wise mod(5), we get blocked mating design for PTC. We denote this design $D(p, b, k)$ with parameter $v = p(p - 1)$, $b = p - 1$ and $k = p$ by d_2 .

Example 2 Consider first columns as 4 initial blocks and then developing cyclically (mod5), we get the following blocked design for PTC, which satisfy the condition of PTC.

B_1 $(2 \times 1) \times 0$ $(3 \times 2) \times 1$ $(4 \times 3) \times 2$ $(0 \times 4) \times 3$ $(1 \times 0) \times 4$

B_2 $(4 \times 2) \times 0$ $(0 \times 3) \times 1$ $(1 \times 4) \times 2$ $(2 \times 0) \times 3$ $(3 \times 1) \times 4$

B_3 $(1 \times 3) \times 0$ $(2 \times 4) \times 1$ $(3 \times 0) \times 2$ $(4 \times 1) \times 3$ $(0 \times 2) \times 4$

B_4 $(3 \times 4) \times 0$ $(4 \times 0) \times 1$ $(0 \times 1) \times 2$ $(1 \times 2) \times 3$ $(2 \times 3) \times 4$

4 Optimality and analysis of the designs

Optimal designs are a class of experimental designs that are optimal with respect to some statistical criterion. In the design of experiments for estimating statistical models, optimal designs allow parameters to be estimated without bias and with minimum variance. A non-optimal design requires a greater number of experimental runs to estimate the parameters with the same precision as an optimal design. In practical terms, optimal experiments can reduce the costs of experimentation (Hinkelmann and Kempthorne, 2005). In this section (4.1) we first show that design d_2 , constructed in method two is optimal in the sense of Das and Gupta (1997).

4.1 BLOCKED MODELS

We consider triallel cross experiments involving p inbred lines, giving rise to a total of $n_c = p(p-1)(p-2)/3$ possible distinct crosses. Let r_{di} denote the number of times the i^{th} cross appears in a design d , ($i = 1, 2, \dots, p(p-1)(p-2)/3$) and, similarly, let s_{dj} denote the total number of times that the j^{th} line occurs among the crosses in the design d , ($j = 1, 2, \dots, p$). Evidently, $\sum r_{di} = bk$ and $\sum s_{dj} = 3bk$. Also, $n = bk$ is the total number of observation generated by d . Further, define r_d and s_d to be $r_d = (r_{d1}, \dots, r_{dnc})'$, $s_d = (s_{d1}, \dots, s_{dnc})'$, and let n denote the total number of crosses (observations) in the design d_2 .

Following Das and Gupta (1997), Sharma and Sileshi (2012) we propose the following model for the data obtained from our proposed design d_2 .

The following model for a blocked situation:

$$\text{ModelM1: } \mathbf{y} = \mu \mathbf{1}_n + \Delta_1' \mathbf{g} + \Delta_2' \boldsymbol{\beta} + \boldsymbol{\varepsilon} \quad (4.1.1)$$

Where \mathbf{Y} is the $n \times 1$ vector of observations responses, μ is a general mean effect, $\mathbf{1}_n$ denotes an n - component column vector of all ones, $\mathbf{g}_{p \times 1}$ and $\boldsymbol{\beta}_{b \times 1}$ are vectors of p general combining ability effects⁴ and b block effects respectively, $\Delta_1'_{n \times p}$ and $\Delta_2'_{n \times b}$ are the corresponding design matrices, that is, the (h, l) th element of Δ_1' (respectively, of Δ_2') is 1 if the

h^{th} Observation pertains to the l^{th} line (respectively, to the l^{th} block), and is zero otherwise; $\boldsymbol{\varepsilon}$ is the vector of random error components, these components being distributed with mean zero and constant variance σ^2 .

Under the model (4.1.1), blocked situation we have not included the specific combining ability effects. It can be shown that the coefficient matrix of the reduced normal equations for estimating linear functions of general combining ability effects, using a design d_2 , is

$$C_d = G_d - N_d K_d^{-1} N_d' = c_{ij} \quad (i, j = 1, 2, \dots, p) \quad (4.1.2)$$

Where $G_d = \Delta_1 \Delta_1' = (g_{dii'})$, $g_{dii'} = sd_i = 3(p-1)$ and for $i \neq i'$, $g_{dii'}$ is the number of crosses in d in which line i and i' appear together. $N_d = \Delta_1 \Delta_2' = (n_{dij})$, (n_{dij}) is the number of times the line i occurs in block j of d and $K_d = \Delta_2 \Delta_2'$ is the diagonal matrix of block sizes.

⁴ **Combining Ability**

Combining ability studies provide the information on the genetic mechanism controlling quantitative traits and enable us to select suitable parents for further improvement or used in hybrid combinations for commercial purpose. General ability is a good estimate of additive gene action, where as specific combining ability is a measure of non-additive gene action.

Following Dey and Midha (1996), Sharma and Sileshi (2012) we have the following identities which are useful for obtaining the information matrix C_{d_2} .

- i. $\sum_{l=1}^b n_{dil} = 3(p - 1)$
- ii. $\sum_{l=1}^b n_{dil}^2 = 9(p - 1)$ (4.1.3)
- iii. $\sum_{l=1, i \neq i'}^b n_{dil} n_{di'l} = 6$

Where n_{dil} is the number of times ith line occurs in lth blocks in d and $n_{di'l}$ is the number of times ith and $i' th$ lines occur in lth block in d .

Using above identities (4.1.3), the information matrix C_d is given by

$$C_{d_2} = \frac{1}{p} \begin{bmatrix} 3(p-1)(p-3) & 3(3-p) & \dots & 3(3-p) \\ 3(3-p) & 3(p-1)(p-3) & \dots & 3(3-p) \\ \vdots & \vdots & \dots & \vdots \\ \vdots & \vdots & \dots & \vdots \\ 3(3-p) & 3(3-p) & \dots & 3(p-1)(p-3) \end{bmatrix}$$

A design d_2 is said to be connected if and only if $\text{rank}(C_{d_2}) = (p - 1)$ or equivalently, if all elementary contrast among general combining ability effects are estimable using design d_2 . A connected design d_2 is variance balanced if and only if the information matrix C_{d_2} is completely symmetric i.e., the matrix C_{d_2} has all the diagonal elements equal and all the off diagonal elements are equal.

Theorem 4.1.1 for proposed design d_2 , the rank of information matrix (C_{d_2}) is equal to $(p - 1)$.

Since the rank $(C_{d_2}) = (p - 1)$. The design d_2 is connected and also variance balanced because the matrix C_{d_2} has all diagonal elements equal and all the off diagonal elements equal. Now the trace of C_{d_2} is

$$\text{Trace}(C_{d_2}) = 3(p - 3)(p - 1) \quad (4.1.4)$$

The criterion for the optimality is the constancy of the variance for all pair wise comparisons of the lines together with the minimization of this variance.

Theorem 4.1.2 let $d \in D(p, b, k)$, be a block design for triallel crosses satisfying the following where $D(p, b, k)$ denotes the class of all connected block designs with positive integers p lines, b blocks, k block size, theorem given by Das and Gupta (1997), Sharma and Sileshi (2012).

- i. $\text{Trace}(C_{d_2}) \leq k^{-1}b\{3k(k - 1 - 2x) + px(x + 1)\}$, and
- ii. C_{d_2} is completely symmetric, where $x = \lfloor \frac{2k}{p} \rfloor$, greatest integer

Then d_2 is universally optimal in the relevant class of competing design in $D(p, b, k)$ and in particular is A-optimal.

Now consider $d_2 \in D(p, b, k)$ constructed by using the initial blocks with parameters $v = p(p - 1)$, $b = p - 1$, $k = p$ and applying Theorem 4.1.2 we see that.

$$\begin{aligned} \text{Trace}(C_d) &= k^{-1}b\{3k(k - 1 - 2x) + px(x + 1)\} \\ &= 3(p - 1)(p - 3), \text{ which is equal to the value given at (4.1.4)} \end{aligned}$$

Now we state the following theorem.

Theorem 4.1.3 Let $d \in D(p, b, k)$, be a block for triallel crosses constructed by initial blocks orthogonal Latin square satisfying.

- i. Trace $(C_{d_2})=3(p-1)(p-3)$
- ii. C_{d_2} is completely symmetric
- iii. $3k/p$ is an integer

Then d is universally optimal in the relevant class of competing design in $D(p, b, k)$ and in particular is A-optimal .

Example three.

For $p = 5$ we construct a block designs for the PTC in Chapter three.

$$B_1 \quad (2 \times 1) \times 0 \quad (3 \times 2) \times 1 \quad (4 \times 3) \times 2 \quad (0 \times 4) \times 3 \quad (1 \times 0) \times 4$$

$$B_2 \quad (4 \times 2) \times 0 \quad (0 \times 3) \times 1 \quad (1 \times 4) \times 2 \quad (2 \times 0) \times 3 \quad (3 \times 1) \times 4$$

$$B_3 \quad (1 \times 3) \times 0 \quad (2 \times 4) \times 1 \quad (3 \times 0) \times 2 \quad (4 \times 1) \times 3 \quad (0 \times 2) \times 4$$

$$B_4 \quad (3 \times 4) \times 0 \quad (4 \times 0) \times 1 \quad (0 \times 1) \times 2 \quad (1 \times 2) \times 3 \quad (2 \times 3) \times 4$$

$$C_{d_2} = G_d - \frac{N_d N_d'}{K_d}$$

$$G_d = \begin{bmatrix} 12 & 6 & 6 & 6 & 6 \\ 6 & 12 & 6 & 6 & 6 \\ 6 & 6 & 12 & 6 & 6 \\ 6 & 6 & 6 & 12 & 6 \\ 6 & 6 & 6 & 6 & 12 \end{bmatrix},$$

$$N_d = \begin{bmatrix} 3 & 3 & 3 & 3 \\ 3 & 3 & 3 & 3 \\ 3 & 3 & 3 & 3 \\ 3 & 3 & 3 & 3 \\ 3 & 3 & 3 & 3 \end{bmatrix}, N_d N_d' = \begin{bmatrix} 36 & 36 & 36 & 36 & 36 \\ 36 & 36 & 36 & 36 & 36 \\ 36 & 36 & 36 & 36 & 36 \\ 36 & 36 & 36 & 36 & 36 \\ 36 & 36 & 36 & 36 & 36 \end{bmatrix}$$

Having this the information matrix (C_{d_2}) is

$$C_{d_2} = \frac{1}{5} \begin{bmatrix} 24 & -6 & -6 & -6 & -6 \\ -6 & 24 & -6 & -6 & -6 \\ -6 & -6 & 24 & -6 & -6 \\ -6 & -6 & -6 & 24 & -6 \\ -6 & -6 & -6 & -6 & 24 \end{bmatrix}$$

We note that the matrix C is determined entirely by the specific design, that is, by the incidence matrix N_d . Here the information matrix (C_d) matrix is symmetric, and the sum of any row or any column of C_d add to zero, that is, $C_d \mathbf{1}_d = 0$ which implies that the matrix C is not of full rank. The rank of $C_d = p - 1 = 4$. Therefore the design is connected and the diagonal and the off diagonal elements of C_d are the same thus the design is also variance balanced.

4.2 Unblocked Model

For the data obtained from our proposed design d_1 , we propose the following model given by Hinkelmann(1965).

Model for an unblocked (completely randomized) situation:

$$\text{ModelM2: } \mathbf{y} = \mu \mathbf{1}_n + \Delta_1' \mathbf{g} + \varepsilon \quad (4.2.1)$$

We now take up the optimality aspects in unblocked situation of our design d_1 . The optimality criterion chosen is the minimization of the average variance of the best linear unbiased estimators of all elementary contrasts among first line of effects and also among second line of effects. According to Kiefer (1958), a design $D^* \in D$, is universally optimal in a relevant class of competing designs if:

- a. The information matrix C_{d^*} of the design is completely symmetric means has all its diagonal elements equal and all its off-diagonal elements equal; and

b. $\text{Trace}(C_{d^*}) = \max_{d \in D} \text{Trace}(C_d)$, then d^* is universally optimal in D .

Consider now any PTC design $d^*_1 \in D(p, n)$ with $2n/p = r_{d^*_1 I}$ is an integer for those lines which belongs to first kind of effect and $n/p = r_{d^*_1 II}$ is also an integer for those lines which belongs to second kind of effect.

Now $\text{trace } C_{d^*} = \text{trace}(R - 1/n r_{d^*_1} r_{d^*_1}')$ where R is a diagonal matrix with diagonal elements given by the replication numbers for the lines which belong to first kind of effect and $r_{d^*_1 I}$ is the column vector of replication for the lines which belong to first kind of effect.

$C_{d^*} = \text{Trace}(n - \sum_{i=1}^p r_{d^*_{II}}^2)$, here minimization of $\sum_{i=1}^p r_{d^*_{II}}^2$ subject to $\sum_{i=1}^p r_{d^*_{II}} = n$ is attained when $r_{d^*_{II}} = 2n/p$. So a design d^* with $r_{d^*_{II}} = 2(p-1)$ is universally optimal in $D(p, n)$.

Hence $\text{trace } C_{d^*_1} = 2(p-1)(p-2)$ for lines which belongs to first kind of effect.

For design d_1 , $C_{11} = 2(p-2)A$ is an information matrix for lines which belong to first kind of effect and has trace equal to $2(p-1)(p-2)$. Hence design d_1 is universally optimal for first kind of effects.

Similarly we can show that $\text{trace } C_{d^*_1} = (p-1)^2$ for lines which belong to second kind of effect.

For design d_1 , $C_{22} = (p-1)A$ is an information matrix for lines which belong to second kind of effect and has trace equal to $(p-1)^2$. Hence design d_1 is universally optimal for second of kind of effects.

Now we state the following result.

Theorem 4.2. Let d_1 be a PTC mating design constructed by using Latin square of order p bordered by its transversal, where p is a prime or power of a prime. Then the design d_1 is universally optimal for estimating first and second kind of effects for PTC in $D(p, n)$.

4.3 HOW WE ANALYSIS THE CONSTRUCTED PTC DESIGNS?

4.3.1 Introduction

Statistical analysis was performed according to the method of experimental design used to test the nature of the differences between parents, single crosses and three-way crosses each alone, and Duncan's Multiple Range Test used to compare the differences between the means (Gomez and Gomez, 1983). A statistical model is actually a linear relation of effect different levels of a number of factors involved in an experiment along with one or more terms of representing error effects. For complete trialallel crosses evaluated in complete blocks, Rawlings and Cockerham (1962a) presented a linear model leading to an orthogonal analysis of variance and expectation of various mean squares in terms of statistical variance components, relationships between of genetic and statistical variance components, and presented tests for a number of genetic hypotheses.

Model for a three-way cross: The genetic part of the three –way cross $(ij)k$ can be represented by the following model

$$y_{(ij)k} = \mu + h_i + h_j + g_k + d_{ij} + s_{(i)k} + s_{(j)k} + t_{(ij)k} + e_{(ij)k} \quad (4.3.1)$$

Where μ is the overall mean, h and g are the general effects, d and s are two-line specific effects, and t is a three-line specific effect.

For the purpose of analysis of a PTC we are led by (4.3.1) to adopt the reduced model, In case of *PTC* , the above model is further simplified by retaining only single line effects as follows for an *RCBD* situation,

$$y_{(ij)k} = \mu + h_i + h_j + g_k + e_{(ij)k} \quad (4.3.2)$$

($i, j, k=1,2,\dots,p, i \neq j \neq k$) where μ , h and g have the same meaning as above and the $e_{(ij)k}$ are considered to be independent random variable with mean zero and variance σ^2 . For definiteness we shall call g the general effect of the first kind and h the general effect of the second kind.

Following Sharma et.al (2011) the matrix notation of model (4.3.1) can be written as:

$$y = \mu \mathbf{1}_n + \mathbf{X}h + \mathbf{Z}g + e \quad (4.3.3)$$

Where y is the column vector of the n observations, $\mathbf{1}_n$ is an $n \times 1$ vector of unity elements, $\mathbf{h}' = (h_1, h_2, h_3, \dots, h_p)$, $\mathbf{g}' = (g_1, g_2, \dots, g_p)$, $\mathbf{X} = (x_{ui})$ is an $n \times p$ matrix with $x_{ui} = 1$ if i_h occurs in the u th cross otherwise $x_{ui} = 0$, $\mathbf{Z} = (z_{vk})$ is an $n \times p$ matrix with $z_{vk} = 1$ if k_F occurs in the v -th cross otherwise $z_{vk} = 0$, e is the $pr \times 1$ vector of the residuals.

The normal equations for $\hat{\mu}, \hat{h}$, and \hat{g} are

$$\begin{aligned} n\hat{\mu} + \mathbf{1}'_n \mathbf{X} \hat{h} + \mathbf{1}'_n \mathbf{Z} \hat{g} &= \mathbf{1}'_n y \\ \mathbf{X}' \mathbf{1}_n \hat{\mu} + \mathbf{X}' \mathbf{X} \hat{h} + \mathbf{X}' \mathbf{Z} \hat{g} &= \mathbf{X}' y \\ \mathbf{Z}' \mathbf{1}_n \hat{\mu} + \mathbf{Z}' \mathbf{X} \hat{h} + \mathbf{Z}' \mathbf{Z} \hat{g} &= \mathbf{Z}' y \end{aligned} \quad (4.3.4)$$

From the first equation (4.3.4) we obtain

$$\hat{\mu} = \bar{y} - 1/n \mathbf{1}'_n \mathbf{X} \hat{h} - 1/n \mathbf{1}'_n \mathbf{Z} \hat{g}$$

Where \bar{y} denotes the mean of all n observations. After substituting the value of $\hat{\mu}$ into the last two equations in (4.3.4) we get the following normal equations.

$$\begin{aligned} \mathbf{X}'(I_n - 1/n \mathbf{1}_n \mathbf{1}'_n) \mathbf{X} \hat{h} + \mathbf{X}'(I_n - 1/n \mathbf{1}_n \mathbf{1}'_n) \mathbf{Z} \hat{g} &= Q_1 \\ \mathbf{Z}'(I_n - 1/n \mathbf{1}_n \mathbf{1}'_n) \mathbf{X} \hat{h} + \mathbf{Z}'(I_n - 1/n \mathbf{1}_n \mathbf{1}'_n) \mathbf{Z} \hat{g} &= Q_2 \end{aligned} \quad (4.3.5)$$

Where $Q_1 = (Q_{11}, \dots, Q_{1p}) = \mathbf{X}'(Y - \mathbf{1}_n \bar{y})$, and $Q_2 = (Q_{22}, \dots, Q_{2p}) = \mathbf{Z}'(Y - \mathbf{1}_n \bar{y})$

Let us denote $C_1 = \mathbf{X}'\mathbf{X}$, $C_2 = \mathbf{X}'\mathbf{Z}$, and $C_3 = \mathbf{Z}'\mathbf{Z}$

$$C_{11} = \mathbf{X}'(I_n - 1/n \mathbf{1}_n \mathbf{1}'_n) \mathbf{X}, C_{12} = \mathbf{X}'(I_n - 1/n \mathbf{1}_n \mathbf{1}'_n),$$

and $C_{22} = \mathbf{Z}'(I_n - 1/n \mathbf{1}_n \mathbf{1}'_n) \mathbf{Z}$

Then the coefficient matrix of (5.4) becomes

$$C = \begin{bmatrix} C_{11} & C_{12} \\ C_{12} & C_{22} \end{bmatrix} \quad (4.3.6)$$

Further it is not hard to see that

$$C_1 = 2(P - 2)I_p + 2\mathbf{1}_p \mathbf{1}'_p, C_2 = -2I_p + 2\mathbf{1}_p \mathbf{1}'_p'$$

$$C_3 = \mathbf{Z}'\mathbf{Z} = (P - 1)I_p, C_{11} = 2(P - 2)(I_p - 1/P \mathbf{1}_p \mathbf{1}'_p) = 2(P - 2)A$$

$$C_{12} = C_{21} = -2(I_p - 1/p \mathbf{1}_p \mathbf{1}'_p) = -2A, \text{ And } C_{22} = (p - 1)(I_p - 1/p \mathbf{1}_p \mathbf{1}'_p) = (p - 1)A$$

Where $(I_p - 1/P \mathbf{1}_p \mathbf{1}'_p) = A$, I_p is an identity matrix of order p and $\mathbf{1}_p$ is a $p \times 1$ vector of unity elements.

Now the reduced normal equations at (4.3.4) can be expressed as, solution of the normal equations.

$$C \begin{pmatrix} \hat{h} \\ \hat{g} \end{pmatrix} = \begin{pmatrix} Q_1 \\ Q_2 \end{pmatrix} \quad (4.3.7)$$

Where $C = \begin{bmatrix} C_{11} & C_{12} \\ C_{12} & C_{22} \end{bmatrix}$, is a coefficient matrix of the reduced normal equations.

Following Sharma et.al (2011) we will show that the proposed designs are connected, where connectedness is defined as follows.

Definition 4.3.1: A PTC is said to be connected if all contrasts $h_i - h_j$ ($i, j = 1, 2, \dots, p$) and $g_k - g_l$ ($k, l = 1, 2, \dots, p$) are estimable.

This leads to the following theorem.

Theorem 4.3.1: The necessary condition for a PTC to be connected is that the rank $(C) = 2(p - 1)$.

Theorem 4.3.2: All linear contrasts among the general effects of the first and second kind i.e.

$h_i - h_j$ ($i, j = 1, 2, \dots, p$) and $g_k - g_l$ ($k, l = 1, 2, \dots, p$) are estimable if and only if the rank $(A) = p - 1$.

Now solving (4.3.6) we obtain the following estimators for \mathbf{h} and \mathbf{g}

$$\hat{h} = [(p - 1)Q_1 + 2Q_2]/2p(p - 3) \quad (4.3.8)$$

$$\hat{h} = Q_h/2p(p - 3), \text{ where } Q_h = (p - 1)Q_1 + 2Q_2$$

$$\hat{g} = [Q_1 + (p - 2)Q_2]/p(p - 3) \quad (4.3.9)$$

$$\hat{g} = Q_g/p(p - 3), \text{ where } Q_g = Q_1 + (p - 2)Q_2$$

Now

$$\text{var}(\hat{h}) = \sigma^2(p-1)/2p(p-3)I_p \quad (4.3.10)$$

And

$$\text{var}(\hat{g}) = \sigma^2(p-2)/p(p-3)I_p \quad (4.3.11)$$

From Eqs (4.3.10) and (4.3.11) it is clear that the all elementary contrasts among general effects of the first and second kind are estimable with the same variance, respectively. Thus, the proposed PTC mating design is variance balanced. We thus have the following definition and result.

Definition 4.2.2: Consider an arrangement of the $v = pr$ triallel crosses in a connected design.

- (a) The design is balanced for the general first kind of effects if the covariance matrix of first kind effects is a constant times the identity matrix.
- (b) The design is balanced for the general second kind of effects if the covariance matrix of second kind effects is a constant times the identity matrix.

Theorem 4.2.3 The mating design d_1 for PTC obtained by using Latin square and its transversal is variance balanced for general effects of the first and second kind for PTC, if the covariance matrices of first and second kind effects are a constant times the identity matrices, respectively.

4.3.2 HYPOTHESES TESTING

For fixed effects model the parameterization in (4.3.3) is not orthogonal, hence it does not lead to an orthogonal partitioning of the between –crosses-SS according to the different types of parameters in (4.3.3). Furthermore, for the random effects which is not orthogonal and this leads to covariance between some of the parameters in (4.3.3) as was shown by Hinkelmann (1965) using finite population and randomization approach.

The analysis for the fixed effects model (4.3.3), including appropriate sum of squares, was given by Ponnuswamy and Das (1973).

The sum of squares due to general effect of first kind (**h**) and second kind (**g**) are given by

$$SS(h) = \hat{h}Q_h = Q_h \mathbf{Q}_h / 2p(p - 3) \quad (4.3.12)$$

$$SS(g) = \hat{g}Q_g = Q_g \mathbf{Q}_g / p(p - 3) \quad (4.3.13)$$

Now let us suppose that a design d_1 is replicated s times. We then consider the following model

$$y_{(ij)kl} = \mu + b_l + y_{(ij)k} + \varepsilon_{(ij)kl} \quad (4.3.14)$$

Where $y_{(ij)kl}$ is the phenotypic value in l -th replication on j -th cross mated to k -th parent, μ is the general mean, b_l is the effect of l -th replication, $y_{(ij)k}$ is the cumulative effect of the triallel cross $(ij)k$ and is equal to $h_i + h_j + g_k + \varepsilon_{(ij)k}$ with h and g are fixed effects and $\varepsilon_{(ij)kl}$ are independent random variables with mean zero and variance σ_ε^2 .

The analysis of variance (ANOVA) is then given in Tables 4.3.1 and 4.3.2 on the basis of model (4.3.14) and (4.3.3).

Table 4.3.1

. Analysis of variance of proposed PTC mating design in s replicates

Source	D.F	S.S	M.S	E (M.S)
Replicates	$s - 1$	$pr \sum_{l=1}^s (\bar{y}_l - \bar{y})^2$	$SSR/s - 1$	
Three-way crosses	$pr - 1$	$s \sum_{i=1}^p \sum_{j=1}^p \sum_{k=1}^p (\bar{y}_{(ij)k} - \bar{y})^2$	$SST/pr - 1$	
Error	$(s - 1)(pr - 1)$	subtraction	$SSE/(s - 1)(pr - 1)$	σ_ε^2
Total	$spr - 1$	$\sum_{l=1}^s \sum_{i=1}^p \sum_{j=1}^p \sum_{k=1}^p (y_{(ij)kl} - \bar{y})^2$		

Where \bar{y} is the overall mean, \bar{y}_l the mean of all three-way crosses in the l -th replicate, mean of $\bar{y}_{(ij)k}$ the three-way crosses $(ij)k$ over all replicates. The sum of square for three-way crosses can be decomposed as given in the following table 4.3.2.

Table 4.3.2 Analysis of variance for three – way crosses

Source	Degrees of freedom	Sum of squares	Expected mean squares
h elim g	$(p - 1)$	$\hat{h}Q_h$	$\sigma_\varepsilon^2 + s\sigma^2 + s(p - 1)\sigma_h^2$
g ign h	$(p - 1)$	$\sum_{l=1}^s \sum_{k=1}^p y_{ij..}^2 - spr\bar{y}^2$	$\sigma_\varepsilon^2 + s\sigma^2 + 2s\sigma_h^2 + sr\sigma_g^2 + 2s\sigma_h^2$
g elim h	$(p - 1)$	$\hat{g}Q_g$	$\sigma_\varepsilon^2 + s\sigma^2 + s(p - 1)\sigma_g^2$
h ign g	$(p - 1)$	difference	$\sigma_\varepsilon^2 + s\sigma^2 + 2s\sigma_g^2 + sr\sigma_h^2 + 2s\sigma_h^2$
remainder	$p(r - 2) + 1$	R=difference	$\sigma_\varepsilon^2 + s\sigma^2$
three-way crosses	$pr - 1$	$s \sum_{i=1}^p \sum_{j=1}^p \sum_{k=1}^p (\bar{y}_{(ij)k} - \bar{y})^2$	

5 ILLUSTRATIVE EXAMPLE

5.1 Introduction

For illustrating the theory given in this paper, we show the essential steps of analysis of a triallel cross experiment, using mating design proposed in this paper. For this purpose, we take data from an experiment on grain yields per plant in three way hybrids, reported by Singh and Chaudhary (1997) on page 169. The author used a randomized complete block design with $p = 6$ as he considered all possible 60 triallel crosses among $p = 6$ inbred lines. On the purpose of illustration, we take the data of relevant crosses from this experiment.

The most commonly used statistical software for statistical design and analysis of experiments such as R, GENSTAT, SAS, STATA, SPSS, MS-Excel, which we are not going to employ in our analysis directly. We employed MS-Excel and R statistical soft wares for calculating analysis of variance and estimation of model parameters.

The design chosen is d_1 , there are 20 crosses and the design is RBD with two replications.

The observations are given below in Table 5.1.

Table 5.1.

Data from an experiment on grain yields per plant in three way hybrids

Cross	Replication I	Replication II	Total	Mean
(0 × 1) 2	83.62	99.88	183.50	91.75
(0 × 1) 4	66.86	64.24	131.10	65.55
(0 × 2) 3	99.04	92.28	191.32	95.66
(0 × 2) 4	107.38	84.56	191.94	95.97
(0 × 3) 1	89.30	95.08	184.38	92.19
(0 × 3) 2	89.52	97.10	186.62	93.31
(0 × 4) 1	76.28	79.84	156.12	78.06
(0 × 4) 3	93.56	85.46	178.02	89.01
(1 × 2) 0	86.52	71.36	157.88	78.94
(1 × 2) 3	97.46	106.56	204.02	102.01
(1 × 3) 0	100.36	101.86	202.22	101.11
(1 × 3) 4	67.76	72.62	140.38	70.19
(1 × 4) 2	81.34	82.30	163.62	81.82
(1 × 4) 3	77.64	77.60	155.24	77.62
(2 × 3) 1	63.06	59.96	123.02	61.51
(2 × 3) 4	93.16	99.36	192.52	96.26
(2 × 4) 0	111.70	114.76	226.46	113.23
(2 × 4) 1	78.92	82.64	161.56	80.78
(3 × 4) 0	62.86	67.48	130.34	65.17
(3 × 4) 2	96.64	102.76	199.22	99.61

5.2. PRESENTATION, INTERPRETATION AND IMPLICATIONS OF RESULTS

The following exhibit the vectors $Q_1, Q_2, Q_h, Q_g, \hat{h}$ and \hat{g} are given in this section also the matrices X, Z and C are given, R software are used to calculate or analyze those all vectors and matrices. The simple RBD analysis for triallel cross design and the analysis of variance for three way crosses on grain yields per plant in the three way hybrids are given in Table 5.3 and 5.4 respectively. The estimates of first and second kind of general effects are shown in Table 5.5.

Table 5.2

Line with half and full parents.

Crosses	Line (with half parents, X)					Line (with full parents, Z)				
	0	1	2	3	4	0	1	2	3	4
$(0 \times 1) \times 2$	1	1	0	0	0	0	0	1	0	0
$(0 \times 1) \times 4$	1	1	0	0	0	0	0	0	0	1
$(0 \times 2) \times 3$	1	0	1	0	0	0	0	0	1	0
$(0 \times 2) \times 4$	1	0	1	0	0	0	0	0	0	1
$(0 \times 3) \times 1$	1	0	0	1	0	0	1	0	0	0
$(0 \times 3) \times 2$	1	0	0	1	0	0	0	1	0	0
$(0 \times 4) \times 1$	1	0	0	0	1	0	1	0	0	0
$(0 \times 4) \times 3$	1	0	0	0	1	0	0	0	1	0
$(1 \times 2) \times 0$	0	1	1	0	0	1	0	0	0	0
$(1 \times 2) \times 3$	0	1	1	0	0	0	0	0	1	0
$(1 \times 3) \times 0$	0	1	0	1	0	1	0	0	0	0
$(1 \times 3) \times 4$	0	1	0	1	0	0	0	0	0	1
$(1 \times 4) \times 2$	0	1	0	0	1	0	0	1	0	0

$(1 \times 4) \times 3$	0	1	0	0	1	0	0	0	1	0
$(2 \times 3) \times 1$	0	0	1	1	0	0	1	0	0	0
$(2 \times 3) \times 4$	0	0	1	1	0	0	0	0	0	1
$(2 \times 4) \times 0$	0	0	1	0	1	1	0	0	0	0
$(2 \times 4) \times 1$	0	0	1	0	1	0	1	0	0	0
$(3 \times 4) \times 0$	0	0	0	1	1	1	0	0	0	0
$(3 \times 4) \times 2$	0	0	0	1	1	0	0	1	0	0

Now let we check whether the design is connected or not which is given in the above, here we considered only for unblocked cases. The coefficient matrix is given by

$$C = \begin{bmatrix} C_{11} & C_{12} \\ C_{12} & C_{22} \end{bmatrix}$$

Where $C_{11} = 2(P - 2)(I_P - 1/P \mathbf{1}_P \mathbf{1}'_P)$

$$= \begin{bmatrix} 4.8 & -1.2 & -1.2 & -1.2 & -1.2 \\ -1.2 & 4.8 & -1.2 & -1.2 & -1.2 \\ -1.2 & -1.2 & 4.8 & -1.2 & -1.2 \\ -1.2 & -1.2 & -1.2 & 4.8 & -1.2 \\ -1.2 & -1.2 & -1.2 & -1.2 & 4.8 \end{bmatrix}$$

$C_{12} = C_{21} = -2(I_P - 1/P \mathbf{1}_P \mathbf{1}'_P)$

$$= \begin{bmatrix} -1.6 & 0.4 & 0.4 & 0.4 & 0.4 \\ 0.4 & -1.6 & 0.4 & 0.4 & 0.4 \\ 0.4 & 0.4 & -1.6 & 0.4 & 0.4 \\ 0.4 & 0.4 & 0.4 & -1.6 & 0.4 \\ 0.4 & 0.4 & 0.4 & 0.4 & -1.6 \end{bmatrix}$$

And $C_{22} = (P - 1)(I_P - 1/P \mathbf{1}_P \mathbf{1}'_P)$

$$= \begin{bmatrix} 3.2 & -0.8 & -0.8 & -0.8 & -0.8 \\ -0.8 & 3.2 & -0.8 & -0.8 & -0.8 \\ -0.8 & -0.8 & -3.2 & -0.8 & -0.8 \\ -0.8 & -0.8 & -0.8 & 3.2 & -0.8 \\ -0.8 & -0.8 & -0.8 & -0.8 & 3.2 \end{bmatrix}$$

Thus the coefficient matrix is

$$C = \begin{bmatrix} 4.8 & -1.2 & -1.2 & -1.2 & -1.2 & -1.6 & 0.4 & 0.4 & 0.4 & 0.4 \\ -1.2 & 4.8 & -1.2 & -1.2 & -1.2 & 0.4 & -1.6 & 0.4 & 0.4 & 0.4 \\ -1.2 & -1.2 & 4.8 & -1.2 & -1.2 & 0.4 & 0.4 & -1.6 & 0.4 & 0.4 \\ -1.2 & -1.2 & -1.2 & 4.8 & -1.2 & 0.4 & 0.4 & 0.4 & -1.6 & 0.4 \\ -1.2 & -1.2 & -1.2 & -1.2 & 4.8 & 0.4 & 0.4 & 0.4 & 0.4 & -1.6 \\ -1.6 & 0.4 & 0.4 & 0.4 & 0.4 & 3.2 & -0.8 & -0.8 & -0.8 & -0.8 \\ 0.4 & -1.6 & 0.4 & 0.4 & 0.4 & -0.8 & 3.2 & -0.8 & -0.8 & -0.8 \\ 0.4 & 0.4 & -1.6 & 0.4 & 0.4 & -0.8 & -0.8 & 3.2 & -0.8 & -0.8 \\ 0.4 & 0.4 & 0.4 & -1.6 & 0.4 & -0.8 & -0.8 & -0.8 & 3.2 & -0.8 \\ 0.4 & 0.4 & 0.4 & 0.4 & -1.6 & -0.8 & -0.8 & -0.8 & -0.8 & 3.2 \end{bmatrix}$$

The C matrix is symmetric, and the sum of any row or any column of C add to zero, that is,

$C_{10 \times 10} \mathbf{1}_{10 \times 1} = \mathbf{0}$, which implies that the matrix C is not of full rank i.e. $2(C-1) = 8$ which follows the design is connected.

The adjusted total for first and second effects

$$Q_1 = \mathbf{X}'(\mathbf{Y} - \mathbf{1}_N \bar{Y})$$

$$Q_2 = \mathbf{Z}'(\mathbf{Y} - \mathbf{1}_N \bar{Y})$$

$$= \begin{pmatrix} 9.86 \\ -23.15 \\ 32.22 \\ -12.70 \\ -6.25 \end{pmatrix}$$

$$= \begin{pmatrix} -12.38 \\ -33.53 \\ 20.51 \\ 18.73 \\ -18.10 \end{pmatrix}$$

And

$$Q_h = (p - 1)Q_1 + 2Q_2$$

$$Q_g = Q_1 + (p - 2)Q_2$$

$$= \begin{pmatrix} 64.22 \\ -159.64 \\ 169.92 \\ -13.32 \\ -61.18 \end{pmatrix}$$

$$= \begin{pmatrix} 47.01 \\ -123.73 \\ 93.76 \\ 43.50 \\ -60.54 \end{pmatrix}$$

Table 5.3

Simple RBD analysis for triallel crosses design.

Source	D.F	S.S	M.S	F
Replication	1	5.417	5.417	
Three way cross	19	7546.416	397.180	10.299
Error	19	732.746	38.566	
Total	39	8284.581		

Table 5.4

Analysis of variance for three way crosses

Source	S.S	M.S	D.F	M.S	S.S	source
<i>h</i> elim <i>g</i>	3120.118	780.030	4	796.592	3186.370	<i>g</i> elim <i>h</i>
<i>g</i> ign <i>h</i>	1188.348	297.087	4	280.524	1122.096	<i>h</i> ign <i>g</i>
Remainder	3237.950	294.359	11	294.359	3237.950	Remainder
Three-way crosses	7546.416	397.180	19	397.180	7546.416	3-way Crosses

Estimates for first and second kind of general effects.

$$\hat{h} = [(p - 1)Q_1 + 2Q_2]/2p(p - 3), \hat{g} = [Q_1 + (p - 2)Q_2]/ p(p - 3)$$

$\begin{pmatrix} 3.211 \\ -7.982 \\ 8.496 \\ -0.666 \\ -3.059 \end{pmatrix}$	$\begin{pmatrix} 4.701 \\ -12.373 \\ 9.376 \\ 4.350 \\ -6.054 \end{pmatrix}$
--	--

Table 5.5

Estimates of first and second kind of general effects.

Line	first kind effect h_i	second kind effects g_k	standard error of h_i	standard error of g_k
0	3.211	4.701	± 5.058	± 6.194
1	-7.982	-12.373	± 5.058	± 6.194
2	8.496	9.376	± 5.058	± 6.194
3	-0.666	4.350	± 5.058	± 6.194
4	-3.059	-6.054	± 5.058	± 6.194

5.3. DISCUSSION

The constructed design in blocked cases i.e. the information matrix (C_d) matrix is symmetric, and the sum of any row or any column of C_d add to zero, that is, $C_d \mathbf{1}_d = \mathbf{0}$ which implies that the matrix C is not of full rank, The rank of $C_d = P - 1 = 4$. Therefore the design is connected and the diagonal and the off diagonal elements of C_d are the same thus the design is also variance balanced.

From the result chapter 4, for the constructed PTC design we observe that the information matrix (C) is symmetric, and the sum of any row or any column of C add to zero, that is, $C \mathbf{1}_n = \mathbf{0}$,

which implies that the matrix \mathbf{C} is not full rank. In fact the rank of \mathbf{C} is $2(p - 1) = 8$, which is essential condition for the design to be connected. Therefore the design is connected for estimating the general effects for both cases.

REFERENCES

- [1] Arora, B.S. and Aggarwal, K.R. (1984). Confounded trialallel experiments and their applications, *Sankhya* **B46**, 54–63.
- [2] Arora, B.S. and Aggarwal, K.R. (1989). Trialallel experiments with reciprocal effects, *Jour Ind Soc Agril Stat* **41**, 91–103.
- [3] Batra ,P.K. Sreenath ,P.R. and Parsad ,R.(1997) . Studies on robustness of block designs against interchange of treatments, *J .Ind. Soc. Agril. Statist.*, vol. **41**, pp. 91-103.
- [4] Calvin, L.D. (1954). Doubly Balanced Incomplete Block Designs in which treatment effects are correlated, *Biometrics*, **10**, 61-88.
- [5] Ceranka, B. Chudzik, H. Dobek, A. and Kielczewska , H. (1990)Estimation of parameters for trialallel crosses compared in block designs, *Statist Appl* **2**, 27–35.
- [6] Das, A. and Gupta, S. (1997). Optimal block designs for trialallel cross experiments, *Commu Stat-theory and Methods* **26(7)**, 1767–1777.
- [7] Den´es, J. and Keedwell, A. D. (1974). Latin squares and their applications. Akad´emiai Kiad´o, Budapest
- [8] Dey, A. and Midha, K, Chand. (1996). Optimal designs for diallel crosses, *Biometrika* **83(2)**, 484–489.

- [9] Dharmalingam,M. (2002). Construction of Partial Triallel Crosses based on Trojan square design, *Jour of Applied Stat* **29**(5) , 695–702.
- [10] Falconer, D. S. and Mackay TFC (1996). Introduction to Quantitative Genetics. Fourth edition. Addison Wesley Longman, Harlow, Essex, UK.
- [11] Fisher, R.A. (1918). The correlation between relatives on the supposition of Mendelian inheritance. *Trans.Roy. Soc. Edinburgh* **52**, 399-433
- [12] Gomez, W. A. and Gomez, R. A. (1976). *Statistical Procedures for Agricultural Research*. John Wiley and Sons, New York.
- [13] Griffings,B. (1956). Concepts of general and specific combining ability in relation to diallel crossing system, *Aust J Bio Sci* **9** , 463–493.
- [14] Gupta,S . and Kageyama,S. (1994) .Optimal complete diallel crosses, *Biometrika* **81** , 420 424.
- [15] Hinkelmann, K. (1963) Design and analysis of multi – way genetic cross experiments. Dissertation,Lowa State University.
- [16] Hinkelmann,K. (1965) Partial triallel crosses, *Sankhya* **A 27** , 173–196
- [17] Hinkelmann, K. (1975) *A Survey of Statistical Design and Linear Models*, Amsterdam; North Hollond, pp. 243–269.
- [18] Hinkelmann,K. and Kempthorne, O. (1963). Two classes of group divisible partial Diallel crosses. *Biometrics* **27**, 183-190.

- [19] Hinkelmann, K. and Kempthorne, O. (2005) *Design and Analysis of Experiments*, Volume 2: Advanced Experimental Design, (First Ed.) , Wiley.
- [20] Kiefer, J. (1975). Construction and optimality of generalized Youden designs, in *a survey of Statistical Design and Linear Models*, J.N. Srivastava, ed., Amsterdam: North Holland, pp. 33–53.
- [21] Lynch, M. and B. Walsh. 1998. Genetics and analysis of quantitative traits. Sinauer Associates Inc., Sunderland, Massachusetts.
- [22] Narain, P. (1990) *Statistical Genetics*. Wiley Eastern Limited, New Delhi.
- [23] Panda, D.K., Sharma, V.K. and Parsad, R. (2001). Robustness of optimal block designs for triallel crosses experiments against exchange of a cross. *J. Ind. Soc. Agril. Statist.*, **54(3)**, 328-341.
- [24] Peterson R.G. (1985). *Design and Analysis of Experiments*, Marcel Dekker.
- [25] Ponnuswamy, K. N., Das, M. N. and Handoo, M. I. (1974). Combining ability type analysis of triallel crosses in Maize. *Theor. Appl. Genet.* , **45**, 170-175.
- [26] Ponnuswamy, K.N and Subbarayan, A. (1990) Construction of partial triallel mating designs and estimation of variance components. *Design of Experiments. Proc. Symp. Thiruvnanthapuram/India, Publ Cent Math Sci Trivandrum* **19**, 55–71.
- [27] Ponnuswami, K.N. and Srinivasan, M.R. (1991). Construction of partial triallel crosses (PTC) using a class of balanced incomplete block designs (BIBD). *Commu Stat-Theory and Methods* **A20**, 3315–3323.

- [28] Rao, C.R.(1956). A General class of quasifactorial and related designs, *Sankhya* **17**(Pt-2), 165–174.
- [29] Rawlings, J.O. and Cockerham, C.C.(1962) . Trialallel analysis, *Crop Sci* **2** , 228–231.
- [30] Sharma, M. K. (2000). Application of PBIB Designs in CDC Method IV. *Journal of Applied Statistics*, **27(8)**, 1013-1019.
- [31] Sharma, M. K. (2004). Optimal diallel cross. *Recent advances in mating designs*. Dhanpat Rai and Company (p) Ltd. New Delhi.
- [32] Sharma, M.K. and Fanta, S. (2010). Optimal block designs for diallel crosses. *Metrika*, **71**, 361-372.
- [33] Sharma, M.K. *et al* (2011). Optimal partial trialallel cross designs, *MASA*, **6**, 1-8.
- [34] Sharma, M.K. and Fanta, S. (2012). Optimal partial trialallel cross designs, **66(2)**, 1-6
- [35] Singh, R.K. and Chaudhary , B.D. (1997). *Biometrical Methods in Quantitative Genetic Analysis*, Kalyani Publishers, New Delhi, India.
- [36] Subbarayan, A. (1992). On the applications of pure cyclic triple system for plant breeding experiments, *Jour of Applied Stat* **19(4)**, 489–500.
- [37] Yates, F. (1936). Incomplete Randomized Blocks, *Annals Eugenics*, **7**, 121-140.

DECLARATION

I, the undersigned, declare that the thesis is my original work, has not been presented for a degree in any university and that all sources of material used for the thesis have been duly acknowledged.

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