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Significance of Experimental Designs in Agricultural Research

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PREFACE

Experimentation is an integral component of almost every agricultural and other scientific endeavour. Properly designed experiments not only answer all the questions of the researcher but also make efficient use of available resources while doing so. Thus, careful and efficient designing of an experiment is both an art and science. It is a common phenomenon both in this country and abroad that experimenters are able to state the research problems clearly they are after, but are often unable to devise a suitable experiment because they are not sure which design to apply to conduct the experiment and even if s/he knows the design, then how to analyse the resulting data from such an experiment is another roadblock in the process. Thus, any form of a quick handy guidance to proper designing experiments and analysing data thereof would be of immense benefit to the experimenters.

The purpose of this manuscript is to expose the readers to some modern, sophisticated, efficient designs actually used by the agricultural researchers in conducting their experiments and also to introduce the readers to some useful web resources for generating layout of the designs and for analysing data from designed experiments. We have taken a case study approach for describing the designs. In other words, real life situations for conducting an experiment are described first and then a suitable design for the situation is suggested. We hope that this pocket diary will be immensely useful to the experimenters. Criticisms and suggestions for improvements are always welcome.

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CONTENTS

1. INTRODUCTION

Designing an experiment is an inevitable component of every research endeavour in agricultural sciences. The data generated through designed experiments exhibit a lot of variability. The variability may be wanted and desirable, or unwanted and undesirable, but controllable in the sense that it can be accounted for. There is also some more variability, which is unwanted, undesirable and uncontrollable. The reason for its presence is unknown. For instance, the experimental units (plots) subjected to the same treatment give rise to different observations and thus create variability. These plots are expected to give same response, but actually the responses are different; reasons unknown. The variation may arise because of variable fertility/soil moisture/soil depth, etc. of the two plots. The statistical methodologies, in particular the theory of linear estimation and analysis of variance, enable us to partition the total variability in the data into two major components. The first major component comprises of that part of the total variability to which we can assign causes or reasons. The second component comprises of that part of the total variability to which we cannot assign any cause or reason. This part of variability is known as *experimental error*. This variability arises because of some factors unidentified as a source of variation. Even a careful planning of the experiment cannot ensure total elimination of this component. The observations obtained from experimental units identically treated allow the estimation of this experimental error. Ideally one should select a design that will give experimental error as small as possible. There is, though, no rule of thumb to describe what amount of experimental error is small and what amount of it can be termed as large. A popular measure of the experimental error is the percent Coefficient of Variation (CV). Generally the researcher desires the CV to be small, though there is no degree of smallness defined. A very low value of percent CV may also be viewed with suspicion and must be ascertained by using other parameters like variability across replications for all the treatments or otherwise.

The explainable part of the total variability has two major components. One major component is the conditions to study or the treatments. This part of the variability is wanted or desirable. There is always a deliberate attempt on the part of the experimenter to create variability by the application of several treatments in the experiment. So treatments are one component in every designed experiment that causes variability. The other component of the explainable part of variability is the experimental units itself. This variability is unwanted and undesirable. The factors that cause this variability are called *nuisance factors*. This part of the variability is accounted for by using the principle of local control. Before planning the experiment, the experimenter must have a complete knowledge about the experimental units on which the experiment would be conducted and the sources and nature of variability in the experimental units. If this variability is substantial and is not accounted for by proper designing of experiment, then this component would sit in the experimental error and make it unduly large. The end result would be a bad experiment. There could be many ways of accounting for the variability due to experimental units. The remedy depends upon the sources and nature of the factors causing variability in the experimental units. As a matter of fact, the way to account for the variability in the experimental units will dictate what type of design is to be used. One may adopt one blocking system, two blocking systems, nested blocks or nested rows and columns, etc. for accounting for the variability. Many a time, depending upon practical constraints, a naive design is the best design.

Treatments in the design could be unstructured *i.e.*, treatments are the levels of a single factor *e.g.* 10 different tree species; 6 different varieties of wheat; 8 different formulations of chemicals, 4 different grazing systems, 9 different nutrition scenarios, 4 different power levels for running a machine, etc. In this case the interest of the experimenter is to make all the possible pair wise comparisons among treatments.

Treatments in the design could be structured *i.e.*, treatments are all possible combinations of levels of several factors (factorial experiment); *e.g.* combinations of three levels of nitrogen, three levels of phosphorous, two levels of potash, two different spacings, two different dates of sowing making a total of 72 treatment combinations. In this case the interest of the experimenter is to estimate the main effects of the factors and interactions among factors.

Once the data has been generated through the design, the model to explain the data is defined. A typical model could be

response = constant + explainable part of variability + unexplainable part of variability (or error)

Utilizing the fact that the explainable part of the variability has two major components *viz.*, due to treatments and due to experimental units, the model may be rewritten as response = constant + treatments effect + experimental units effect + error

It, therefore, follows that the model is defined by the way the experiment is designed and the way the data are analyzed follows from the model defined. Thus we have the following flow chart:

$$\left.\begin{array}{r}\textit{Treatments}\\ \\ \textit{Experimental Units}\end{array}\right] \rightarrow \textit{design} \quad \rightarrow \textit{model} \quad \rightarrow \textit{analysis of data.}$$

The data generated are then analyzed. The broad analysis of variance from a total of *n* observations is given below. The degrees of freedom can be appropriately found for a given design.

ANOVA				
Source	**DF**	**SS**	**MS**	**F-ratio**
Model or explainable part of variability	s	SSM	MSM = SSM/s	MSM/ MSE
Unexplainable part of variability or Error	n–s–1	SSE	MSE = SSE/ (n–s–1)	
Total	n - 1	SST		

The analysis of variance can further be restructured as

Treatments Unstructured	
Source	**DF**
Treatments	a
Experimental units	b
Error	n–a–b–1
Total	n–1

Treatments Structured	
Source	**DF**
Treatments	a
Main Effects	a1
All Interactions	a-a1
Experimental units	b
Error	n–a–b–1
Total	n–1

It may be seen from above that there is no difference in the analysis if the treatments are unstructured or structured. In case the treatments are structured, then the treatment sum of squares can be further partitioned into the sum of squares due to main effects and interactions.

However, the important question to be addressed is the choice of a design for a particular experimental setting. The choice would be determined by (a) the nature of treatments (treatments structured or unstructured); (b) variability in the experimental units and the way it is accounted for; (c) questions to be answered or inference problem.

The purpose of this document is to demonstrate how experiments have been designed for different experimental situations in agricultural sciences. These designs have actually been adopted by the experimenters in agricultural sciences and the data generated have been analyzed. No claim is being made for this coverage to be exhaustive. More emphasis has been laid on efforts made by IASRI, New Delhi in last 10-15 years in suggesting more appropriate, modern and sophisticated experimental designs to the experimenters. At places some designs have been described that have a strong potential of applications in those situations where illustrated. (For detail, the readers may also like to see "A *Profile of Design of Experiments at IASRI,*" and "*Glimpses of Basic Research in Design of Experiments at IASRI*"; in "IASRI: An Era of Excellence" available at http://www.iasri.res.in).

Sections 2, 3 and 4 respectively highlight the usefulness of designs with unstructured treatments in conducting experiments while sections 5, 6, 7 and 8 respectively describe the usefulness of designs with structured treatments through some real life experiments. Section 9 describes various web resources that help in disseminating knowledge about designed experiments and analysis of data, which can be fruitfully used by the experimenters.

2. INCOMPLETE BLOCK DESIGN

If there is only one source of variability in the experimental units, and it is not possible to have number of homogeneous experimental units equal to the number of treatments, then recourse is made to incomplete block designs. As the name itself suggests, these designs do not accommodate all the treatments in a block. The experimental units are grouped into blocks in such a way that the experimental units within a block are as homogeneous as possible and the variability between the blocks is very large. Some examples of incomplete block designs are given in the sequel.

Blocks	**$v = 13 = b, r = 4 = k, \lambda = 1, n = 52$**			
Block 1	1	5	6	8
Block 2	2	6	7	9
Block 3	3	7	8	10
Block 4	4	8	9	11
Block 5	5	9	10	12
Block 6	6	10	11	13
Block 7	7	11	12	1
Block 8	8	12	13	2
Block 9	9	13	1	3
Block 10	10	1	2	4
Block 11	11	2	3	5
Block 12	12	3	4	6
Block 13	13	4	5	7

This experiment is generally run as a randomized complete block (RCB) design. But a RCB design would require blocks of size 13. These would be large blocks and the experimental units within a block may not be homogeneous. On the other hand, the design suggested has blocks of size 4. There is a reduction in the block size to the order of 1/3. Obviously, small blocks are expected to have more homogeneity among units within blocks leading thereby to smaller intra block variance compared to larger (and therefore, heterogeneous) blocks where the intra block variance is expected to be large.

This design, indeed, is a balanced incomplete block (BIB) design and would require 52 experimental units. However, if the experimenter wants to cut down on the total number of observations so as to economize the cost of running the experiment, an alternative but A-efficient design could be the following:

An alternate design 2 for $v = 13, b = 8, k = 4, r_1 = r_3 = r_5 = r_6 = r_7 = r_{10} = 3$**; all other treatments are replicated twice,** $n = 32$

Block 1	**Block 2**	**Block 3**	**Block 4**	**Block 5**	**Block 6**	**Block7**	**Block 8**
5	1	3	1	3	6	1	2
7	4	4	2	7	8	10	5
8	6	5	3	9	9	12	6
11	7	10	11	13	10	13	12

The randomization of treatments to the blocks in an incomplete block design involves the following steps: (a) Randomize the treatment labels; (b) Randomize the blocks; (c) Randomize the treatments within each block.

Incomplete block designs like BIB designs have been adopted by All India Coordinated Research Project on Integrated Farming Systems, IIFSR, Modipuram and at several other places. But these designs are necessarily equi-replicated and have common block size. But other incomplete block designs, as described above, are also useful alternative to balanced incomplete block designs because these may not be equi-replicated or have common block size and could be available for fewer experimental units. These designs have a strong potential of application in similar experimental situations and could be a good alternative to balanced incomplete block designs because these lead to saving of scarce resources. A-efficient incomplete block designs are available at Design Resources Server at http://iasri.res.in/design/ibd/ibd.htm.

3. RESOLVABLE DESIGN

In National Agricultural Research and Education System (NARES), many experiments are conducted throughout the country for improvement of crop varieties. These trials have large number of crop varieties and, therefore, require use of incomplete block designs for maintaining homogeneity of experimental units within blocks. It has also been seen that in many crop improvement programmes initial varietal trials and advanced varietal trials are generally conducted using a randomized complete block (RCB) design. The analysis of data generated from such trials reveals that the CV in many of these experiments is high and as a consequence the precision of varieties comparisons is low. This amounts to saying that the error sum of squares is unduly high compared to the sum of squares attributable to the model. Hence, small differences among variety effects may not be detected as significant. A large number of such trials are rejected due to high CV. Incomplete block designs described in Section 2 can be used in such experiments. But from experimenters' considerations, in order to demonstrate the effect of all the treatments at one place, incomplete block designs may not be an appropriate alternative. So to circumvent this problem, recourse is made to resolvable designs. A block design is said to be *resolvable* if the set of b incomplete blocks can be partitioned into r groups (or replicates) containing x blocks, with the property that each treatment is assigned to one unit in each group. In other words, the x blocks in each group form a complete replication. The number of experimental units in each block is k and the number of blocks in each replicate is $x = b/r$. Obviously, the number of treatments is $v = xk$. In resolvable block designs, the between blocks within replication variation helps in reducing the experimental error (provided the experimental units within blocks are homogeneous and between blocks within replication there is heterogeneity), increasing thereby the precision of estimation of treatment contrasts of interest. Among the class of resolvable designs, alpha (α) - designs are very useful class of designs and have now been used extensively by agricultural scientists. A special class of resolvable block designs is square lattice and rectangular lattice. These designs are, however, a particular case of alpha designs. Resolvable designs are also nested designs in which the experimental units are divided into bigger blocks, which are complete replicate. Within each bigger block are smaller blocks, called sub-blocks, which are incomplete blocks. The treatments are allocated to the experimental units within sub-blocks of bigger block.

Resolvable block designs allow performing an experiment one replication at a time. For example, in an agricultural experiment, for example, the land may be divided into a number of large areas corresponding to the replications and then each area is subdivided into blocks. These designs have been adopted for varietal trials in the NARES. These designs are also useful for the field trials with large number of treatments/crop varieties which may not always be laid out in a single location or a single season. Therefore, it is desired that variation due to location or time periods may also be controlled along with controlling within location or time period variation. This can also be handled by using resolvable block designs. Here locations or time periods may be taken as replications and the variation within a location or a time period can be taken care of by blocking.

An Initial Varietal Trial was conducted at S.K. Nagar on mustard crop with 30 entries (treatments). An alpha design in 15 blocks and block size 6 and 3 replications was recommended. The layout of the design is the following:

Replication I				
B_1	**B_2**	**B_3**	**B_4**	**B_5**
30	19	6	1	4
23	5	24	29	25
16	21	26	13	11
2	8	15	22	27
14	28	17	9	18
10	12	3	20	7

Replication II				
B_1	**B_2**	**B_3**	**B_4**	**B_5**
3	19	20	17	26
18	9	15	2	16
13	4	30	12	6
23	29	5	22	1
28	14	10	27	11
8	24	25	7	21

Replication III				
B_1	**B_2**	**B_3**	**B_4**	**B_5**
24	19	30	25	26
28	7	17	3	5
20	1	21	12	22
11	23	13	29	14
8	27	4	16	6
2	15	10	9	18

The analysis of variance of the data generated from an alpha design (resolvable design) is given in the sequel.

ANOVA for RCB design	
Source	**DF**
Treatments	29
Replications	2
Error	58
Total	89

ANOVA for resolvable design	
Source	**DF**
Treatments	29
Blocks	14
Replications	2
Blocks within Replications	12
Error	46
Total	89

It may be seen from the ANOVA table that 12 degrees of freedom due to blocks within replication are taken away from the error. This is the advantage that one gets because of the nested structure. Further, since the blocks are small, it is expected that the intra block variance would also be small.

In another situation an experimenter was planning to compare 40 exotic and indigenous collections / cultivars of early sown Cauliflower. The experimenter was advised to use an alpha design in 40 treatments arranged in 12 blocks of size 10 each and 3 replications with the following layout:

Replication - I										
Block 1	1	5	9	13	17	21	25	29	33	37
Block 2	2	6	10	14	18	22	26	30	34	38
Block 3	3	7	11	15	19	23	27	31	35	39
Block 4	4	8	12	16	20	24	28	32	36	40
Replication - II										
Block 1	1	7	12	16	18	22	25	31	36	39
Block 2	2	8	9	13	19	23	26	32	33	40
Block 3	3	5	10	14	20	24	27	29	34	37
Block 4	4	6	11	15	17	21	28	30	35	38
Replication - III										
Block 1	1	7	10	13	20	23	27	30	36	38
Block 2	2	8	11	14	17	24	28	31	33	39
Block 3	3	5	12	15	18	21	25	32	34	40
Block 4	4	6	9	16	19	22	26	29	35	37

Alpha designs were recommended for four experiments to be conducted with cotton varieties. First two of these experiments were to be conducted to morphologically characterize varieties and hybrids with respect to about 45 morphological characters both qualitative and quantitative and document the database as a part of DUS test for PVP. The parameters of the designs are:

(1) $v = 70$; $b = 20$; $k = 14$; $r = 4$; (2) $v = 70$; $b = 28$; $k = 10$; $r = 4$; (3) $v = 84$; $b = 24$; $k = 14$; $r = 4$;

(4) $v = 28$; $b = 12$; $k = 7$; $r = 3$; (5) $v = 14$; $b = 6$; $k = 7$; $r = 3$;

Besides, these designs have also been recommended and adopted at (a) 22 centres of AICRP on Rapeseed and Mustard, Bharatpur; NBPGR, New Delhi; CSK HPKV, Palampur; IARI, New Delhi; CCS HAU, Hisar; All India Sorghum Crop Improvement Project, Hyderabad.

The randomization of treatments in a resolvable deign is done in the following way: (i) Randomize the treatments, *i.e.*, randomly allocate the treatments to treatment numbers; (ii) Randomize the replications; (iii) Randomize the blocks within each replication; (iv) Separate randomization should be done for each replication; (v) Randomize the treatments to experimental units within each block; (vi) Separate randomization should be done for each of the blocks.

Randomized layout of alpha design is available at Design Resources Server at (number of treatments ≤ 150) http://iasri.res.in/design/Alpha/catalogue.htm. In 2007, a monograph on alpha design was also published at ICAR-IASRI.

4. AUGMENTED DESIGN

A typical experimental setting could be one where the v treatments in the experiment could be assumed to be composed of two disjoint sets, (a) first set contains w treatments called test treatments or tests, (b) second set contains u treatments called control treatments or controls, $u \geq 1$; $w + u = v$. The interest of the experimenter is in making tests *versus* controls comparisons. The comparisons among tests and among controls are of no consequence to the experimenter.

Two types of set ups have been studied in the literature, (a) Single replication of tests; controls replicated; (b) Tests replicated; controls replicated. Designs for situation (a) are generally termed as augmented designs while designs for second situation (b) are termed as reinforced designs.

Suppose that $v = w + u$ (w tests and u controls) treatments are arranged in b blocks with block sizes as $k_1, k_2, \ldots, k_b$, such that $k_1 + k_2 + \ldots + k_b = n$. The analysis of variance of the data generated from such an experiment would be the following:

ANOVA	
Source	**DF**
Blocks (Adjusted)	b – 1
Treatments (Adjusted)	v – 1
Among Tests	w – 1
Among Controls	u – 1
Tests vs Controls	1
Error	n – v – b + 1
Total	n –1

The randomization of treatments for the case when the tests and the controls are replicated is like any other block design. However, the randomization, when the tests have single replication, is tricky and the experimenters generally commit mistake in doing a proper randomization. The steps involved are the following: (a) Follow the standard randomization procedure for the known design in control treatments or check varieties; (b) Test treatments or new varieties are randomly allotted to the remaining experimental units, as in a completely randomized design; (c) If a new treatment appears more than once, assign the different entries of the treatment to a block at random with the provision that no treatment appears more than once in a block until that treatment appears once in each of the blocks.

A question generally asked by the experimenters for running this experiment with single replication of tests is as to how many times each control should be replicated in each block. This question has been answered and optimum replication of controls on each block has been obtained by minimizing the information per observation. Software has been developed and is available online at Design Resources Server for generating randomized layout of an augmented design (http://iasri.res.in/design/Augmented Designs/home.htm).

Augmented designs for both the setups when the tests have single replication and the tests can be replicated have been used immensely by the experimenters. This software has helped in generating the randomized layout of the design with optimum replication for the situation when the tests have single replication. On the other hand for the second situation when the tests are also replicated, reinforced designs have been used. The design for tests may be an alpha design or any other incomplete block design or a block design with treatments structure as factorial in nature. In the sequel are described examples for both the experimental settings.

For the purpose of illustration a design is obtained using the software for number of tests, $w = 54$, number of controls, $u = 4$, number of blocks, $b = 6$, block size, $k = 13$ $(9 + 4)$, number of plots required = $54 + (4\times6) = 78$. The optimum replication of controls in this example is one. The randomized layout of the design is (T# denotes the test treatment and C# denotes the control treatment):

Randomized layout of an augmented design					
B_1	**B_2**	**B_3**	**B_4**	**B_5**	**B_6**
T44	C2	C1	T54	C4	T6
T31	C3	T45	T13	T37	T17
C1	T38	C4	T9	C1	C1
C4	T36	T10	T16	T5	C3
T33	C1	C2	C1	C3	T2
C2	T21	T18	C4	C2	T25
T35	T4	T39	T49	T7	C2
T40	C4	T26	T53	T23	T14
T11	T52	T46	C3	T3	C4
C3	T42	C3	T8	T12	T43
T15	T32	T20	T48	T1	T50
T27	T24	T29	C2	T41	T34
T51	T47	T22	T28	T19	T30

Consider another example for situation (b) where the tests are also replicated. A trial is conducted with 21 new strains of Toria *vis-a-vis* 3 controls (checks) using an α - design for new strains and the three checks augmented in each of the blocks once. This is a reinforced alpha design. The details of the experiment are (a) α - design for 21 new strains has the parameters $w = 21$, $b = 9$, $r = 3$ and $k = 7$; (b) After augmenting the $u = 3$ checks, the resulting design has parameters $v = 21 + 3$; $b = 9$; $r_1 = 3$; $r_0 = 9$; and $k = 10$; $n = 90$.

Replication I		
B_1	**B_2**	**B_3**
1	2	3
4	5	6
7	8	9
10	11	12
13	14	15
16	17	18
19	20	21

Replication II		
B_1	**B_2**	**B_3**
1	2	3
5	6	4
8	9	7
12	10	11
13	14	15
17	18	16
21	19	20

Replication III		
B_1	**B_2**	**B_3**
1	2	3
5	6	4
9	7	8
11	12	10
15	13	14
18	16	17
20	21	19

Replication I		
A	B	C
B	C	A
C	A	B

Replication II		
A	B	C
B	C	A
C	A	B

Replication III		
A	B	C
B	C	A
C	A	B

Alternatively, one could have used an α - design in 24 treatments. The two options possible are (a) $v = 24$; $b = 9$; $r = 3$; $k = 8$; $n = 72$; (b) $v = 24$; $b = 16$; $r = 4$; $k = 6$; $n = 96$. But these designs are not good for this experimental setting. As stated earlier, inference problem decides what design to use.

Another reinforced alpha design in 55 treatments (50 tests and 5 check varieties of Tomato) arranged in 10 blocks each of size 15 and 2 replications was recommended for Germplasm Evaluation at NBPGR, New Delhi. The replications of the check varieties are 10 each. The layout of the design recommended is as given:

Replication I				
B_1	**B_2**	**B_3**	**B_4**	**B_5**
21	12	18	9	25
31	47	48	14	45
16	22	28	19	35
26	37	23	34	20
36	17	3	39	40
41	32	8	24	15
11	42	33	4	30
6	27	38	49	5
46	7	13	44	50
1	2	43	29	10
55	51	51	54	53
52	54	55	55	51
54	52	53	53	54
51	53	54	51	55
53	55	52	52	52

Replication II				
B_1	**B_2**	**B_3**	**B_4**	**B_5**
38	27	9	36	24
34	35	49	50	44
7	20	31	4	37
25	8	15	29	6
19	41	3	23	46
1	2	22	32	30
45	48	42	10	5
13	14	40	17	18
26	21	28	43	33
47	39	16	11	12
53	52	54	52	55
51	54	52	55	51
55	51	53	53	53
52	55	51	54	52
54	53	55	51	54

5. BLOCK DESIGN WITH FACTORIAL STRUCTURE

These are the designs for experiments where treatments are structured. These are muti-factor experiments with each factor having several levels. In the sequel we describe an experimental situation called crop sequence experiments, where these designs have been used effectively. The underlying idea of conducting crop sequence experiments is to improve cropping intensity; soil nutrients; and returns to farmers.

Category I experiments

A crop sequence experiment comprises of two crops, one grown in kharif season followed by another grown in rabi season. Two sets of treatments are applied in succession; m treatments are applied in kharif crop and n treatments are applied in rabi crop. On application of n treatments in rabi crop, mn treatment combinations appear. The treatment structure is factorial in nature (two factors at m and n levels respectively). It is indeed possible that the m kharif and / or n rabi treatments also have factorial structure. The observations are recorded in both the seasons. The interest of the experimenter is in (a) direct effects of kharif and rabi treatments, (b) residual effects of kharif treatments, and (c) interaction between residual effects of kharif treatments and direct effects of rabi treatments.

These experiments are generally conducted as: (a) Randomized Complete Block (RCB) design for m kharif treatments; (b) For application of rabi treatments, the plots of kharif experiment are subdivided into n sub-plots (number of treatments in the rabi crop); (c) Resulting design is split-plot design for rabi treatments.

As in split plot designs, (a) main-plots provide residual effect of m kharif treatments; (b) sub-plots give direct effects of n rabi treatments; (c) main-plot × sub-plot interaction provides the interaction of residual effect of kharif and direct effect of rabi treatments.

It is assumed that residual effects persist for one season, *i.e.*, treatments applied to kharif crop have residual effect on rabi crop and treatments applied to rabi crop have residual effect on the following kharif crop. Usually experiments are continued for two or more number of years. In second year, m kharif treatments are again applied on main plots (*same randomization*) and observations are recorded sub-plot wise. Sub-plots now give residual effects of rabi treatments and main-plots give direct effects of kharif treatments and the procedure is continued.

But this design has some limitations, *viz.*, (a) precision of main plot treatments (representing the residual effects) is generally low, (b) each block is a complete replicate and with large number of treatment combinations it is difficult to maintain homogeneity within block, (c) because of two error components, combined analysis of experiments conducted over years is a problem particularly when the error variances are heterogeneous, and (d) identification of best treatment combination is a problem.

What is the alternative? An *Incomplete Block design with orthogonal factorial structure* (*OFS*) *and / or balance* is an alternative. *Extended Group Divisible* (*EGD*) *designs* have OFS with balance. In view of practical problems one needs a *resolvable solution.* But the choice of design depends upon the main effects efficiency. Keeping in mind the experimenter's interest, one needs a design with full main-effects efficiency and controlled or high two-factor efficiency. The design should also be valid even when the treatments in both or any crop also have a factorial structure.

A rice – wheat crop sequence experiment was to be planned by the Division of Agronomy, ICAR-IARI, New Delhi. The kharif crop (rice) had five herbicidal treatments (1, 2, 3, 4, 5) and 3 replications. The rabi crop (wheat) had four herbicidal treatments (a, b, c, d) applied. An extended group divisible design (or a rectangular design) 5×4 in 10 plots per block was advised and adopted at Division of Agronomy, IARI, New Delhi. The kharif crop rice used 5 herbicidal treatments, while the rabi crop wheat uses 4 herbicidal treatments. The layout of the design is given below:

Replication I	
Block 1	**Block 2**
1 a	1 c
5 b	5 c
3 b	5 d
4 a	4 c
5 a	2 d
1 b	1 d
2 b	2 c
3 a	3 d
4 b	4 d
2 a	3 c

Replication II	
Block 1	**Block 2**
5 d	3 b
2 d	2 c
3 a	1 b
4 d	4 b
5 a	4 c
1 d	1 c
2 a	2 b
3 d	5 b
4 a	3 c
1 a	5 c

Replication III	
Block 1	**Block 2**
5 d	4 a
2 d	2 a
3 b	3 a
4 b	3 c
2 b	5 a
1 d	1 c
5 b	2 c
3 d	1 a
4 d	4 c
1 b	5 c

The analysis of variance is given below. The analysis of data using RCB design for kharif treatments and Split-plot design after giving rabi treatments is also given for comparison purpose only.

<table>
<tr><th colspan="4">Analysis for first year Kharif</th></tr>
<tr><th colspan="2">RCBD (Kharif)</th><th colspan="2">EGD (Kharif)</th></tr>
<tr><td>Source</td><td>DF</td><td>Source</td><td>DF</td></tr>
<tr><td>Replications</td><td>2</td><td>Blocks</td><td>5</td></tr>
<tr><td>Treatments</td><td>4</td><td>Direct (Kharif)</td><td>4</td></tr>
<tr><td>Error</td><td>8</td><td>Error</td><td>50</td></tr>
<tr><td>Total</td><td>14</td><td>Total</td><td>59</td></tr>
</table>

<table>
<tr><th colspan="4">Analysis for first year Rabi</th></tr>
<tr><th colspan="2">Split – Plot (Rabi)</th><th colspan="2">EGD (Rabi)</th></tr>
<tr><td>Source</td><td>DF</td><td>Source</td><td>DF</td></tr>
<tr><td>Replications</td><td>2</td><td>Blocks</td><td>5</td></tr>
<tr><td>Main-Plot [Residual kharif]</td><td>4</td><td>Replications</td><td>2</td></tr>
<tr><td>Error (a)</td><td>8</td><td>Blocks within Replications</td><td>3</td></tr>
<tr><td>Sub-Plot [Direct rabi]</td><td>3</td><td>Residual (kharif)</td><td>4</td></tr>
<tr><td>MP × SP</td><td>12</td><td>Direct (rabi)</td><td>3</td></tr>
<tr><td>Error (b)</td><td>30</td><td>Residual * Direct</td><td>12</td></tr>
<tr><td>Total</td><td>59</td><td>Error</td><td>35</td></tr>
<tr><td></td><td></td><td>Total</td><td>59</td></tr>
</table>

As can be seen from the above ANOVAs, there is only one error in the design used as against two error components in the ANOVA of a split-plot design. This is a clear advantage of using the suggested design. Moreover, the design adopted is an incomplete block design. That has its own advantages.

The ANOVA for the second year is the following:

Analysis for second year (EGD)			
Kharif Season		**Rabi Season**	
Source	DF	Source	DF
Blocks	5	Blocks	5
Replications	2	Replications	2
Blocks within replications	3	Blocks within replications	3
Residual (Rabi)	3	Residual (Kharif)	4
Direct (Kharif)	4	Direct (Rabi)	3
Residual * Direct	12	Residual * Direct	12
Error	35	Error	35
Total	59	Total	59

Using this analysis, the best treatment can be picked up easily.

Motivated by the use of block designs with factorial structure by the experimenters, resolvable designs have been obtained at IASRI with any number of replications that allow estimation of main effects (direct and residual effects) with full efficiency and interactions with high efficiency. The parameters of these designs are

number of treatments, $v = m \times n = f_1 \times f_2 \times \ldots \times f_p \times h_1 \times h_2 \times \ldots \times h_q$; $n > m$, and have a common factor $1 < f < m$, *i.e.*, $m = g_1 f$ and $n = g_2 f$; number of blocks, $b = rf$; replication = r; (minimum block size) $k = g_1 g_2 f$.

It is indeed possible that some interactions are also estimated with full efficiency. Randomized layout of these designs is available at Design Resources Server (www.iasri.res.in/design/factorial/factorial.htm). However, when m and n are co-primes, then there is a problem of obtaining a block design with OFS. This is an open problem.

An extended group divisible design 4×2^2 in 8 plots per block has been adopted at Kanpur Centre of AICRP on CSR (now AICRP on IFS), PDFSR (now ICAR-IIFSR), Modipuram, Kanpur Centre. The kharif rice uses 4 levels of P_2O_5, while the rabi crops use 2 doses of P_2O_5 in each of the two-summer crops *viz. Sasbenia* for green manure and *Green gram.*

A reinforced extended group divisible design 13 (12+1)×2 in 13 plots per block has been adopted at Division of Agronomy, IARI, New Delhi. The kharif crop rice uses13 treatments (combination of 3 doses of N from 4 different formulations and one control), while the rabi crop mustard uses 2 doses of fertilizers.

A reinforced extended group divisible design $3^2 \times 2+1$ in 7 plots per block has been adopted by PDFSR (now ICAR-IIFSR), Modipuram at 32 Research Stations across the country. The treatment combinations are levels of three factors, *viz.*, Nitrogen (40, 80 and 120 kg/ha), Phosphorous (0, 40 and 80 kg/ha) and Potassium (0 and 40 kg/ha) and one absolute control.

Further, a reinforced extended group divisible design $4 \times 2^2 + 8$ in 12 plots per block has been adopted by Division of Agronomy, ICAR-IARI, New Delhi, in Rainfed Agriculture. The treatments are combinations of 4 levels of biofertilizers and 2 levels each of P_2O_5 and 2 method of placement. The 8 control treatments are the combinations of 4 biofertilizers given with and without water.

Category II experiments

In these experiments, once again two sets of treatments are applied in succession; *m* treatments are applied in kharif crop and *n* treatments are applied in rabi crop. On application of *n* treatments in rabi crop, instead of *mn* treatment combinations only a proper subset of *mn* treatment combinations appear. The interest of the experimenter is in (a) direct effects of kharif and rabi treatments, and (b) residual effects of kharif treatments. The interaction between residual effects of kharif treatments and direct effects of rabi treatments is not of interest to the experimenter.

An experiment was conducted at Indore centre of AICRP on Cropping Systems Research (On-Stations) to study the effect of frequency of Phosphorous (P) application for judicious use of P fertilizers in soybean – wheat sequence. The treatments tried in the experiment are given in the table below:

Treatment	Soybean (Kharif)	Wheat (Rabi)
1	No Phosphorus	No Phosphorus
2	No Phosphorus	100% P
3	50% P	50% P
4	50% P	100% P
5	100% P	No Phosphorus
6	100% P	50% P
7	100% P	100% P
8	50% P + FYM	50% P + FYM
9	50% P + FYM	50% P
10	50% P + FYM + PSM	50% P + FYM + PSM

The observations are recorded in both the seasons. Strictly speaking, at the end of the second season this becomes bivariate data, which is ultimately converted into univariate data by creating an index of the type gross returns or net returns or energy equivalent or protein equivalent, etc. The univariate data in the form of index is then analyzed.

A close look at treatment structure shows that although there are 10 treatments applied in each season, the number of distinct treatment in each season is 5 only. After giving the treatments in the second season, there are 10 distinct treatment combinations, although there should have been 25 treatment combinations. This means that only a fraction of 25 treatments have been tried in the experiment. The distinct treatment with repetitions are

Distinct Treatment (Phosphorus)		Repetitions in	
		Kharif crop	Rabi crop
No Phosphorus	T_1	2	2
50% P	T_2	2	3
100% P	T_3	3	3
50 % P + FYM	T_4	2	1
50% P + FYM + PSM	T_5	1	1

For studying the direct effects of treatments applied to Soybean, the data are analyzed as a general block design with 5 treatments, 3 blocks and replications of treatments as given in the table above. For testing the residual effect of Phosphorus treatments of soybean on wheat and the direct effect of Phosphorus treatments applied to wheat, a connection is developed between the structurally incomplete row–column design and designs for Category II experiments.

The row-column design has the (a) replications of the original designs as rows; (b) treatments applied to Soybean crop (first set of treatments) as columns; (c) cells of the array have the treatments applied to the wheat crop (second set of treatments). The following table is generated from Table of treatments:

30 Observations	**First Set of Treatments**				
	1	**2**	**3**	**4**	**5**
Replication I	1, 3	2, 3	1, 2, 3	2, 4	5
Replication II	1, 3	2, 3	1, 2, 3	2, 4	5
Replication III	1, 3	2, 3	1, 2, 3	2, 4	5

This design is disconnected and, therefore, it is not possible to answer all the questions that needed to be answered. So the choice of treatment combinations or the fraction is not proper.

One may, therefore, add one more treatment combination <u>50% Recommended P + FYM</u> (T_4) during kharif followed by <u>50% Recommended P + FYM + PSM</u> (T_5) during rabi to the existing 10 making it 11 treatment combinations. The structure of row-column design now is the following:

33 Observations	**First Set of Treatments**				
	1	**2**	**3**	**4**	**5**
Replication I	1, 3	2, 3	1, 2, 3	2, 4, 5	5
Replication II	1, 3	2, 3	1, 2, 3	2, 4, 5	5
Replication III	1, 3	2, 3	1, 2, 3	2, 4, 5	5

This is a connected design and all the questions can be answered using this design. However, addition of a treatment combination leads to generation of 3 more observations. On the other hand, the experimenter may not like to add observations because of cost constraint. The problem can be remedied without increasing the number of observations. The experimenter may replace the combination 50% Recommended P + FYM (T_4) during kharif and 50% P (T_2) during rabi with 50% Recommended P + FYM (T_4) during kharif and 50% P + FYM + PSM (T_5) during rabi in any one of the three replications. It is not desirable to make this change in all the replications. It can be made in any one replication as well. Thus, no additional resources are required to run the experiment. If the replacement is done in third replication only, then the structure of row-column design is the following:

33 Observations	**First Set of Treatments**				
	1	**2**	**3**	**4**	**5**
Block I	1, 3	2, 3	1, 2, 3	2, 4	5
Block II	1, 3	2, 3	1, 2, 3	2, 4	5
Block III	1, 3	2, 3	1, 2, 3	4, 5	5

The design is connected and all the questions can now be answered using this design.

6. RESPONSE SURFACE METHODOLOGY

Experiments under AICRP on Soil Test Crop Response correlations are conducted on a soil with a wide range of soil fertility in terms of available nitrogen (N), phosphorus (P) and potassium (K). For getting wide ranges of soil fertility, normally fertility gradients are created in the previous season. The whole area is divided into four equal strips. On each strip four different fertilizer treatments *viz.* 0X, 0.5X, X and 2X are applied. Here X denotes the recommended dose of N, P and K. This is followed by sowing of an exhaust crop, preferably a crop that is not to be taken as a test crop in the next season. Demarcation of strips is maintained after harvest of the exhaust crop so as to facilitate the laying out of the soil test crop response correlation experiment in next season.

The main objectives of the soil test crop response experiment are (a) to establish relationship between resultant crop yield and soil test values (N, P and K available in the soil, before the crop is sown) and the fertilizer doses (added N, P, K after the crop is sown); (b) to obtain optimum levels of fertilizer doses for maximum crop yield for given soil test values; and (c) to obtain balanced fertilizer recommendations for the specific target yields.

The design for fitting response surface should have design points that (a) enable to study the response due to N, P, and K; (b) enable to study the accumulation behaviour of N, P, and K; (c) enable to study the dilution behaviour of N, P, and K; (d) includes treatment combination corresponding to nearly optimum fertilizer dose of N, P, and K; (e) includes a treatment combination corresponding to highest level of N, P, and K. Since these experiments have continued for long time, the experimenters have sufficient knowledge of the optimum levels of N, P and K from the results of previous experiments. Therefore, such experiments may be run with 3 levels of N, P and K each, one above, one below and one about the expected optimum level, respectively. The 3 levels of N, P, and K may not include zero application (or no application) of the nutrient as one of the levels. Absolute control treatments, however, may be incorporated in the design.

The design proposed is the following;

Sl. No.	N	P	K
1.	1	2	2
2.	2	2	2
3.	3	2	2
4.	2	1	2
5.	2	3	2
6.	2	2	1
7.	2	2	3
8.	1	1	1
9.	2	1	1
10.	1	2	1
11.	1	1	2
12.	2	3	3
13.	3	3	3
14.	3	3	2
15.	3	2	3
16.	0	0	0
17.	0	0	0
18.	0	0	0
19.	3	1	1
20.	3	2	1
21.	3	3	1
22.	2	2	0
23.	2	0	2
24.	0	2	2

In this design 1, 2, 3 denote, respectively the three levels of N, P, K. Points 22-24 were included for studying dilution behaviour of N, P and K on the request of Project Co-ordinator. However, some agronomists may feel that P and K should not be used with 0 level of N.

Points 1, 2, 3 enable to study the response to N at middle levels of P, K. Similarly, points 2, 4, 5 enable to study the response to P at middle levels of N and K and points 2, 6, 7 enable to study the response to K at middle levels of N and P.

This design has been adopted by all the co-ordinating centres of AICRP on STCR.

This experiment also aimed at evolving basis for conjoint use of organic manures (OM) and bio-fertilizers (BF) efficiently in providing integrated nutrient supply system. For 3 levels of OM and 3 strips the design recommended is the following:

	Strip 1	**Strip 2**	**Strip 3**
OM1	A	B	C
OM2	B	C	A
OM3	C	A	B

Here A, B and C are 8 design points each with following structure:

A	**B**	**C**
0 0 0	0 0 0	0 0 0
3 1 1 and 2 2 0	3 2 1 and 2 0 2	3 3 1 and 0 2 2
Any 5 points from 1–15	Any of the 5 points from the remaining 10 points (from 1–15)	Remaining 5 points

This is a semi-Latin Square type of arrangement. All treatment combinations are tried on each level of OM. All treatment combinations are also tried on all the strips. All the three groups *viz*., A, B, C appear with every level of OM and also in all the strips precisely once.

This design may alternatively be viewed as reinforced resolvable block design with 3 resolvable groups. Each group is a complete replicate. The 3 levels of OM are the 3 replications or the 3 resolvable groups. There are 3 blocks within each replication. The 3 strips on each level of OM are the 3 blocks. In all there are 9 blocks. There are 7 treatment combinations in each block. Each block is reinforced with a control treatment.

Designs for fitting response surfaces have been used in different situations in agricultural research. It may indeed be possible that the doses of levels of all the factors are generally equispaced. In that case the designing of an experiment is quite involved. In the sequel we shall describe designs that have actually been used in some situations:

To obtain the optimum combination of levels of the factors given in the table below (osmotic dehydration of banana), an experiment was conducted at the Division of Agricultural Engineering, ICAR-IARI, New Delhi.

Sl. No.	Factor	Levels
1.	Concentration of sugar Solution	40%, 50%, 60%, 70% and 80%
2.	Solution to sample ratio	1:1, 3:1, 5:1, 7:1 and 9:1
3.	Temperature of Osmosis	25^{0}c, 35^{0}c, 45^{0}c, 55^{0}c and 65^{0}c

A modified second order rotatable response surface design for 3 factors each at 5 *equi-spaced* levels in 36 design points was recommended. The design points with levels coded as -2, -1, 0, 1, 2 and factors coded as X1, X2 and X3 are given in the table below:

No.	X1	X2	X3
1	-1	-1	-1
2	-1	-1	1
3	-1	1	-1
4	1	-1	-1
5	-1	1	1
6	1	-1	1
7	1	1	-1
8	1	1	1
9	-1	-1	-1
10	-1	-1	1
11	-1	1	-1
12	1	-1	-1
13	-1	1	1
14	1	-1	1
15	1	1	-1
16	1	1	1
17	-2	0	0
18	2	0	0
19	0	-2	0
20	0	2	0
21	0	0	-2
22	0	0	2
23	0	0	0
24	0	0	0
25	0	0	0
26	0	0	0
27	0	0	0
28	0	0	0
29	0	0	0
30	0	0	0
31	0	0	0
32	0	0	0
33	0	0	0
34	0	0	0
35	0	0	0
36	0	0	0

An experiment was conducted to study production of low fat meat products using fat replacers at Division of Biotechnology, ICAR-IVRI, Izatnagar.

The experimenter was interested in obtaining optimum combination of fat replacers. The factors and the levels tried in the experiment were:

Sl. No.	**Factor**	**Levels**
1.	Whey protein	2%, 3%, 4%, 5% and 6%
2.	Guar Gum	0.50%, 0.75%, 1.00%, 1.25% and 1.50%
3.	Starch	2.0%, 2.5%, 3.0%, 3.5% and 4.0%

A modified second order rotatable response surface design for 3 factors, each at 5 equi-spaced levels in 28 design points was recommended. The 28 design points were essentially the same as that of design points 1- 28 in the above experiment on banana.

An experiment was conducted with an objective to obtain optimum storability conditions of Instant Pigeon Pea (Dal) at the Division of Agricultural Engineering, ICAR-IARI, New Delhi. The factors and the levels tried in the experiment were:

Sl. No.	**Factor**	**Levels**
1.	Soaking Solution Concentration	0.5, 1.0, 1.5%
2.	Cooking Time	8, 10, 12 minutes
3.	Flaking Thickness	0.5, 0.75, 1.0 mm

A modified second order response surface design (Box-Behnken design with 4 center points, the number of center points decided on the basis of the modified second order response surface design) for 3 factors each at 3 *equi-spaced* levels in 16 design points was recommended. The design points with coded levels as –1, 0, 1 are given in the table below:

X1	**X2**	**X3**
1	1	0
1	-1	0
-1	1	0
-1	-1	0
1	0	1
1	0	-1
-1	0	1
-1	0	-1

X1	**X2**	**X3**
0	1	1
0	1	-1
0	-1	1
0	-1	-1
0	0	0
0	0	0
0	0	0
0	0	0

The Division of Post Harvest Technology, ICAR-IARI, New Delhi planned an experiment related to osmotic dehydration of Aonla with the objective to obtain optimum combination of levels of solution to sample ratio, concentration of sugar solution, revolutions per minute and temperature of osmosis. The factors and levels tried in the experiment were:

Sl. No.	**Factor**	**Levels**
1.	Solution to sample ratio	1:1, 2:1, 3:1, 4:1 and 5:1
2.	Concentration of sugar solution	30%, 40%, 50%, 60% and 70%
3.	Revolutions per minute	100, 150, 200, 250 and 300
4.	Temperature of osmosis	25^0C, 30^0C, 35^0C, 40^0C and 45^0C

A second order rotatable response surface design with orthogonal blocking for 4 factors each at 5 equi-spaced levels in 30 design points arranged in three blocks each of size 10 was suggested. The layout of design with levels coded as -2, -1, 0, 1, 2 and factors coded as X1, X2, X3, X4 is as given below:

Block - I				**Block - II**				**Block - III**			
X1	X2	X3	X4	X1	X2	X3	X4	X1	X2	X3	X4
-1	-1	-1	-1	-1	-1	-1	1	2	0	0	0
-1	-1	1	1	-1	-1	1	-1	-2	0	0	0
-1	1	-1	1	-1	1	-1	-1	0	2	0	0
1	-1	-1	1	1	-1	-1	-1	0	-2	0	0
1	-1	1	-1	-1	1	1	1	0	0	2	0
1	1	-1	-1	1	-1	1	1	0	0	-2	0
-1	-1	1	1	1	1	-1	1	0	0	0	2
1	1	1	1	1	1	1	-1	0	0	0	-2
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0

7. MIXED ORTHOGONAL ARRAYS (FOR FRACTIONAL FACTORIALS)

Orthogonal arrays (or mixed orthogonal arrays) of strength two are fractional factorial designs or a symmetric (or asymmetric) factorial experiment that permit orthogonal estimation of general mean and all main effects under the assumption that all interactions are absent. Mixed orthogonal arrays have been recommended and used in an experiment at ICAR-IARI, New Delhi.

A Scientist from Division of Agricultural Chemicals, ICAR-IARI, New Delhi, wished to conduct an experiment for preparation of super absorbent composites with maximum water absorption characteristics and enhanced stability and moisture absorption behaviour in plant growth media with an aim to achieve maximum and fast rate of absorbency utilizing minimum possible concentration of monomer, cross linker and alkali. The various factors along with their levels were

Factor	Levels
Nature of alkali (A)	3
Duration of alkali exposure(B)	3
Temperature of reaction(C)	3
Concentration of Alkali (D)	5
Backbone: Clay ratio (E)	5

Factor	Levels
Monomer Concentration (F)	5
Cross linker Concentration(G)	5
Initiator Concentration (H)	5
Volume of Water (I)	5

This is a typical $3^3 \times 5^6$ asymmetric factorial experiment and even a single replication would require 421,875 runs (or design points or treatment combinations). No experimenter can afford so many runs for an experiment.

The interest of the experimenter was to identify the important factors out of the 9 factors in the experiment and so main effects of the factors were of major concern. The two factor and other interactions were not of much interest and can be assumed to be absent.

Obviously, one has to look for a fractional factorial or a subset of 421,875 design runs. On discussion with the experimenter it was found that only 70-80 design runs could be affordable.

Mixed orthogonal arrays of strength two can be used as a design for this experiment. This would allow the estimation of general mean and all main effects without correlations. But the factors have 3 and 5 levels and these numbers are co-prime. A mixed orthogonal array of strength two for a $3^3 \times 5^6$ experiment would need at least 225 runs. But it is not sure that such an array is existent. The number of design points is another issue if such an array exists. But it was required to suggest to the experimenter a fractional factorial plan in 72 runs arranged in $\underline{4}$ blocks each of size 18 for a $3^3 \times 5^6$ factorial experiment. The blocking was required because it would not be possible to maintain homogeneity among the experimental units. Blocking also adds to the complexity of the problem.

However, a mixed orthogonal array of strength two exists for a $3^3 \times 5^6$ factorial experiment in 72 runs. The desired fractional factorial design for a $3^3 \times 5^6$ factorial experiment can be obtained from a $3^3 \times 5^6$ mixed orthogonal array of strength two by collapsing one of the levels of all the factors with 6 levels. The resulting design thus obtained is a fractional factorial design for a $3^3 \times 5^6$ experiment in 72 runs with four blocks of size 18 each. This, however, is not an orthogonal array. The use of this fractional factorial ensures inter effect orthogonality, but the intra effect orthogonality was not possible. This was the small cost the experimenter had to bear. This design was adopted at all the centres in the country.

The suggested design had unequal replications of the levels of factors with five levels. This was not acceptable to the experimenter. So in the following year the experimenter changed the levels of the factors to 3 and 6 respectively. With this change, a design was advised with 72 design runs only and four blocks of size 18 each. But with this change the experimenter was able to accommodate more than 9 factors in the experiment, in fact 13 factors, 7 factors with 3 levels and 6 factors with 6 levels. The new factors and levels tried in the experiment were:

Factor	**Levels**
A (clay type)	6
B (backbone clay ratio)	6
C (maonomer conc)	6
D (crosslinker conc)	6
E (Initiator conc.)	3
F (volume of water)	6
G (duration of reaction-1)	3

Factor	**Levels**
H (Temperature of reaction-1)	3
I (Nature of alkali)	3
J (duration of exposure)	6
K (Conc. Of alkali)	6
L (Temperature of reaction-2)	3
M (volume of alkali)	6

Being motivated with the adoption of fractional factorials for mixed factorial experiments obtained from mixed orthogonal array, resolvable mixed orthogonal arrays of strength two have been obtained and are available at Design Resources Server at http://iasri.res.in/design/Oarray/oa/OA/catalogue.htm. These designs are very useful as fractional factorial designs for asymmetric factorial experiments.

8. MIXTURE EXPERIMENTS

An experiment was conducted at Division of Fruits and Horticultural Technology, ICAR-IARI, New Delhi, with an objective to study feasibility of blending of fruit juice/pulp of lime, aonla (Indian Gooseberry or Emblica Officinalis) mixed in different ratios *viz.* 0:100; 5:95; 10:90; 15:85; 20:80; 25:75; 50:50; 75:25; 80:20; 85:15; 90:10; 95:5; 100:0; for preparation and standardization of Ready to Serve (RTS) beverages for improving the aroma, taste and nutrients of the beverages.

A panel of 9 judges adopting 9-point hedonic scale organoleptically evaluated the prepared beverages. The experiment was replicated three times. The data collected were analyzed as a one-way classified data (which was later analyzed as mixture experiments).

Discussion with experimenters and a close scrutiny of the data revealed that there was a wide difference in the age of judges. Intuition suggests that the age of the respondent influences the evaluation of the characteristics of the beverages prepared from different fruit juice/pulp blends.

The data generated were thus divided into three groups based on the age of the respondents, *viz.* 22 – 34 years; 35 – 44 years; 45 – 55 years. The data were analyzed as a two-way classified data and significant differences were observed among the age groups.

A very strong recommendation that emerged from this study was that "RTS beverages should be age specific and not uniform for all age groups for better acceptability of the product." This recommendation has been adopted now.

Preparation and standardization of ready to serve (RTS) fruit beverages (judges for organoleptical evaluation differ in age groups, different recommendations for different age groups) has been used in Division of Post Harvest Technology, ICAR-IARI, New Delhi.

An innovative application of mixture experiments is to determine the optimum split of fixed quantity of fertilizer to be applied at different crop growth stages. This application has been successfully demonstrated by using the data taken from an experiment on Paddy trial conducted at ANGRAU, Hyderabad by giving split-doses of fixed quantity of fertilizer at different crop-growth stages. This data is available in Agricultural Field Experiments Information System (AFEIS).

Another innovative application of mixture methodology is to determine optimum proportions of fixed area to be allocated to different crops so to maximize gross returns or crop equivalent yields. This methodology has been successfully developed by analyzing data taken from replacement series intercropping experiments conducted by PDKV, Akola with an objective to determine optimum proportions of area to be allocated to different crops to maximize gross returns or crop equivalent yields.

This methodology has a strong potential of application in experiments conducted with an objective to study the effect of soil amendments: (soil: gravel as 80:20, 50:50, 75:25 and 100:0) on the growth and yield behaviour of horticultural trees. The volume of soil in pit is constant. Data for such experiments may be available at ICAR-CSWCRTI (now ICAR-Indian Institute of Sil and Water Conservation, Dehradun.

This methodology has also been used in animal nutritional trials where the total amount of feed is constant, but the feed is a combination in different proportions of several different kinds of inputs.

Some other experimental situations where the experiments with mixtures methodology have a strong potential of application are enumerated in the sequel.

One potential application could be in experiments conducted on Chemometrics with an objective to study natural food antioxidant ingredients profile for antioxidant activity. Different proportions of Rosmarinus, Papaya and Alfalfa can be tried in the experiment.

Another experiment where mixtures methodology can be used effectively is the evaluation of irrigation water from different quality sources. The amount of irrigation water used is fixed. This can also be used in experiments where fixed dose of insecticides or pesticides from different formulations has been used. The mixtures methodology has also been used successfully in food processing experiments.

Remark 8.1. Many experiments conducted in NARES are generally run and analyzed as one would run experiments for comparing treatments and picking the best treatments from among the treatments tried in the experiment. Actually these are mixture experiments and more often than not the optimum combination may be the one not tried in the experiment. If these experiments are run as mixtures experiments and then analyzed as mixtures methodology, the quality of experimentation would enhance. But the problem is to know the design to be adopted (in other words, the proportions to be tried in the experiment). The choice has to be based on statistical considerations. The other associated problem is the choice of an appropriate model and the analysis of data generated to obtain the optimum proportion. These problems are not independent but are intertwined. So role of statistics is indispensible.

9. WEB RESOURCES

Several web resources have been created for the benefit of the experimenters. The basic purpose of these resources is to provide e-learning and e-advisory to the experimenters. Some popular e-resources are described in the sequel:

9.1. Design resources server (www.iasri.res.in/design)

This web resource has been created as an International Public Good to disseminate research in Design of Experiments among the scientists of NARES in particular and researchers all over globe in general. This web resource is open to everyone all over the globe. Anyone can join this and add information to the site to strengthen it further with the permission of the developers. The goal of this web resource is to help experimenters in agricultural, biological and social sciences, industry, etc. in planning and designing their experiments and then analyzing the data generated. It also targets at spreading advances in theoretical, analytical and applicational aspects of Design of Experiments among mathematicians and statisticians both in academia and also involved in advisory and consultancy services.

The server is matter-of-factly mobile library on Design of Experiments. It is dynamic in nature and new additions are posted on it from time to time. Ultimate objective of this server is to provide E-advisory services and E-learning. The contents of the server are divided into four broad categories *viz.* (a) Useful for Experimenters, (b) Useful for Statisticians, (c)

Other Useful Links, (d) Site Information.

- **Useful for Experimenters:** Electronic Books, online generation of randomized layout of designs including completely randomized designs, randomized complete block designs both for single and multi-factor experiments, Latin Square designs for single factor experiments, augmented designs, alpha designs, square lattice designs, online analysis of data, analysis of data using various statistical packages, Statistical Genomics.
- **Useful for Statisticians:** Literature and catalogues of BBB designs, efficient incomplete block designs, designs for making test treatments-Control treatment comparisons, designs for bioassays, supersaturated designs, online generation of designs for experiments with mixtures, designs for factorial experiments, response surface designs, online generation of Hadamard matrices, MOLS and mixed orthogonal arrays.
- **Other Useful Links:** Discussion Board, Ask a Question, Archive of Questions, Who-is-Where, Important Links, Books on Design of Experiments.
- **Site Information:** Feedback, How to Quote Design Resources Server, Copyright, Disclaimer, Contact us and Site map.

"Ask a Question" is an important feature of the server through which any user can ask a question on design and / or analysis of data or seek any other clarification. E-mails automatically go to developers of the site who in turn answer the query. On an average three to four questions are asked every week. The server also provides an archive of questions asked and answers given for the benefit of stakeholders. It also provides a Discussion Board for sharing any useful piece of research or idea with any other scientist over the globe. Electronic books on "Design and Analysis of Agricultural Experiments" and "Advances in Data Analytical Techniques available on the server" provide online learning material. Analysis of data using SAS, SPSS and MS EXCEL is a very important feature of the server. This link provides the analysis of data generated from almost all the possible experimental designs used by the experimenters. The data used in the analysis are generally the actual experiments conducted in NARES. Online generation of randomized layout of design and in some cases the generation of a field book are the added features of this web resource. DRS is a virtual library on Design of Experiments in particular and Statistics in general. The server is dynamic in nature and new links on various topics are added to it regularly. It is very helpful for the agricultural researchers and statisticians across NARES for e-learning and e-advisory. The server is hosted at the home page of IASRI, New Delhi and can be reached at www.iasri.res.in/design. The Website of the ICAR also provides a link of the server under 'important links'. ***It is a copyright of IASRI (ICAR): L46452/2013.*** This web resource has become very popular with scientists of NARES and is also being used in the 6 continents throughout the globe. Some other Web Resources on Designed Experiments would also be discussed.

9.2. Strengthening statistical computing in NARS

Recently through a mega project of National Agricultural Innovation Project (NAIP) on "*Strengthening Statistical Computing in NARS*," a healthy statistical computing environment has been created and the capacity building of the agricultural scientists in the usage of high end statistical computing and statistical techniques has been taken up on a large scale. This has been a unique project in which all NARES organizations have signed an MOU with each other for better implementation and harnessing the benefits of statistical computing environment created for NARES. An Indian NARS Statistical Computing Portal has been developed to provide IP authenticated online analysis of data. At present 24 different analysis modules are available on this resource. A user from Indian NARES can upload the *.xls, *.xlsx, *.csv and *.txt data files and get the results in real time environment as *.rtf or *.pdf files without having statistical package installed on his /her Desktop while working on a computer working on static IP address of any Indian NARES organization. This resource is very popular among the researchers in NARES and a large number of users log in daily on this server. ***It is a copyright of IASRI (ICAR): SW-7397/2013 and L-55719/2013.*** This portal and capacity development has helped in saving time and resources for different analysis of data and publication of research papers in high impact factor journals. This has also enabled in creating a network of researchers and statisticians in NARES for pursuing large research agenda.

The fusion of statistical computing environment created by the mega project on Strengthening Statistical Computing in NARS with the Design Resources Server provides a single window solution to the agricultural scientists for conducting their experiments and for analysis of data generated. This has in fact revolutionized the synthesis of statistical and agricultural sciences and has enabled to improve the quality of agricultural research, make it globally visible and acceptable. These efforts need to be further pursued in continuous manner for capacity building both for researchers and implementing team, updating and upgrading the statistical packages, maintaining and adding new modules on Indian NARS Statistical Computing Portal by making an appropriate provision of funds.

9.3. Web generation of experimental designs balanced for indirect effects of treatments (www.iasri.res.in/webdbie)

For easy accessibility and quick reference to neighbour balanced designs (NBDs) and crossover designs (CODs) by the experimenters, a software *Web Generation of Experimental Designs Balanced for Indirect Effects of Treatments* has been developed. The software generates five classes of neighbour balanced block designs (v treatments, b blocks, r replications and block size k) and eight classes of crossover designs (v treatments, p periods and n units/sequences). The webpage displays the layout plan along with the randomized layout for given number of treatments. The parameters of the designs so generated are also displayed. An online catalogue of neighbour balanced block designs (v treatments, b blocks, r replications and k block size) and crossover designs (v treatments, p periods and n units/sequences) for $v \leq 20$ is also available in the online generation module software. Search facility of all designs and designs for some particular value of parameters has been provided along with showing the layout of the design.

9.4 Other web resources

Other web resources developed for generating experimental designs for possible application in some other experimental situations are outlined in the sequel.

WS-PBIBD (http://nabg.iasri.res.in/pbibweb/) is a web based solution for generation of partially balanced incomplete block (PBIB) designs. An online catalogue of three-associate class PBIB designs existing in the literature has been developed which facilitates the selection of an appropriate design just by choosing the parameters from the catalogue. Filter facility has been added to the catalogue for an easy selection.

On-line analysis module for block design with complete/incomplete blocks, equal/ unequal block sizes and equal/ unequal replications has been developed for easy accessibility of the users to perform on-line analysis. This is hosted at http://www.iasri.res.in/WebAnalysis/index.aspx

On-line analysis of row column designs is also made available at http://iasri.res.in/css/home.aspx.

Polycross nursery is a specific type of field design to ensure random mating among the genotypes and is commonly used in the breeding of wind-pollinated species. In a polycross trial, each genotype gets an equal chance of pollinating, or being pollinated by, any of the others. Different types of designs for polycross trials are conducted for different situations like neighbour balanced polycross designs, designs for directional wind system and neighbour restricted polycross designs. For easy accessibility and quick reference to polycross trials by the experimenters, the designs are generated online (http://design.iasri.res.in/webpd/index1.html.

Diallel crosses have been used in plant and animal breeding trials to investigate the genetic properties and potentials of inbred lines or individuals. In a complete diallel cross (CDC) plan, as the number of lines increases, the number of crosses increases rapidly resulting in difficulty to handle all of them effectively. Hence, it is desirable to go for a subset or sample of all possible crosses, which is known as partial diallel crosses (PDC). One of the ways to obtain these sample crosses is through the association schemes of known PBIB designs. Web Based Generation and Analysis of Partial Diallel Crosses (*webPDC*) is user friendly web based software for generation and analysis of PDCs obtained using association schemes of PBIB designs. This software is useful for breeders to a considerable extent for generation and analysis of PDC plans. This software is hosted at http://nabg.iasri.res.in/webpdc/login.aspx.

9.5. Sample survey resources server (http://sample.iasri.res.in/ssrs/)

This is a web resource created with a goal to disseminate research in theory, application and computational aspects of sample survey among the statisticians in academia, practicing statisticians involved in advisory and consultancy services, scientists in the NARES and the statisticians involved in conducting large scale sample surveys, particularly in the National Statistical System with focus on agricultural statistical system. This resource focuses on propagating research in sample survey including designing a survey, estimation procedures with support of online software for computing purposes, analysis of survey data, e-learning, etc. This resource is useful to surveyors in agricultural sciences, biological sciences, social sciences, industry and in statistical organizations in the centre and the states in planning and designing surveys and in analyzing the complex survey data generated. One important feature of the resource is the Discussion Forum that aims at providing online advisory and consultancy to the surveyors in general and surveyors in agriculture in particular. This forum also provides a platform to establish a network of statisticians, survey statisticians and survey practitioners.

E-learning material in the form of electronic book and e-lectures are available for the benefit of survey statisticians. This server also provides online software for small area estimation using Fay-Harriot Model.

A common problem faced by the agricultural scientists is the determination of sample size. Even in designed experiments, the scientists resort to sampling from plots. This web resource provides an online calculator for sample size determination for the use by scientists in NARES. This calculator determines the sample size for estimation of population mean or population proportion for simple random sampling with and without replacement when CV is known or CV is unknown.

10. CONCLUSION

The purpose of this document has been to expose the stakeholders with the designs actually adopted by the researchers in NARES. The choice of a design depends upon the structure of treatments, the questions to be answered by the researcher, the variability in the experimental units and the resources available. In factorial experiments, the choice of treatments also assumes importance. Therefore, there cannot be any thumb rule to decide what design to use in a given experimental setting. The choice of a design needs to be made in consultation with a statistician. However, broad guidelines are given in the sequel.

- For single factor experiments or varietal trials, incomplete block designs, particularly resolvable incomplete block designs like alpha designs may be useful.

- For experiments with factorial structure of treatments to be run in an incomplete block designs, block designs with orthogonal factorial structure and balance with full efficiency on main effects may be useful.
- For making test treatments vs control treatments comparisons when the tests have single replication, augmented designs may be used. But the researcher has to be very careful about the proper randomization.
- For making test treatments vs control treatments comparisons when the tests have more than one replication, reinforced designs may be used. The design in test treatments could be an alpha design or a block design with factorial structure or any other design.
- In multi-factor experiments, if the interest is to obtain the combination of input factors so as to optimize the response, designs for fitting second order response surfaces should be used. However, in case the levels in the experiment are equi-spaced, then the choice of a proper design becomes more difficult.
- In multi-factor experiments, if the interest is to obtain the combination of input factors so as to optimize the response, but the input factors are such that in every combination the sum of levels is constant (fixed), then designs for mixture experiments should be used.

NOTE