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भारतीय कृषि अनुसंधान परिषद
Agrisearch with a human touch

एन. ऐ .इ. योजना– भा.कृ.अनु.प. के तत्वाधान मे उत्पाद एवं प्रसंस्करण विकासोन्मुख परीक्षणों हेतु आधुनिक अभिकल्पनाओं पर अप्रत्यक्ष प्रशिक्षण कार्यक्रम

(16-17 मार्च, 2021)

Virtual Training Programme On Advanced Designs for Product and Process Development Oriented Experiments Under the aegis of NAE Scheme of ICAR

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संदर्भ पुस्तिका
Reference Manual



भा.कृ.अनु.प. – भारतीय कृषि सांख्यिकी अनुसंधान संस्थान
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प्राक्कथन

भा.कृ.अनु.प.–भारतीय कृषि सांख्यिकी अनुसंधान संस्थान देश में कृषि सांख्यिकी एवं सूचना विज्ञान का एक अग्रणी संस्थान है। संस्थान में कृषि सांख्यिकी में शोध कार्य के साथ–साथ अध्यापन एवं प्रशिक्षण कार्यक्रम भी चलाए जाते हैं और इसमें परीक्षणात्मक अभिकल्पनाओं, प्रतिचयन तकनीकों, सांख्यिकीय अनुवंशिकी, फसल पूर्वानुमान तकनीकों, जैव–सूचना और संगणक अनुप्रयोग पर विशेष महत्त्व दिया जाता है। संस्थान की कृषि सांख्यिकी एवं संगणक अनुप्रयोग से संबंधित परामर्शदात्री सेवा के कारण राष्ट्रीय कृषि अनुसंधान एवं शिक्षा प्रणाली व राष्ट्रीय कृषि सांख्यिकी प्रणाली में संस्थान का महत्वपूर्ण स्थान है। राष्ट्रीय कृषि अनुसंधान एवं शिक्षा प्रणाली के वैज्ञानिकों के लिए उपयोगी सांख्यिकीय सॉफ्टवेयर पैकेज विकसित करने में भी संस्थान अग्रणी है।

सांख्यिकी रूप से वैध निष्कर्ष ही उत्कृष्ट कृषि अनुसंधान की नींव बनाते हैं। विश्व स्तर पर कृषि अनुसंधान को प्रतिस्पर्धी बनाने के लिए जरूरी है कि आँकड़ों का संकलन एवं विश्लेषण उचित सांख्यिकीय विधियों द्वारा किया जाए। संस्थान द्वारा आयोजित प्रशिक्षण कार्यक्रम सांख्यिकीय तकनीकों में विकास को कृषि एवं संबद्ध अनुसंधान में कार्यरत वास्तविक प्रयोक्ताओं को स्थानांतरित करने में बहुत उपयोगी सिद्ध हुए हैं।

वर्तमान प्रशिक्षण कार्यक्रम "**उत्पाद एवं प्रसंस्करण विकासोन्मुख परीक्षणों हेतु आधुनिक अभिकल्पनाएं**" की संकल्पना, कृषि विशेषज्ञों एवं सांख्यिकीविद्ों को एक साथ लाने के उद्देश्य से की गई है ताकि सहभागियों में आपस में एवं संकाय सदस्यों के साथ अधिक से अधिक विचारों का आदान प्रदान हो सके। मुझे विश्वास है कि यह कार्यशाला सहभागियों को अनुसंधान में प्रभावी रूप से कार्य करने में तो सक्षम बनाएगा ही, साथ ही वैज्ञानिक परीक्षणों के लिए अभिकल्पनाओं का सही उपयोग करके एवं उनकों आधुनिक सांख्यिकी पद्धतियों की सहायता से वैध निष्कर्ष निकालने में भी सहायक होगा ।

प्रशिक्षण कार्यक्रम इस प्रकार तैयार किया गया है कि इसमें सैद्धान्तिक एवं अनुप्रयोग दोनों पहलु सम्मिलित है। प्रशिक्षण में सम्मिलित विषयों इस प्रकार हैः (क) एकल कारक परीक्षणों के लिए अभिकल्पना (ख) बहु कारक परीक्षणों के लिए अभिकल्पना (ग) बहु–अनुक्रिया परीक्षणों हेतु अभिकल्पनाएँ (घ) बायो–असे परीक्षणों के लिए अभिकल्पना एवं (ड़) आँकड़ों के विष्लेषण हेतु सांख्यिकीय वेब रिसोर्सेज ।

संदर्भ पुस्तिका में दिये गए व्याख्यान, विषय का विस्तृत विवरण प्रदान करते हैं। आशा है कि प्रशिक्षण कार्यक्रम के दौरान बताई गई धारणाओं को समझने में सहभागियों के लिए यह मैनुअल उपयोगी होगा। मैं संपूर्ण संकाय सदस्यों को इस कार्य के लिए हार्दिक धन्यवाद देता हूँ। डॉ. सीमा जग्गी, प्रभागाध्यक्ष (का.), परीक्षण अभिकल्पना तथा इस प्रशिक्षण कार्यक्रम के संयोजक डॉ अनुपमा सिंह, डॉ. सुकान्त दाश एवं डॉ अनिल कुमार के इस बहुमूल्य दस्तावेज़ को समय से तैयार करने के लिए प्रयत्न सराहनीय हैं।

21/3/2021

(राजेन्द्र प्रसाद)
निदेशक, भा.कृ.अनु.प.–भा.कृ.सां.अ.सं.

नई दिल्ली
15 मार्च, 2021

आमुख

भा.कृ.अनु.प.–भारतीय कृषि सांख्यिकी अनुसंधान संस्थान, कृषि सांख्यिकी एवं सूचना विज्ञान का एक अग्रणी संस्थान है। यह संस्थान भा.कृ.अ.प. के शिक्षा प्रभाग के मानव संसाधन विकास कार्यक्रम के तत्वावधान में कृषि सांख्यिकी एवं संगणक अनुप्रयोग में उच्च संकाय प्रशिक्षण केन्द्र के रूप में भी कार्य कर रहा है। उच्च संकाय प्रशिक्षण केन्द्र के अन्तर्गत आयोजित किये जाने वाले प्रशिक्षण कार्यक्रमों के अतिरिक्त संस्थान भारतीय कृषि अनुसंधान परिषद् के शिक्षा विभाग द्वारा प्रायोजित ग्रीष्मकालीन/शीतकालीन स्कूल तथा विभिन्न राष्ट्रीय एवं अर्न्तराष्ट्रीय संस्थानों की आवश्यकतानुसार अन्य प्रशिक्षण कार्यक्रम भी आयोजित करता है। वर्तमान प्रशिक्षण कार्यक्रम भा.कृ.अनु.प.––भारतीय कृषि सांख्यिकी अनुसंधान संस्थान एवं कृषि रसायन संभाग, भा.कृ.अनु.प.– भारतीय कृषि अनुसंधान संस्थान द्वारा युग्म रूप से आयोजित किया गया है।

उपयुक्त सांख्यिकीय तकनीकों का अनुप्रयोग, कृषि अनुसंधान की नींव है। कृषि अनुसंधान की गुणवत्ता बनाये रखने एवं बढ़ाने के लिए यह अत्यन्त महत्वपूर्ण एवं आवश्यक है कि आँकड़ों के संकलन, विश्लेषण एवं परिणामों की व्याख्या के लिए उचित सांख्यिकीय पद्धतियों को अपनाया जाये। कृषि सांख्यिकी से संबंधित शोध कार्यो में संस्थान ने सांख्यिकी की दुनिया में अपना उच्च स्थान बना रखा है।

कृषि शोधकर्ताओं को सांख्यिकीय परामर्श सेवाएँ देने में संस्थान बहुत अनुभवी है। संस्थान अब राष्ट्रीय कृषि अनुसंधान प्रणाली में कार्यरत कृषि विशेषज्ञों को ई–परामर्श सेवाएं प्रदान कर रहा है। इसी उद्देश्य से संस्थान में एक डिज़ाईन रिसोर्स सर्वर (http://drs.icar.gov.in) का विकास किया है। संस्थान ने NARES के लिए स्टेटिस्टिकल कम्प्यूटिंग पोर्टल (http://stat.iasri.res.in/sscnarsportal) का भी विकास किया है जिसमें अलग–अलग प्रसंग सर्विस ऑरीएन्टेड कम्प्यूटिंग के रुप में दिये गए है । स्थिति विशिष्ट वैब समाधान भी संस्थान में विकसित किये गए हैं और इन्हें संस्थान के वैबपृष्ठ पर सामान्य जन कार्यक्षेत्र में उपलब्ध किया गया है । संस्थान को कृषि वैज्ञानिकों के लिए, कृषि अनुसंधान में उचित सांख्यिकीय तकनीकों के अनुप्रयोग को बढ़ाने के उद्देश्य को लेकर प्रशिक्षण कार्यक्रम आयोजित करने का भी विस्तृत अनुभव है।

वर्तमान प्रशिक्षण कार्यक्रम " **उत्पाद एवं प्रसंस्करण विकासोन्मुख परीक्षणों हेतु आधुनिक अभिकल्पनाएं** " शोधकर्ताओं को दक्ष परीक्षण अभिकल्पनाओं एवं परीक्षणात्मक आँकड़ों के विश्लेषण की परिष्कृत सांख्यिकीय विश्लेषण तकनीकों में विकास से अवगत कराना है। परिणामों की व्याख्या करने एवं प्रस्तुतिकरण पर विशेष बल दिया जायेगा। प्रशिक्षण कार्यक्रम को इस प्रकार तैयार किया गया है कि यह सिद्धान्त एवं अनुप्रयोग का मिला–जुला रूप है। इस प्रशिक्षण कार्यक्रम के दौरान, सहभागी एसएएस/एमएस–एक्सेल/आर सॉफ्टवेयर इत्यादि सांख्यिकीय पैकेजों के अनुप्रयोग द्वारा अनेक आँकड़ों के सैटों का विश्लेषण करेंगे। इसके परिणामस्वरूप, इस संस्थान के वैज्ञानिकों और भा.कृ.अनु.प. – भारतीय कृषि अनुसंधान संस्थान में कार्यरत कृषि वैज्ञानिकों के बीच सहयोग बढ़ेगा।

हम सभी संकाय सदस्यों का हार्दिक धन्यवाद करतें हैं जिन्होंने इस कार्यशाला को सार्थक एवं सफल बनाने में अपना अमूल्य समय दिया है। इस संहिता को तैयार करने में त्रुटियों को कम करने का हर सम्भव प्रयास किया गया है, फिर भी कमियाँ रह सकती हैं । भारतीय कृषि सांख्यिकी अनुसंधान संस्थान या लेखक, इस संहिता में दी गई सामग्री के प्रयोग द्वारा हुई किसी भी प्रकार की हानि के लिए उत्तरदायी नहीं होंगे। हम कृषि शिक्षा बिभाग एवं निदेशक, भा.कृ.अनु.प.– भारतीय कृषि अनुसंधान संस्थान का आभार प्रकट करते हैं जिन्होंने इस कार्यशाला के लिए सहभागियों को प्रायोजित किया। हम डॉ. राजेंद्र प्रसाद, निदेशक, भा.कृ.अनु.प.–भा.कृ.सां.अ.सं. के आभारी हैं जिन्होंने हमारा मार्गदर्शन किया और इस कार्यशाला में सतत् रूचि बनाए रखी व हमें सभी आवश्यक सुविधाएँ उपलब्ध कराई। हम डॉ. सीमा जग्गी, प्रभागाध्यक्ष (का.), परीक्षण अभिकल्पना का विशेष उल्लेख करना चाहेंगें जिन्होंने विभिन्न गतिविधियों में हमारा मार्गदर्शन एवं सहयोग किया। हम उन सबके प्रति भी आभारी हैं जिन्होंने अपने अथक प्रयासों से इस संहिता को तैयार करने में मदद की है।

नई दिल्ली	**डॉ अनुपमा सिंह**	**अनिल कुमार**	**सुकान्त दाश**
15 मार्च, 2021	**पाठ्यक्रम निदेशक**	**पाठ्यक्रम संयोजक**	**पाठ्यक्रम संयोजक**

FOREWORD

The ICAR-Indian Agricultural Statistics Research Institute is a premier Institute in the discipline of Agricultural Statistics and Informatics in the country. The Institute has been engaged in conducting research, teaching and organizing training programmes in Agricultural Statistics and Informatics with special emphasis on Experimental Designs, Sampling Techniques, Statistical Genetics, Crop Forecasting Techniques, Bioinformatics and Computer Applications. The Institute has been very actively pursuing advisory services that have enabled the institute to make its presence felt both in National Agricultural Research & Education System (NARES) and National Agricultural Statistics System (NASS). The Institute has taken a lead in developing Statistical Software Packages useful for Agricultural Research.

Statistically valid inferences lay the foundations of quality agricultural research. In order to make research globally competitive, it is essential that sound statistical methodologies be adopted for the collection and analysis of data. The training programmes organized by the Institute are very useful in appraising the advances in statistical techniques to the actual users in the agricultural and allied sciences.

The present online training "**Advanced Designs for Product and Process Development Oriented Experiments**" jointly organized by ICAR-IASRI and Division of Agricultural Chemicals, ICAR- IARI, New Delhi has been especially designed to bring faculty members and students in NARES together so as to derive the maximum academic advantage through interaction with the faculty and among the fellow participants. I am sure that the experience gained from this training will enable the participants to conduct their research more effectively and draw valid conclusions by adopting suitable experimental designs for their scientific experimentation and using appropriate and modern statistical methodologies for analyzing data generated from such experiments.

The training contents are intertwining of theory and application. The topics are covered are as follows: (a)Designs for Single Factor Experiments, (b)Designs for Multi-Factor Experiments, (c)Response Surface Designs and Applications, (d)Designs for Bioassay and (e) web resources on designed experiments.

The lecture notes given in the reference manual provide a detailed exposition of the subject. I hope that the reference manual will be quite useful to the participants. I take this opportunity to thank the entire faculty for doing a wonderful job. I wish to complement Dr. Seema Jaggi, Head of Division (A), Design of Experiments, Dr. Sukanta Dash, Course Co-Coordinators Dr. Anil Kumar, Course Coordinator, for bringing out this valuable document on time. We look forward to suggestions from every corner in improving this manual.

21-3-2021

Rajender Parsad
DIRECTOR, ICAR-IASRI

New Delhi
15 March, 2021

PREFACE

The ICAR-Indian Agricultural Statistics Research Institute has been and continues to be a premier Institute in the discipline of Agricultural Statistics and Informatics. The institute is also functioning as a Centre of Advanced Faculty Training in Agricultural Statistics and Computer Applications under the aegis of Human Resource Development Programme of the Education Division of ICAR. Besides organizing the training programme under Centre of Advanced Faculty Training (CAFT), Institute also takes lead in conduct of summer/ winter school sponsored by Education Division of ICAR and tailor made training programmes for different national and international organizations. The present online training programme is being organized jointly by by ICAR-IASRI and Division of Agricultural Chemicals, ICAR- IARI, New Delhi

Application of appropriate statistical techniques forms the backbone of any research endeavour in agriculture and allied sciences. In order to maintain and improve the quality of agricultural research, it is of paramount importance that sound and modern statistical methodologies are used in the collection and analysis of data and then in the interpretation of results. The Institute has established itself in the world of Statistics so far as research in Agricultural Statistics is concerned.

The Institute has also become a veteran in rigorously undertaking the consultancy and advisory services for the agricultural research personnel. Institute now plans to start E-advisory services for the research personnel in NARES. With this aim, Institute has taken a lead in the development of a Design Resources Server (http://drs.icar.gov.in). The Institute has also developed Indian NARES Statistical Computing Portal (http://stat.iasri.res.in/sscnarsportal) in which analysis of different events is provided as a service oriented computing. Situation specific web solutions have also been developed in the institute and made available in public domain at institute webpage. The Institute has a vast experience of organizing several training programmes for the agricultural scientists with a view to improve the use of sound statistical techniques in agricultural research.

The present online training "**Advanced Designs for Product and Process Development Oriented Experiments**" is designed to acquaint the participants with the advances in experimental designs and statistical analytical techniques related to product and process development oriented experiments. The emphasis will also be laid on interpretation and presentation of results. The training programme is planned in such a way that it is blend of theory and applications. The participants will be familiarized with experimental designs for product and process development oriented experiments. This training programme will also enable to build a bridge between this Institute and the faculty and students of ICAR-IARI, New Delhi.

We take this opportunity to thank all the faculty members who have devoted their time and energy in making this training programe meaningful and successful. Although every editorial care has been taken in preparing the manual, errors and omissions are likely to occur. ICAR-Indian Agricultural Statistics Research Institute or the authors are, however, in no way responsible for any liability arising out of the use of the contents of this reference manual. We are thankful to Division of Agriculture Education, ICAR for providing all necessary support to organize this training programme under NAE scheme of ICAR. We are thankful to Director, ICAR-IARI for all necessary support provided us to organize this online workshop. We are also grateful to Dr. Rajender Parsad, Director, ICAR-IASRI for his guidance and continuous interest in this workshop and making all necessary facilities available to us. We would like to make a special mention of Dr. Seema Jaggi, Head (A), Design of Experiments for her guidance and support. We are also thankful to one and all for their untiring efforts and help in preparing this manual.

New Delhi
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CONTENTS

Planning and Designing of Agricultural Experiments

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An experiment is usually associated with a scientific method for testing certain phenomena. An experiment facilitates the study of such phenomena under controlled conditions and thus creating controlled condition is an essential component. Scientists in the biological fields who are involved in research constantly face problems associated with planning, designing and conducting experiments. Basic familiarity and understanding of statistical methods that deal with issues of concern would be helpful in many ways. Researchers who collect data and then look for a statistical technique that would provide valid results will find that there may not be solutions to the problem and that the problem could have been avoided first by a properly designed experiment. Obviously it is important to keep in mind that we cannot draw valid conclusions from poorly planned experiments. Second, the time and cost involved in many experiments are enormous and a poorly designed experiment increases such costs in time and resources. For example, an agronomist who carries out fertilizer experiment knows the time limitation of the experiment. He knows that when seeds are to be planted and harvested. The experimenter plot must include all components of a complete design. Otherwise what is omitted from the experiment will have to be carried out in subsequent trials in the next cropping season or next year. The additional time and expenditure could be minimized by a properly planned experiment that will produce valid results as efficiently as possible. Good experimental designs are products of the technical knowledge of one's field, an understanding of statistical techniques and skill in designing experiments.

Any research endeavor may entail the phases of Conception, Design, Data collection, Analysis and Dissemination. Statistical methodologies can be used to conduct better scientific experiments if they are incorporated into entire scientific process, i.e., From inception of the problem to experimental design, data analysis and interpretation. When planning experiments, we must keep in mind that large uncontrolled variations are common occurrences. Experiments are generally undertaken by researchers to compare effects of several conditions on some phenomena or in discovering an unknown effect of particular process. An experiment facilitates the study of such phenomena under controlled conditions. Therefore, the creation of controlled condition is the most essential characteristic of experimentation. How we formulate our questions and hypotheses are critical to the experimental procedure that will follow. For example, a crop scientist who plants the same variety of a crop in a field may find variations in yield that are due to periodic variations across a field or to some other factors that the experimenter has no control over. The methodologies used in designing experiments will separate with confidence and accuracy a varietal difference of crops from the uncontrolled variations.

The different concepts in planning of experiment can be well explained through chapati tasting experiment.

Consider an experiment to detect the taste difference in chapati made of wheat flour of

c306 and pv 18 varieties. The null hypothesis we can assume here is that there is no taste difference in chapatis made of c306 or pv18 wheat flours. After the null hypothesis is set, we have to fix the level of significance at which we can operate. The pv18 is a much higher yielding variety than c306. Hence a false rejection may not help the country to grow more pv18 and the wheat production may decrease while a false acceptance may give more production of pv18 wheat and the consumption may be less or practically nil. Thus the false acceptance or false rejection are of practically equal consequence and we agree to choose the level of significance at $\alpha = 0.05$. Now to execute the experiment, a subject is to be found with extrasensory powers who can detect the taste differences. The colors of c306 and pv18 are different and anyone, even without tasting the chapatis, can distinguish the chapatis of either kind by a mere glance. Thus the taster of the chapatis has to be blindfolded before the chapatis are given for tasting. Afterwards, the method is to be decided in which the experiment will be conducted. The experiment can be conducted in many ways and of them three methods are discussed here:

- Give the taster equal number of chapatis of either kind informing the taster about it.
- Give the taster pairs of chapatis of each kind informing the taster about it.
- Give the taster chapatis of either kind without providing him with any information. Let us use 6 chapatis in each of these methods.

Under first method of experimentation, if the null hypothesis is true, then the experimenter cannot distinguish the two kinds of chapaties and he will randomly select 3 chapatiS out of 6 chapaties given to him, as made of pvl8 wheat. In that case, all correct guesses are made if selection exactly coincides with the exactly used wheat variety and the probability for such an occurrence is:

$$1 \Big/ \binom{6}{3} = 1 \Big/ 20 = 0.05$$

Under second method,the pv18 wheat variety chapaties are selected from each pair given if the null hypothesis is true. Furthermore, independent choices are made of pv18 variety chapaties from each pair. Thus the probability of making all correct guesses is

$$1/(2)^3 = 1/8 = 0.125.$$

In third method the experimenter has to make the choice for each chapati and the situation is analogous at calling heads or tails in a coin tossing experiment. The probability of making all correct guesses would then be:

$$1/2^6 = 1/64 = .016.$$

If the experimenter makes all correct guesses in third method as its probability is smaller than the selected $\alpha = 0.05$, we can reject the null hypothesis and conclude that the two wheat varieties give different tastes at chapaties. In other methods the probability of making all correct guesses does not exceed $\alpha = 0.05$ and hence with either method, we cannot reject the null hypothesis even if all correct guesses are made.
However, if 8 chapaties are used by first method and if the taster guesses all of them, we can reject the null hypothesis, at 0.05 level of significance, as the probability of making all

correct guesses would then be $1/\binom{8}{3} = 1/56$ which is smaller than 0.05. 8 chapaties will not enable us to reject the null hypothesis even if all correct guesses are made by second method as the probability of making all correct guesses is $\left(\frac{1}{4}\right)^4 = \frac{1}{16} = 0.06$ it is easy to see that if 10 chapaties are given by second method and if all correct guesses are made, then we can reject the null hypothesis at 0.05 level of significance. Not to unduly influence the taster in making guesses, we should also present the chapaties in a random order rather than systematically presenting them for tasting.

The above discussed chapati tasting experiment brings home the following salient features of experimentation:

- All the extraneous variations in the data should be eliminated or controlled excepting the variations due to the treatments under study. One should not artificially provide circumstances for one treatment to show better results than others.
- Far a given size of the experiment, though the experiment can be done in many ways, even the best results may not turn out to be significant with some designs, while some other design can detect the treatment differences. Thus there is an imperative need the choose the right type of design, before the commencement of the experiment, lest the results may be useless.
- If for some specific reasons related to the nature .of the experiment, a particular method has to be used in experimentation, then adequate number of replications of each treatment have to be provided in order to get valid inferences.
- The treatments have to be randomly allocated to the experimental units.

The terminologies often used in planning and designing of experiments are listed below.

Treatment

Treatment refers to controllable quantitative or qualitative factors imposed at a certain level by the experimenter. For an agronomist several fertilizer concentrations applied to a particular crop or a variety of crop is a treatment. Similarly, an animal scientist looks upon several concentrations of a drug given to animal species as a treatment. In agribusiness we may look upon impact of advertising strategy on sales a treatment. To an agricultural engineer, different levels of irrigation may constitute a treatment.

Experimental Unit

An experimental unit is an entity that receives a treatment e.g., for an agronomist or horticulturist it may be a plot of a land or batch of seed, for an animal scientist it may be a group of pigs or sheep, for a scientist engaged in forestry research it may be different tree species occurring in an area, and for an agricultural engineer it may be manufactured item. Thus, an experimental unit maybe looked upon as a small subdivision of the experimental material, which receives the treatment.

Experimental Error

Differences in yields arising out of experimental units treated alike are called Experimental Error.

Controllable conditions in an experiment or experimental variable are terms as a factor. For example, a fertilizer, a new feed ration, and a fungicide are all considered as factors. Factors may be qualitative or quantitative and may take a finite number of values or type. Quantitative factors are those described by numerical values on some scale. The rates of application of fertilizer, the quantity of seed sown are examples of quantitative factors. Qualitative factors are those factors that can be distinguished from each other, but not on numerical scale e.g., type of protein in a diet, sex of an animal, genetic make up of plant etc. While choosing factors for any experiment researcher should ask the following questions, like What treatments in the experiment should be related directly to the objectives of the study? Does the experimental technique adopted require the use of additional factors? Can the experimental unit be divided naturally into groups such that the main treatment effects are different for the different groups? What additional factors should one include in the experiment to interact with the main factors and shed light on the factors of direct interest? How desirable is it to deliberately choose experimental units of different types?

Basic Principles of Design of Experiments

Given a set of treatments which can provide information regarding the objective of an experiment, a design for the experiment, defines the size and number of experimental units, the manner in which the treatments are allotted to the units and also appropriate type and grouping of the experimental units. These requirements of a design ensure validity, interpretability and accuracy of the results obtainable from an analysis of the observations.

These purposes are served by the principles of:

- Randomization
- Replication
- Local (Error) control

Randomization

After the treatments and the experimental units are decided the treatments are allotted to the experimental units at random to avoid any type of personal or subjective bias, which may be conscious or unconscious. This ensures validity of the results. It helps to have an objective comparison among the treatments. It also ensures independence of the observations, which is necessary for drawing valid inference from the observations by applying appropriate statistical techniques.

Depending on the nature of the experiment and the experimental units, there are various experimental designs and each design has its own way of randomization. Various speakers while discussing specific designs in the lectures to follow shall discuss the procedure of random allocation separately.

Replication

If a treatment is allotted to r experimental units in an experiment, it is said to be replicated r times. If in a design each of the treatments is replicated r times, the design is said to have r replications. Replication is necessary to

- Provide an estimate of the error variance which is a function of the differences among observations from experimental units under identical treatments.
- Increase the accuracy of estimates of the treatment effects.

Though, more the number of replications the better it is, so far as precision of estimates is concerned, it cannot be increased infinitely as it increases the cost of experimentation. Moreover, due to limited availability of experimental resources too many replications cannot be taken.

The number of replications is, therefore, decided keeping in view the permissible expenditure and the required degree of precision. Sensitivity of statistical methods for drawing inference also depends on the number of replications. Sometimes this criterion is used to decide the number of replications in specific experiments.

Error variance provides a measure of precision of an experiment, the less the error variance the more precision. Once a measure of error variance is available for a set of experimental units, the number of replications needed for a desired level of sensitivity can be obtained as below.

Given a set of treatments an experimenter may not be interested to know if two treatment differ in their effects by less than a certain quantity, say, d. In other words, he wants an experiment that should be able to differentiate two treatments when they differ by d or more.

The significance of the difference between two treatments is tested by t-test where

$$t = \frac{\bar{y}_i - \bar{y}_j}{\sqrt{2s^2 / r}},$$

Here, $\bar{y}_i$, and $\bar{y}_j$ are the arithmetic means of two treatment effects each based on r replications, s^2 is measure of error variation.

Given a difference d, between two treatment effects such that any difference greater than d should be brought out as significant by using a design with r replications, the following equation provides a solution of r.

$$t = \frac{|d|}{\sqrt{2s^2 / r}},$$

$$r = \frac{t_0^2}{d^2} x 2s^2 \qquad \ldots(1)$$

where t_0 is the critical value of the t-distribution at the desired level of significance, that is, the value of t at 5 or 1 per cent level of significance read from the t-table. If s^2 is known or based on a very large number of observations, made available from some pilot pre-experiment investigation, then t is taken as the normal variate. If s^2 is estimated with n

degree of freedom (d.f.) then t_0 corresponds to n d.f.

When the number of replication is r or more as obtained above, then all differences greater than d are expected to be brought out as significant by an experiment when it is conducted on a set of experimental units which has variability of the order of s^2. For example, in an experiment on wheat crop conducted in a seed farm in Bhopal, to study the effect of application of nitrogen and phosphorous on yield a randomized block design with three replications was adopted. There were 11 treatments two of which were (i) 60 Kg/ha of nitrogen (ii) 120 Kg/ha of nitrogen. The average yield figures for these two application of the fertilizer were 1438 and 1592 Kg/ha respectively and it is required that differences of the order of 150 Kg/ha should be brought out significant. The error mean square (s^2) was 12134.88. Assuming that the experimental error will be of the same order in future experiments and t_0 is of the order of 2.00, which is likely as the error d.f. is likely to be more than 30 as there are 11 treatments; Substituting in (1), we get:

$$r = \frac{2t_0^2 s^2}{d^2} = \frac{2x2^2 x2134.88}{150^2} = 4 \text{ (approx.)}$$

Thus, an experiment with 4 replications is likely to bring out differences of the order of 150 Kg/ha as significant.

Another criterion for determining r is to take a number of replications which ensures at least 10 d.f. for the estimate of error variance in the analysis of variance of the design concerned since the sensitivity of the experiment will be very much low as the F test (which is used to draw inference in such experiments) is very much unstable below 10 d.f.

Local Control

The consideration in regard to the choice of number of replications ensure reduction of standard error of the estimates of the treatment effect because the standard error of the estimate of a treatment effect is $\sqrt{s^2 / r}$, but it cannot reduce the error variance itself. It is, however, possible to devise methods for reducing the error variance. Such measures are called *error control* or local control. One such measure is to make the experimental units homogenous. Another method is to form the units into several homogenous groups, usually called blocks, allowing variation between the groups.

A considerable amount of research work has been done to divide the treatments into suitable groups of experimental units so that the treatment effect can be estimated more precisely Extensive use of combinatorial mathematics has been made for formation of such group treatments. This grouping of experiment units into different groups has led to the development of various designs useful to the experimenter. We now briefly describe the various term used in designing of an experiment

Blocking

It refers to methodologies that form the units into homogeneous or pre-experimental subject-similarity groups. It is a method to reduce the effect of variation in the experimental material on the Error of Treatment of Comparisons. For example, animal scientist may

decide to group animals on age, sex, breed or some other factors that he may believe has an influence on characteristic being measured. Effective blocking removes considerable measure of variation nom the experimental error. The selection of source of variability to be used as basis of blocking, block size, block shape and orientation are crucial for blocking. The blocking factor is introduced in the experiment to increase the power of design to detect treatment effects.

The importance of good designing is inseparable from good research (results). The following examples point out the necessity for a good design that will yield good research. First, a nutrition specialist in developing country is interested in determining whether mother's milk is better than powdered milk for children under age one. The nutritionist has compared the growth of children in village A, who are all on mother's milk against the children in village B, who use powdered milk. Obviously, such a comparison ignores the health of the mothers, the sanitary-conditions of the villages, and other factors that may have contributed to the differences observed without any connection to the advantages of mother's milk or the powdered milk on the children. A proper design would require that both mother's milk and the powdered milk be alternatively used in both villages, or some other methodology to make certain that the differences observed are attributable to the type of milk consumed and not to some uncontrollable factor. Second, a crop scientist who is comparing 2 varieties of maize, for instance, would not assign one variety to a location where such factors as sun, shade, unidirectional fertility gradient, and uneven distribution of water would either favor or handicap it over the other. If such a design were to be adopted, the researcher would have difficulty in determining whether the apparent difference in yield was due to variety differences or resulted from such factors as sun, shade, soil fertility of the field, or the distribution of water. These two examples illustrate the type of poorly designed experiments that are to be avoided.

Analysis of Variance

Analysis of Variance (ANOVA) is a technique of partitioning the overall variation in the responses into different assignable sources of variation, some of which are specifiable and others unknown. Total variance in the sample data is partitioned and is expressed as the sum of its non-negative components is a measure of the variation due to some specific independent source or factor or cause. ANOVA consists in estimation of the amount of variation due to each of the independent factors (causes) separately and then comparing these estimates due to ascribable factors (causes) with the estimate due to chance factor the latter being known as experimental error or simply the error.

Total variation present in a set of observable quantities may, under certain circumstances, be partitioned into a number of components associated with the nature of classification of the data. The systematic procedure for achieving this is called *Analysis of Variance*. The initial techniques of the analysis of variance were developed by the statistician and geneticist R. A. Fisher in the 1920s and 1930s, and is sometimes known as Fisher's analysis of variance, due to the use of Fisher's F-distribution as part of the test of statistical significance.

Thus, ANOVA is a statistical technique that can be used to evaluate whether there are differences between the average value, or mean, across several population groups. With this model, the *response variable is continuous* in nature, whereas the *predictor variables are*

categorical. For example, in a clinical trial of hypertensive patients, ANOVA methods could be used to compare the effectiveness of three different drugs in lowering blood pressure. Alternatively, ANOVA could be used to determine whether infant birth weight is significantly different among mothers who smoked during pregnancy relative to those who did not. In a particular case, where two population means are being compared, ANOVA is equivalent to the independent two-sample *t*-test.

The fixed-effects model of ANOVA applies to situations in which the experimenter applies several treatments to the subjects of the experiment to see if the response variable values change. This allows the experimenter to estimate the ranges of response variable values that the treatment would generate in the population as a whole. In it factors are fixed and are attributable to a finite set of levels of factor eg. Sex, year, variety, fertilizer etc.

Consider for example a clinical trial where three drugs are administered on a group of men and women some of whom are married and some are unmarried. The three classifications of sex, drug and marital status that identify the source of each datum are known as factors. The individual classification of each factor is known as levels of the factors. Thus, in this example there are 3 levels of factor drug, 2 levels of factor sex and 2 levels of marital status. Here all the effects are fixed. Random effects models are used when the treatments are not fixed. This occurs when the various treatments (also known as factor levels) are sampled from a larger population. When factors are random, these are generally attributable to infinite set of levels of a factor of which a random sample are deemed to occur *eg.* research stations, clinics in Delhi, sire, etc. Suppose new inject-able insulin is to be tested using 15 different clinics of Delhi state. It is reasonable to assume that these clinics are random sample from a population of clinics from Delhi. It describes the situations where both fixed and random effects are present.

In any ANOVA model, general mean is always taken as fixed effect and error is always taken as random effect. Thus class of model can be classified on the basis of factors, other than these two factors. ANOVA can be viewed as a generalization of *t*-tests: a comparison of differences of means across more than two groups.
The ANOVA is valid under certain assumptions. These assumptions are:

- Samples have been drawn from the populations that are normally distributed.
- Observations are independent and are distributed normally with mean zero and variance σ^2.
- Effects are additive in nature.

The ANOVA is performed as one-way, two-way, three-way, etc. ANOVA when the number of factors is one, two or three respectively. In general, if the number of factors is more, it is termed as multi-way ANOVA.

FUNDAMENTALS OF DESIGN OF EXPERIMENTS

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1. Introduction

Any scientific investigation involves formulation of certain assertions (or hypotheses) whose validity is examined through the data generated from an experiment conducted for the purpose. Thus experimentation becomes an indispensable part of every scientific endeavour and designing an experiment is an integrated component of every research programme. Three basic techniques fundamental to designing an experiment are *replication, local control* (*blocking*), and *randomization.* Whereas the first two help to increase precision in the experiment, the last one is used to decrease bias. These techniques are discussed briefly below.

Replication is the repetition of the treatments under investigation to different experimental units. Replication is essential for obtaining a valid estimate of the experimental error and to some extent increasing the precision of estimating the pairwise differences among the treatment effects. It is different from repeated measurements. Suppose that the four animals are each assigned to a feed and a measurement is taken on each animal. The result is four independent observations on the feed. This is replication. On the other hand, if one animal is assigned to a feed and then measurements are taken four times on that animal, the measurements are not independent. We call them repeated measurements. The variation recorded in repeated measurements taken at the same time reflects the variation in the measurement process, while variation recorded in repeated measurements taken over a time interval reflects the variation in the single animal's responses to the feed over time. Neither reflects the variation in independent animal's responses to feed. We need to know about the latter variation in order to generalize any conclusion about the feed so that it is relevant to all similar animals.

For inferences to be broad in scope, it is essential that the experimental conditions should be rather varied and should be representative of those to which the conclusions of the experiment are to be applied. However, an unfortunate consequence of increasing the scope of the experiment is an increase in the variability of response. Local control is a technique that can often be used to help deal with this problem.

Blocking is the simplest technique to take care of the variability in response because of the variability in the experimental material. To block an experiment is to divide, or partition, the observations into groups called blocks in such a way that the observations in each block are collected under relatively similar experimental conditions. If blocking is done well, the comparisons of two or more treatments are made more precisely than similar comparisons from an unblocked design.

The purpose of randomization is to prevent systematic and personal biases from being introduced into the experiment by the experimenter. A random assignment of subjects or experimental material to treatments prior to the start of the experiment ensures that observations that are favoured or adversely affected by unknown sources of variation are observations “selected in the luck of the draw” and not systematically selected.

Lack of a random assignment of experimental material or subjects leaves the experimental procedure open to experimenter bias. For example, a horticulturist may assign his or her favourite variety of experimental crop to the parts of the field that look the most fertile, or a medical practitioner may assign his or her preferred drug to the patients most likely to respond well. The preferred variety or drug may then appear to give better results no matter how good or bad it actually is.

Lack of random assignment can also leave the procedure open to systematic bias. Consider, for example, an experiment conducted to study the effect of drugs in controlling the blood pressure. There are three drugs available in the market that can be useful for controlling the diastolic blood pressure. There are 12 patients available for experimentation. Each drug is given to four patients. If the allotment of drugs to the patients is not random, then it is quite likely that the experimenter takes four observations on drug 1 from the four patients on whom the onset of the disease is recent; the four observations on drug 2 are taken on four patients on whom the disease is 5-6 years old; and the four observations on drug 3 are taken on four patients on whom the disease is chronic in nature. This arrangement of treatments on patients is also likely if the assignment of drugs to the patients is made randomly. However, deliberately choosing this arrangement could well be disastrous. Duration of illness could be a source of variation and, therefore, response to drug 1 would be better as compared to drug 2 and drug 3. This could naturally lead to the conclusion that drug 1 gives a better response to control blood pressure as compared to drug 2 and drug 3.

There are also analytical reasons to support the use of a random assignment. The process of *randomization ensures independence of observations*, which is necessary for drawing valid inferences by applying suitable statistical techniques. It helps in making objective comparison among the treatment effects. The interested reader is referred to Kempthorne (1977) and Dean and Voss (1999).

To understand the meaning of randomization, consider an experiment to compare the effects on blood pressure of three exercise programmes, where each programme is observed four times, giving a total of 12 observations. Now, given 12 subjects, imagine making a list of all possible assignments of the 12 subjects to the three exercise programs so that 4 subjects are assigned to each program. (There are 12! / (4!4!4!), or 34,650 ways to do this). If the assignment of subjects to programs is done in such a way that every possible assignment has the same chance of occurring, then the assignment is said to be a completely random assignment. Completely randomized designs discussed in section 3, are randomized in this way. It is indeed possible that a random assignment itself could lead to the order 1,1,1,1, 2,2,2,2, 3,3,3,3. If the experimenter expressly wishes to avoid certain assignments, then a different type of design should be used. An experimenter should not look at the resulting assignment, decide that it does not look very random, and change it.

The data generated through designed experiments exhibit a lot of variability. Even experimental units (plots) subjected to same treatment give rise to different observations thus creating variability. The statistical methodologies, in particular the theory of linear estimation, enables us to partition this variability into two major components. The first major component comprises of that part of the total variability to which we can assign causes or reasons while the second component comprises of that part of the total variability to which we cannot assign any cause or reason. This variability arises because of some factors unidentified as a source of variation. Howsoever careful planning is made for the experimentation, this component is always present and is known as experimental error. The

observations obtained from experimental units identically treated are useful for 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 Coefficient of Variation (CV). The other major component of variability is the one for which the causes can be assigned or are known. There is always a deliberate attempt on the part of the experimenter to create variability by the application of several treatments. So treatment is one component in every designed experiment that causes variability. If the experimental material is homogeneous and does not exhibit any variability then the treatments are applied randomly to the experimental units. Such designs are known as zero-way elimination of heterogeneity designs or completely randomized designs (CRD). Besides the variability arising because of the application of treatments, the variability present in the experimental material (plots) is the other major known source of variability. Forming groups called blocks containing homogeneous experimental units can account for this variability if the variability in the experimental material is in one direction only. Contrary to the allotment of treatments randomly to all the experimental units in a CRD, the treatments are allotted randomly to the experimental units within each block. Such designs are termed as one-way elimination of heterogeneity setting designs or the block designs. The most common block design is the randomized complete block (RCB) design. In this design all the treatments are applied randomly to the plots within each block. However, for large number of treatments the blocks become large if one has to apply all the treatments in a block, as desired by the RCB design. It may then not be possible to maintain homogeneity among experimental units within blocks. As such the primary purpose of forming blocks to have homogeneous experimental units within a block is defeated. A direct consequence of laying out an experiment in RCB design with large number of treatments is that the coefficient of variation (CV) of the design becomes large. This amounts to saying that the error sum of squares is large as compared to the sum of squares attributable to the model and hence, small treatment differences may not be detected as significant. It also leads to poor precision of treatment comparisons or estimation of any normalized treatment contrast. High CV of the experiments is a very serious problem in agricultural experimentation. Many experiments conducted are rejected due to their high CV values. It causes a great loss of the scarce experimental resources. It is hypothesized that the basic problem with high CV and poor precision of estimation of treatment contrasts is that the block variations are not significant (block mean square is small as compared to error mean square) in large number of cases. In another research project entitled **A Diagnostic Study of Design and Analysis of Field Experiments**, carried out at Indian Agricultural Statistics Research Institute (IASRI), New Delhi, 5420 experiments were retrieved from Agricultural Field Experiments Information System conducted using a RCB design and were analyzed. The replication effects were found to be not significantly different at 5% level of significance in more than 75% of the cases. A close scrutiny of the results of 1186 experiments conducted in RCB design by the PDCSR, Modipuram on Research Stations during 1990-2001, revealed that the replication effect was not significant in 740 (62.39%) of the experiments. In the varietal trials conducted under the aegis of All India Co-ordinated Research Project on Rapeseed and Mustard, we analyzed the data from different initial varietal trials (IVT) and advanced varietal trials conducted at different locations. Data from a total of 30 locations were analyzed as per procedure of RCB design (design adopted). It was found that the replication effects are not significantly different in 21 (70%) experiments. In any experimentation, non-

significant differences between block effects or a high value of CV may arise due to any of the following causes:

1. Bad management of the controllable factors during experimentation (managerial aspects).
2. Faulty formation of blocks (designing).
3. Lack of identification of ancillary information that could have been used as covariate (Analysis of covariance).

The first point may be taken care of if the experimenter is very cautious and experienced. Analysis of covariance is an analytical procedure and is very effective in controlling the experimental error although it has nothing to do with the designing of the experiment. The most important point which has to be taken care of during the allocation of treatments to different experimental units is to adopt the proper blocking techniques. Therefore, there is a strong need to effectively control the variation through blocking. This necessitates the use of incomplete block designs. A block design is said to be an incomplete block design if the design has at least one block that does not contain all the treatments. Some common incomplete block designs are balanced incomplete block (BIB) design, partially balanced incomplete block (PBIB) design including Lattice designs – square and rectangular, cyclic designs, alpha designs, etc. One may, however, argue that in these designs the purpose of demonstration of a variety effect in the field cannot be done as all the treatments are not appearing in adjacent piece of land. To overcome this problem, it is recommended that resolvable block designs with smaller block sizes may be used.

A resolvable block design is a design in which the blocks can be grouped in such a fashion that each of the treatments occurs in each of the groups exactly once; in other words, each group is a complete replication. One particular class of resolvable incomplete block designs that has been recommended for varietal trials is the class of Lattice designs (square lattice and rectangular lattice). The limitation of these designs is that the varieties is $v = s^2$ or $v = s(s-1)$. Further, the block size in case of square lattice designs is s and in case of rectangular lattice designs is s–1. If the number of genotypes to be assessed does not satisfy these conditions, then either some additional genotypes may be added or exiting genotypes may be deleted. This limitation on the number of genotypes and the block size has been overcome by the introduction of alpha designs in the literature. It is now possible to obtain an alpha design for any composite number of genotypes and for any block size. Only restriction is that the block size must be a factor of number of genotypes. In other words, it is possible to obtain an alpha design in $v = sk$ genotypes, where k denotes the block size and s is a positive integer. A critical look at the experimentation in the NARS reveals that α-designs have not found much favour from the experimenters. It may possibly be due to the fact that the experimenters find it difficult to lay their hands on α-designs. The construction of these designs is not easy. An experimenter has to get associated with a statistician to get a randomized layout of this design. For the benefit of the experimenters, a comprehensive catalogue of α-designs for $6 \le v(= sk) \le 150$, $2 \le r \le 5$, $3 \le k \le 10$ and $2 \le s \le 15$ has been prepared along with lower bounds to A- and D- efficiencies and generating arrays. The layout of these designs along with block contents has also been prepared.

In some experimental situations, the user may be interested in getting designs outside the above parametric range. To circumvent such situations, a β- version of user friendly software module for the generation of α-designs has been developed. This module generates the alpha array along with lower bounds to A and D-efficiency. The α-array and the design is generated once the user enter the number of treatments (v), number of replications (r) and the block size (k). The module generates the design for any v, k, r provided v is a multiple of k. It also gives the block contents of the design generated.

Further, the variability in the experimental material may be in two directions and forming rows and columns can control this variability and the treatments are assigned to the cells. Each cell is assigned one treatment. For the randomization purpose, first the rows are randomized and then the columns are randomized. There is no randomization possible within rows and/or within columns. Such designs are termed as two-way elimination of heterogeneity setting designs or the row-column designs. The most common row-column design is the Latin square design (LSD). The other row-column designs are the Youden square designs, Youden type designs, Generalized Youden designs, Pseudo Youden designs, etc.

In the experimental settings just described, the interest of the experimenter is to make all the possible pairwise comparisons among the treatments. There may, however, be situations where some treatments are on a different footing than the others. The set of treatments in the experiment can be divided into two disjoint groups. The first group comprises of two or more treatments called the test treatments while the second group comprises of a single or more than one treatment called the control treatments or the controls. The single control situation is very common with the experimenters. The test treatments are scarce and the experimenter cannot afford to replicate these treatments in the design. Thus, the tests are singly replicated in the design. Such a design in tests is a disconnected design and we cannot make all the possible pairwise comparisons among tests. Secondly, we cannot estimate the experimental error from such a design. To circumvent these problems, the control treatment(s) is (are) added in each block at least once. Such a design is called an augmented design. There may, however, be experimental situations when the tests can also be replicated. In such a situation the tests are laid out in a standard design like BIB design, PBIB design including Lattice design – square and rectangular, cyclic design, alpha designs, etc. and the control(s) is (are) added in each block once (or may be more than once). In this type of experimental setting the interest of the experimenter is not to make all the possible pairwise comparisons among the treatments, tests and controls together. The experimenter is interested in making pairwise comparisons of the tests with the controls only. The pairwise comparisons among the tests or the controls are of no consequence to the experimenter. These experiments are very popular with the experimenters, particularly the plant breeders.

Another very common experimental setting is the following: An experiment is laid out at different locations/sites or is repeated over years. The repetition of the experiments over locations or years becomes a necessity for observing the consistency of the results and determining the range of geographical adaptability. In these experiments, besides analyzing the data for the individual locations/sites or years, the experimenter is also interested in the combined analysis of the data. For performing combined analysis of data, first the data for each experiment at a given location/site or year is analyzed separately. It is then followed

by testing the homogeneity of error variances using Barttlett's χ^2 -test. The details of the Bartlett's χ^2 -test are given in Example 3. It is same procedure as given in the lecture notes on Diagnostics and Remedial Measures with only difference that the estimated error variances S_i^2 are to be replaced by mean square error and $r_i - 1$ is to be replaced by corresponding error degrees of freedom. If the errors are homogeneous, then the combined analysis of data is carried out by treating the environments (locations/sites and/or years) as additional factors. If, however, the error variances are heterogeneous, then the data needs a transformation. A simple transformation is that the observations are divided by the root mean square error. This transformation is similar to Aitken's transformation. The transformed data is then analyzed in the usual manner. In both these cases, first the interaction between the treatments and environments is tested against the error. If the interaction is significant *i.e.* the interaction is present, then the significance of treatments is tested against the interaction mean square. If the interaction is non-significant *i.e.* interaction is absent then the treatments are tested against the pooled mean squares of treatments × environment interaction and error. This is basically for the situations where the experiment is conducted using a RCB design. However, in general if the interaction is absent, then one may delete this term from the model and carry out the analysis using a model without interaction term.

The group of experiments may be viewed as a nested design with locations/years as the bigger blocks and the experiments nested within blocks. For doing the combined analysis, the replication wise data of the treatments at each environment provide useful information. The treatment × site (or year) interactions can also be computed. However, if at each site, only the average value of the observations pertaining to each treatment is given then it is not possible to study the treatment × site (or year) interaction. The different sites or the years are natural environments. The natural environments are generally considered as a random sample from the population. Therefore, the effect of environment (location or year) is considered as random. All other effects in the model that involve the environment either as nested or as crossed classification are considered as random. The assumption of these random effects helps in identifying the proper error terms for testing the significance of various effects.

Some other experimental situations that can be viewed as groups of experiments are those in which it is difficult to change the levels of one of the factors. For example, consider an experimental situation, where the experimenter is interested in studying the long-term effect of irrigation and fertilizer treatments on a given crop sequence. There are 12 different fertilizer treatments and three-irrigation treatments viz. continuous submergence, 1-day drainage and 3-day drainage. It is very difficult to change the irrigation levels. Therefore, the three irrigation levels may be taken as 3 artificially created environments and the experiment may be conducted using a RCB design with 12 fertilizer treatments with suitable number of replications in each of the 3 environments. The data from each of the three experiments may be analyzed individually and the mean square errors so obtained may be used for testing the homogeneity of error variances and combined analysis of data be performed.

In case of artificially created environments, the environment effect also consists of the effect of soil conditions in field experiments. Therefore, it's suggested that the data on some

auxiliary variables may also be collected. These auxiliary variables may be taken as covariate in the analysis.

Besides Aitken's transformation described above, other commonly used transformations are the arcsine transformation, square root transformation and the logarithmic transformation. These transformations are particular cases of a general family of transformations, Box-Cox transformation. The transformations other than Aitken's transformation are basically useful for the analysis of experimental data from individual experiments.

So far we have discussed about the experimental situations when the factors are cross-classified, *i.e.*, the levels of one factor are experimented at all the levels of the other factor. In practical situations it may be possible that one factor is nested within another factor. The variability in the experimental material enables us to form the blocks supposed to comprise of experimental units that are homogeneous. But the experimental units within block may also exhibit variability that can be further controlled by forming sub blocks within blocks. For example, in hilly areas the long strips may form the big blocks while small strips within the long strips may constitute the sub blocks. As another example, the trees are the big blocks and position of the branches on the trees may form the sub blocks. Such designs are called nested designs. The combined analysis of data can also be viewed as a nested design. The sites (or years) may constitute the big blocks and the experiments are nested within each block. The combined analysis of data can also be carried out as a nested design.

The experimental error can be controlled in two ways. As described above, one way of controlling the error is through the choice of an appropriate design by controlling the variability among the experimental units. The other way is through sound analytical techniques. There is some variability present in the data that has not been taken care of or could not be taken care of through the designing of an experiment. Such type of variability can be controlled at the time of analysis of data. If some auxiliary information is available on each experimental unit then this information can be used as a covariate in the analysis of covariance. The covariance analysis results into further reduction in the experimental error. But the auxiliary variable that is being used as a covariate should be such that it is not affected by the application of the treatments. Otherwise a part of the variability will be eliminated while making adjustments for the covariate. There may be more than one covariate also.

The above discussion relates to the experimental situations in which the treatment structure comprises of many levels of a single factor. There are, however, experimental settings in which there are several factors studied together in an experiment. Each factor has several levels. The treatments comprise of all the possible combinations of several levels of all the factors. Such experiments where several factors with several levels are tried and the treatments are the treatment combinations of all the levels of all the factors are known as factorial experiments. Factorial experiments can be laid out in a CRD, RCB design, LSD or any other design. Factorial experiments, in fact, correspond to the treatment structure only. Consider a 3×2×2 experiment in which three levels of *Nitrogen* denoted as n_0, n_1, n_2, two levels of *Phosphorous* denoted as p_0, p_1 and two levels of *Potash* denoted as k_0, k_1 are tried. The 12 treatment combinations are $n_0p_0k_0, n_0p_0k_1, n_0p_1k_0, n_0p_1k_1$, $n_1p_0k_0, n_1p_0k_1, n_1p_1k_0, n_1p_1k_1$, $n_2p_0k_0$, $n_2p_0k_1$, $n_2p_1k_0$, $n_2p_1k_1$. This experiment can be laid out in any design. The advantage of factorial experiments is that several factors can be

studied in one experiment and, therefore, there is a considerable saving of resources. The second advantage is that the precision of comparisons is improved because of the hidden replication of the levels of the factors. In the *12* treatment combinations, each treatment appears only once. But the levels of *N* appear *three* times each, the levels of *P* and *K* appear *six* times each, respectively. These are hidden replications that help in improved precision. The third advantage is that besides studying the main effects of factors we can also study the interactions among factors. The interaction helps us in studying the effect of levels of a factor at constant level of the other factors.

When the number of factors and/or levels of the factors increase, the number of treatment combinations increase very rapidly and it is not possible to accommodate all these treatment combinations in a single homogeneous block. For example, a 2^7 factorial would have 128 treatment combinations and blocks of 128 plots are quite big to ensure homogeneity within them. In such a situation it is desirable to form blocks of size smaller than the total number of treatment combinations (incomplete blocks) and, therefore, have more than one block per replication. The treatment combinations are then allotted randomly to the blocks within the replication and the total number of treatment combinations is grouped into as many groups as the number of blocks per replication.

There are many ways of grouping the treatments into as many groups as the number of blocks per replication. It is known that for obtaining the interaction contrast in a factorial experiment where each factor is at two levels, the treatment combinations are divided into two groups. Such two groups representing a suitable interaction can be taken to form the contrasts of two blocks each containing half the total number of treatments. In such cases the contrast of the interaction and the block contrast become identical. They are, therefore, mixed up and cannot be separated. In other words, the interaction gets confounded with the blocks. Evidently the interaction confounded has been lost but the other interactions and main effects can now be estimated with better precision because of reduced block size. This device of reducing the block size by taking one or more interactions contrasts identical with block contrasts is known as **confounding**. Preferably only higher order interactions with three or more factors are confounded, because these interactions are less important to the experimenter. As an experimenter is generally interested in main effects and two factor interactions, these should not be confounded as far as possible. The designs for such confounded factorials are incomplete block designs. However, usual incomplete block designs for single factor experiments cannot be adopted, as the contrasts of interest in two kinds of experiments are different. The treatment groups are first allocated at random to the different blocks. The treatments allotted to a block are then distributed at random to its different units. When there are two or more replications in the design and if the same set of interactions is confounded in all the replications, then confounding is called **complete** and if different sets of interactions are confounded in different replications, confounding is called **partial**. In complete confounding all the information on confounded interactions is lost. However, in partial confounding, the information on confounded interactions can be recovered from those replications in which these are not confounded. In some experimental situations, some factors require large plot sizes and the effect of these factors is obvious, the experimenter is interested in the main effects of other factor and interaction with high precision. Split plot designs are used for such experimental situations. If the experimenter is interested only in interaction of the two factors and both factors require large plot sizes, the strip plot designs may be used.

In factorial experiments, sometimes, due to constraint on resources and/or time it is not possible to have more than one replication of the treatment combinations. In these situations, a single replicated factorial experiment with or without blocking is used and the higher order interactions are taken as error. To make the exposition clear, consider an experiment that was conducted to study the effect of irrigation (three levels), nitrogen (5 levels), depth (3-6 depths), classes of soil particle sizes (3-5) on organic carbon in rice–wheat cropping system. For each factorial combination, there is only one observation, and the experimenter was interested in studying the main effects and two factor interactions. Therefore, the data was analyzed as per procedure of singly replicated factorial experiment by considering the 3 –factor/4 factor interactions as the error term. In some of the experimental situations, the number of treatment combinations becomes so large that even a single replication becomes difficult. The fractional factorial plans are quite useful for these experimental situations.

The above discussion relates to the experiments in which the levels or level combinations of one or more factors are treatments and the data generated from these experiments are normally analyzed to compare the level effects of the factors and also their interactions. Though such investigations are useful to have objective assessment of the effects of the levels actually tried in the experiment, this seems to be inadequate, especially when the factors are quantitative in nature and cannot throw much light on the possible effect(s) of the intervening levels or their combinations. In such situations, it is more realistic and informative to carry out investigations with the twin purpose:

a) To determine and to quantify the relationship between the response and the settings of a set of experimental factors.
b) To find the settings of the experimental factor(s) that produces the best value or the best set of values of the response(s).

If all the factors are quantitative in nature, it is natural to think the response as a function of the factor levels and data from quantitative factorial experiments can be used to fit the response surface over the region of interest. The special class of designed experiments for fitting response surfaces is called response surface designs.

Through response surface designs one can obtain the optimum combination of levels of input factors. However, there do occur experimental situations where a fixed quantity of inputs, may be same dose of fertilizer, same quantity of irrigation water or same dose of insecticide or pesticide etc. are applied. The fixed quantity of input is a combination of two or more ingredients. For example, fixed quantity of water may be a combination of different qualities of water sources or fixed quantity of nitrogen may be obtained from different sources. In a pesticide trial, a fixed quantity of pesticide may be obtained from four different chemicals. In these experiments the response is a function of the proportion of the ingredient in the mixture rather than the actual amount of the mixture. The experiments with mixture methodology are quite useful for these experimental situations.

Besides controlling the variability in the experimental material by a process of forming blocks, rows and columns, etc. termed as *local control*, there are other techniques. The analysis of covariance technique is one very important way of reducing the experimental error.

2. Contrasts and Analysis of Variance

The main technique adopted for the analysis and interpretation of the data collected from an experiment is the analysis of variance technique that essentially consists of partitioning the total variation in an experiment into components ascribable to different sources of variation due to the controlled factors and error. Analysis of variance clearly indicates a difference among the treatment means. The objective of an experiment is often much more specific than merely determining whether or not all of the treatments give rise to similar responses. For examples, a chemical experiment might be run primarily to determine whether or not the yield of the chemical process increases as the amount of the catalyst is increased. A medical experimenter might be concerned with the efficacy of each of several new drugs as compared to a standard drug. A nutrition experiment may be run to compare high fiber diets with low fiber diets. A plant breeder may be interested in comparing exotic collections with indigenous cultivars. An agronomist may be interested in comparing the effects of biofertilisers and chemical fertilisers. An water technologist may be interested in studying the effect of nitrogen with Farm Yard Manure over the nitrogen levels without farm yard manure in presence of irrigation.

The following discussion attempts to relate the technique of analysis of variance to provide hypothesis tests and confidence intervals for the treatment comparisons among the treatment effects.

2.1 Contrasts

Let $y_1, y_2, \ldots, y_n$ denote n observations or any other quantities. The linear function $C = \sum_{i=1}^{n} l_i y_i$, where l_i's are given number such that $\sum_{i=1}^{n} l_i = 0$, is called a *contrast* of $y_i's$.

Let $y_1, y_2, \ldots, y_n$ be independent random variables with a common mean μ and variance σ^2. The expected value of the random variable C is zero and its variance is $\sigma^2 \sum_{i-1}^{n} l_i^2$. In what follows we shall not distinguish between a contrast and its corresponding random variable.

Sum of squares (s.s.) of contrasts. The sum of squares due to the contrast C is defined as $C^2 / \sigma^{-2} Var(C) = C^2 / \left(\sum_{i=1}^{n} l_i^2 \right)$. Here σ^2 is unknown and is replaced by its unbiased estimate, *i.e. mean square error*. It is known that this square has a $\sigma^2 \chi^2$ distribution with one degree of freedom when the $y_i's$ are normally distributed. Thus the sum of squares due to two or more contrasts has also a $\sigma^2 \chi^2$ distribution if the contrasts are independent. Multiplication of any contrast by a constant does not change the contrast. The sum of squares due to a contrast as defined above is not evidently changed by such multiplication.

Orthogonal contrasts. Two contrasts, $C_1 = \sum_{i=1}^{n} l_i y_i$ and $C_2 = \sum_{i=1}^{n} l_i y_i$ are said to be orthogonal if and only if $\sum_{i=1}^{n} l_i m_i = 0$. This condition ensures that the covariance between C_1 and C_2 is zero.

When there are more than two contrasts, they are said to be mutually orthogonal if they are orthogonal pair wise. For example, with four observations y_1, y_2, y_3, y_4, we may write the following three mutually orthogonal contrasts:

(i) $y_1 + y_2 - y_3 - y_4$

(ii) $y_1 - y_2 - y_3 + y_4$

(iii) $y_1 - y_2 + y_3 - y_4$

The sum of squares due to a set of mutually orthogonal contrasts has a $\sigma^2 \chi^2$ distribution with as many degrees of freedom as the number of contrasts in the set.

Maximum number of orthogonal contrasts. Given a set of *n* values $y_1, y_2, \cdots, y_n$, the maximum number of mutually orthogonal contrasts among them is *n - 1*. One way of writing such contrasts is to progressively introduce the values as below:

(i) $y_1 - y_2$

(ii) $y_1 + y_2 - 2y_3$

: :

: :

(n) $y_1 + y_2 + \cdots + y_{n-1} - (n-1)y_n$.

Another set of orthogonal contrasts for values of *n* is available in the Tables for Biological, Agricultural and Medical Research prepared by Fisher and Yates (1963) under the name of orthogonal polynomials.

To be specific about treatment effects let $\sum l_i t_i$ denote a treatment contrast, $\sum_i l_i = 0$. The BLUE of $\sum l_i t_i$ is $\sum_i l_i \hat{t}_i$ and its variance is denoted by $Var(\sum_i l_i \hat{t}_i)$, where t_i is the parameter pertaining to the treatment effect *i*. The sum of squares due to contrast $\sum_i l_i \hat{t}_i$ is $\left(\sum_i l_i \hat{t}_i\right)^2 / \sigma^{-2} V\hat{a}r\left(\sum_i l_i \hat{t}_i\right)$ where σ^2 is the error variance estimated by the error mean squares, MSE. The significance of the contrast can be tested using the statistic

$$t = \frac{\sum_i l_i \hat{t}_i}{\sqrt{V\hat{a}r\left(\sum_i l_i \hat{t}_i\right)}}$$

which follows the Student's t-distribution with degrees of freedom same as that of error. The null hypothesis is rejected at $\alpha\%$ level of significance if the tabulated value of $t_{(1-\alpha/2,edf)}$ is greater than computed t-value. Here *edf* represents the error degrees of freedom. F-test can be used instead of t-test using the relationship that $t^2_{n_1} = F_{1,n_1}$.

Contrasts of the type $t_i - t_m$ in which experimenters are often interested are obtainable from $\sum_i l_i t_i$ by putting $l_i = 1, l_m = -1$ and zero for the other *l's.* These contrasts are called as elementary contrasts and are useful for pairwise comparisons.

Besides hypothesis testing, the experimenter may also be interested in obtaining a confidence interval. In the sequel, we shall give a formula for a confidence interval for an individual contrast. If confidence intervals for more than one contrast are required, then the multiple comparison methods should be used instead. A-*100 (1 - α)%* confidence interval for the contrast $\sum l_i t_i$ is

$$\sum l_i \hat{t}_i - t_{edf,\alpha/2}\sqrt{\hat{Var}\left(\sum_i l_i \hat{t}_i\right)} \le l_i t_i \le \sum l_i \hat{t}_i + t_{edf,\alpha/2}\sqrt{\hat{Var}\left(\sum_i l_i \hat{t}_i\right)}.$$

We can write this more succinctly as

$$\sum l_i t_i \in \left(\sum l_i \hat{t}_i \pm t_{edf,\alpha/2}\sqrt{\hat{Var}\left(\sum_i l_i \hat{t}_i\right)}\right)$$

where the symbol $\pm$ denotes that the upper limit of the interval is calculated using + and the lower limit using - and *edf* is the number of degrees of freedom for error. The symbol "$\sum l_i t_i \in$" mean that the interval includes the true value of contrast $\sum l_i t_i$ with *100(1 - α)%* confidence.

The outcome of a hypothesis test can be deduced from the corresponding confidence interval in the following way. The null hypothesis $H_0 : \sum_i l_i t_i = h$ will be rejected at significance level α in favor of the two-sided alternative hypothesis $H_1 : \sum_i l_i t_i \neq h$ if the corresponding confidence interval for $\sum_i l_i t_i$ fails to contain *h*.

So far we have discussed experimental situations where one is interested in a single treatment contrast. However, there may be situations when one is interested in a group of treatment contrasts $\mathbf{L}'\mathbf{t}$, where $\mathbf{L}'$ is a $p \times v$ matrix such that $\mathbf{L}'\mathbf{1} = \mathbf{0}$, Rank (**L**) = *p*, and $\mathbf{t} = (t_1, t_2, \cdots, t_v)'$ is a $v \times 1$ vector of treatment effects. The sum of squares due to a set of treatment contrasts $\mathbf{L}'\mathbf{t}$ is $(\mathbf{L}'\hat{\mathbf{t}})'\left(\mathbf{L}'\mathbf{C}^{-}\mathbf{L}\right)^{-}\mathbf{L}'\hat{\mathbf{t}}$ and the dispersion matrix of $\mathbf{L}'\hat{\mathbf{t}}$, the best linear unbiased estimator of $\mathbf{L}'\mathbf{t}$, is $D(\mathbf{L}'\hat{\mathbf{t}}) = \sigma^2\left(\mathbf{L}'\mathbf{C}^{-}\mathbf{L}\right)$ and **C** is the coefficient matrix of reduced normal equations for estimating the linear functions of treatment effects. The null hypothesis of interest say is $H_0 : \mathbf{L}'\mathbf{t} = \mathbf{0}$ against $H_1 : \mathbf{L}'\mathbf{t} \neq \mathbf{0}$. The null hypothesis H_0 is

tested using the statistic $F=\frac{SS(\text{set of Contrasts})}{MSE}$ with *p* and *edf* (error degrees of freedom) degrees of freedom. If $\mathbf{L}'$ comprises of a complete set of (*v-1*) linearly independent parametric functions, *i.e., p = v-1,* then we can get the treatment sum of squares as we get in the ANOVA table. For more details on contrast analysis, a reference may be made to Dean and Voss (1999).

In multi-factor experiments, the treatments are combinations of levels of several factors. In these experimental situations, the treatment sum of squares is partitioned into sum of squares due to main effects and interactions. These sums of squares can also be obtained through contrast analysis. The procedure of obtaining sum of squares due to main effects and interactions is discussed in the sequel.

2.2 Main Effects and Interactions

In general, let there be *n*-factors, say $F_1, F_2, ..., F_n$ and i^{th} factor has s_i levels, $i = 1,...,n$. The $v = (\prod_{i=1}^{n} s_i)$ treatment combinations in the lexico-graphic order are given by $\mathbf{a}_1 \times \mathbf{a}_2 \times ... \times \mathbf{a}_n$ where $\times$ denotes the symbolic direct product and $\mathbf{a}_i' = (0,1,...,s_{i-1})$; $i = 1,2,...,n$. Renumber the treatment combinations from 1 to *v* and analyze the data as per procedure of general block designs for single factor experiments. The treatment sum of squares obtained from the ANOVA is now to be partitioned into main effects and interactions. This can easily be done through contrast analysis. One has to define the set of contrasts for each of the main effects and interactions. Before describing the procedure of defining contrasts for main effects and interactions, we give some preliminaries. The total number of factorial effects (main effects and interactions) is $2^n - 1$. The set of main effects and interactions have a one-one correspondence with $\mathbf{\Omega}$, the set of all *n*-component non-null binary vectors. For example a typical *p*-factor interaction, $F_{g_1}, F_{g_2}, ..., F_{g_p}$ $(1 \le g_1 \le g_2 \le ... \le g_p \le n, 1 \le p \le n)$ corresponds to the element $x = (x_1, ..., x_n)$ of $\mathbf{\Omega}$ such that $x_{g_1} = x_{g_2} = ... = x_{g_p} = 1$ and $x_u = 0$ for $u \ne g_1, g_2, ..., g_p$.

The treatment contrasts belonging to different interactions $F^x, x = (x_1, ..., x_n) \in \mathbf{\Omega}$ are given by

$\mathbf{P}^x\mathbf{t}$, where $\mathbf{P}^x = \mathbf{P}_1^{x_1} \otimes \mathbf{P}_2^{x_2} \otimes ... \otimes \mathbf{P}_n^{x_n}$

where $\mathbf{P}_i^{x_i} = \mathbf{P}_i$ if $x_i = 1$

$= \mathbf{1}'_{s_i}$ if $x_i = 0$

where $\mathbf{P}_i$ is a $(s_i - 1) \times s_i$ matrix of complete set of linearly independent contrasts of order s_i and $\mathbf{1}_{s_i}$ is a $s_i \times 1$ vector of ones. For example, if $s_i = 4$, then $\mathbf{P}_i = \begin{bmatrix} 1 & -1 & 0 & 0 \\ 1 & 1 & -2 & 0 \\ 1 & 1 & 1 & -3 \end{bmatrix}$.

For sum of squares of these contrasts and testing of hypothesis, a reference may be made to section 2.1.

In the sequel we describe some basic designs.

3. Completely Randomized Design

Designs are usually characterized by the nature of grouping of experimental units and the procedure of random allocation of treatments to the experimental units. In a completely randomized design the units are taken in a single group. As far as possible the units forming the group are homogeneous. This is a design in which only randomization and replication are used. There is no use of local control here.

Let there be *v* treatments in an experiment and *n* homogeneous experimental units. Let the i^{th} treatment be replicated r_i times *(i = 1,2,..., v)* such that $\sum_{i=1}^{v} r_i = n$. The treatments are allotted at random to the units.

Normally the number of replications for different treatments should be equal as it ensures equal precision of estimates of the treatment effects. The actual number of replications is, however, determined by the availability of experimental resources and the requirement of precision and sensitivity of comparisons. If the experimental material for some treatments is available in limited quantities, the numbers of their replication are reduced. If the estimates of certain treatment effects are required with more precision, the numbers of their replication are increased.

Randomization

There are several methods of random allocation of treatments to the experimental units. The *v* treatments are first numbered in any order from *1* to *v*. The *n* experimental units are also numbered suitably. One of the methods uses the random number tables. Any page of a random number table is taken. If *v* is a one-digit number, then the table is consulted digit by digit. If *v* is a two-digit number, then two-digit random numbers are consulted. All numbers greater than *v* including zero are ignored.

Let the first number chosen be n_1; then the treatment numbered n_1 is allotted to the first unit. If the second number is n_2 which may or may not be equal to n_1 then the treatment numbered n_2 is allotted to the second unit. This procedure is continued. When the i^{th} treatment number has occurred r_i times, $(i = 1,2,...,v)$ this treatment is ignored subsequently. This process terminates when all the units are exhausted.

One drawback of the above procedure is that sometimes a very large number of random numbers may have to be ignored because they are greater than *v*. It may even happen that the random number table is exhausted before the allocation is complete. To avoid this difficulty the following procedure is adopted. We have described the procedure by taking *v* to be a two-digit number.

Let *P* be the highest two-digit number divisible by *v*. Then all numbers greater than *P* and zero are ignored. If a selected random number is less than *v*, then it is used as such. If it is greater than or equal to *v*, then it is divided by *v* and the remainder is taken to the random number. When a number is completely divisible by *v*, then the random number is *v*. If *v* is

an *n*-digit number, then *P* is taken to be the highest *n*-digit number divisible by *v*. The rest of the procedure is the same as above.

Alternative methods of random allocation

If random number tables are not available, treatments can be allotted by drawing *lots* as below. Let the number of the i^{th} treatment be written on r_i pieces of papers $(i = 1,2,...,v)$. The $\sum_{i=1}^{v} r_i = n$ pieces of papers are then folded individually so that the numbers written on them are not visible. These papers are then drawn one by one at random. The treatment that is drawn in the t^{th} draw is allotted to the t^{th} plot $(t = 1,2,...,n)$.

Random allocation is also possible by using a fair coin. Let there be five treatments each to be replicated four times. There are, therefore, *20* plots. Let these plots be numbered from *1* to *20* conveniently.

When a coin is tossed, there are two events that is, either the head comes up, or the tail. We denote the "head" by H and the "tail" by T. When the coin is tossed twice, there are four events, that is, both times head HH; first head next tail HT: first tail next head TH and both times tail TT. Similarly, when the coin is thrown three times, there are the following eight possible events:

HHH, HHT, HTH, HTT, THH, THT, TTH, TTT.

Similar events can be written easily for four or more number of throws of the coin.

The five treatments are now labeled not by serial numbers as earlier but by any five of the above eight events obtainable by tossing three coins. Let us use the first five events and omit THT, TTH and TTT.

A coin is now thrown three times and the event happened noted. If the event is any of the first five events described above, the treatment labeled by it is allotted to the first experimental unit. If the event happened is any of the last three, it is ignored. The coin is again tossed three times and this event is used to select a treatment for the second experimental unit. If the same event occurs more than once, we are not to reject it until the number of times it has occurred equals the number of replications of the treatment it represents. This process is continued till all the experimental units are exhausted.

Analysis

This design provides a one-way classified data according to levels of a single factor. For its analysis the following model is taken:

$$y_{ij} = \mu + t_i + e_{ij}\ , \qquad i = 1,\cdots,v;\ j = 1,\cdots r_i\ ,$$

where y_{ij} is the random variable corresponding to the observation y_{ij} obtained from the j^{th} replicate of the i^{th} treatment, μ is the general mean, t_i is the fixed effect of the i^{th} treatment and e_{ij} is the error component which is a random variable assumed to be normally and independently distributed with zero means and a constant variance σ^2.

Let $\sum_j y_{ij} = T_i \quad (i = 1,2,...,v)$ be the total of observations from i^{th} treatment. Let further $\sum_i T_i = G$. Correction factor (*C.F.*) $= G^2/n$.

Sum of squares due to treatments $= \sum_{i=1}^{v} \frac{T_i^2}{r_i} - C.F.$

Total sum of squares $= \sum_{i=1}^{v} \sum_{j=1}^{r_i} y_{ij}^2 - C.F.$

ANALYSIS OF VARIANCE

Sources of variation	**Degrees of freedom (D.F.)**	**Sum of squares (S.S.)**	**Mean squares (M.S.)**	**F**
Treatments	$v - 1$	$SST = \sum_{i=1}^{v} \frac{T_i^2}{r_i} - C.F.$	$MST = SST / (v - 1)$	MST/MSE
Error	$n - v$	SSE = by subtraction	$MSE = SSE / (n - v)$	
Total	$n - 1$	$\sum_{ij} y_{ij}^2 - C.F.$		

The hypothesis that the treatments have equal effects is tested by F-test where F is the ratio *MST / MSE* with *(v - 1)* and *(n - v)* degrees of freedom. We may then be interested to either compare the treatments in pairs or evaluate special contrasts depending upon the objectives of the experiment. This is done as follows:

For a completely randomized design, the BLUE of the treatment contrast $\sum l_i t_i$ is $\sum_i l_i \hat{t}_i$

$= \sum_i l_i \bar{y}_i$, where $\bar{y}_i = T_i / r_i$, $Var(\sum_i l_i \hat{t}_i) = \sigma^2 \sum_i \frac{l_i^2}{r_i}$, where σ^2 is the error variance estimated by the error mean squares, MSE. The sum of squares due to contrast $\sum_i l_i \hat{t}_i$ is

$\left(\sum_i l_i \bar{Y}_i \right)^2 / \sum_i \frac{l_i^2}{r_i}$.

The significance of the contrast can be tested by *t* test, where

$$t = \frac{\sum_i l_i \bar{y}_i}{\sqrt{MSE \sum_i \frac{l_i^2}{r_i}}}$$

where $t_{1-\alpha/2,(n-v)}$ is the value of Student's *t* at the level of significance α and degree of freedom *(n - v)*. Contrasts of the type $t_i - t_m$ in which experimenters are often interested are obtainable from $\sum_i l_i t_i$ by putting $l_i = 1, l_m = -1$ and zero for the other *l's*. Such comparisons are known as *pairwise comparisons*.

Sometimes the levels of the treatment factors divide naturally into two or more groups, and the experimenter is interested in the difference of averages contrast that compares the average effect of one group with the average effect of the other group(s). For example, consider an experiment that is concerned with the effect of different colors of exam paper (the treatments) on students' exam performance (the response). Suppose that treatments *1* and *2* represent the pale colors, white and yellow, whereas treatments *3*, *4* and *5* represent the darker colors, blue, green and pink. The experimenter may wish to compare the effects of light and dark colors on exam performance. One way of measuring this is to estimate the contrast $\frac{1}{2}(t_1 + t_2) - \frac{1}{3}(t_3 + t_4 + t_5),$, which is the difference of the average effects of the light and dark colors. The corresponding contrast coefficients are

$$\left[\frac{1}{2}, \frac{1}{2}, -\frac{1}{3}, -\frac{1}{3}, -\frac{1}{3}\right].$$

The BLUE of the above contrast would be $\frac{1}{2}\bar{y}_1 + \frac{1}{2}\bar{y}_2 - \frac{1}{3}\bar{y}_3 - \frac{1}{3}\bar{y}_4 - \frac{1}{3}\bar{y}_5$ with estimated standard error as $\sqrt{MSE(\frac{1}{4r_1} + \frac{1}{4r_2} + \frac{1}{9r_3} + \frac{1}{9r_4} + \frac{1}{9r_5})}$.

A $100(1-\alpha)\%$ confidence interval for the contrast $\sum_i l_i t_i$ is

$$\sum l_i \bar{y}_i - t_{n-v,\alpha/2}\sqrt{MSE\sum \frac{l_i^2}{r_i}} \le \sum l_i t_i \le \sum l_i \bar{y}_i + t_{n-v,\alpha/2}\sqrt{MSE\sum \frac{l_i^2}{r_i}}.$$

4. Randomized Complete Block Design

It has been seen that when the experimental units are homogeneous then a CRD should be adopted. In any experiment, however, besides treatments the experimental material is a major source of variability in the data. When experiments require a large number of experimental units, the experimental units may not be homogeneous, and in such situations CRD can not be recommended. When the experimental units are heterogeneous, a part of the variability can be accounted for by grouping the experimental units in such a way that experimental units within each group are as homogeneous as possible. The treatments are then allotted randomly to the experimental units within each group (or blocks). The principle of first forming homogeneous groups of the experimental units and then allotting at random each treatment once in each group is known as local control. This results in an increase in precision of estimates of the treatment contrasts, due to the fact that error variance that is a function of comparisons within blocks, is smaller because of homogeneous blocks. This type of allocation makes it possible to eliminate from error variance a portion of variation attributable to block differences. If, however, variation between the blocks is

not significantly large, this type of grouping of the units does not lead to any advantage; rather some degrees of freedom of the error variance is lost without any consequent decrease in the error variance. In such situations it is not desirable to adopt randomized complete block designs in preference to completely randomized designs.

If the number of experimental units within each group is same as the number of treatments and if every treatment appears precisely once in each group then such an arrangement is called a ***randomized complete block design.***

Suppose the experimenter wants to study *v* treatments. Each of the treatments is replicated *r* times (the number of blocks) in the design. The total number of experimental units is, therefore, *vr*. These units are arranged into *r* groups of size *v* each. The error control measure in this design consists of making the units in each of these groups homogeneous.

The number of blocks in the design is the same as the number of replications. The *v* treatments are allotted at random to the *v* plots in each block. This type of homogeneous grouping of the experimental units and the random allocation of the treatments separately in each block are the two main characteristic features of randomized block designs. The availability of resources and considerations of cost and precision determine actual number of replications in the design.

Analysis

The data collected from experiments with randomized block designs form a two-way classification, that is, classified according to the levels of two factors, *viz.*, blocks and treatments. There are *vr* cells in the two-way table with one observation in each cell. The data are orthogonal and therefore the design is called an *orthogonal design.* We take the following model:

$$y_{ij} = \mu + t_i + b_j + e_{ij}\ , \qquad \begin{pmatrix} i = 1,2,...,v; \\ j = 1,2,...,r \end{pmatrix},$$

where y_{ij} denotes the observation from i^{th} treatment in j^{th} block. The fixed effects μ, t_i, b_j denote respectively the general mean, effect of the i^{th} treatment and effect of the j^{th} block. The random variable e_{ij} is the error component associated with y_{ij}. These are assumed to be normally and independently distributed with zero means and a constant variance σ^2.

Following the method of analysis of variance for finding sums of squares due to blocks, treatments and error for the two-way classification, the different sums of squares are obtained as follows: Let $\sum_j y_{ij} = T_i \ \ (i = 1,2,...,v)$ = total of observations from i^{th} treatment and $\sum_j y_{ij} = B_j \qquad j = 1,\cdots,r$ = total of observations from j^{th} block. These are the marginal totals of the two-way data table. Let further, $\sum_i T_i = \sum_j B_j = G.$

Correction factor (*C.F.*) = G^2/rv, Sum of squares due to treatments $= \sum_i \frac{T_i^2}{r} - C.F.$,

Sum of squares due to blocks $= \sum_j \frac{B_j^2}{v} - C.F.$, Total sum of squares $= \sum_{ij} y_{ij}^2 - C.F.$

ANALYSIS OF VARIANCE

Sources of variation	**Degrees of freedom (D.F.)**	**Sum of squares (S.S.)**	**Mean squares (M.S.)**	**F**
Blocks	*r - 1*	$SSB = \sum_j \frac{B_j^2}{v} - C.F.$	*MSB = SSB / (r - 1)*	*MSB/MSE*
Treatments	*v - 1*	$SST = \sum_i \frac{T_i^2}{r} - C.F.$	*MST = SST / (v - 1)*	*MST/MSE*
Error	*(r - 1)(v - 1)*	*SSE = by subtraction*	*MSE = SSE / (v - 1)(r - 1)*	
Total	*vr - 1*	$\sum_{ij} y_{ij}^2 - C.F.$		

The hypothesis that the treatments have equal effects is tested by F-test, where F is the ratio *MST / MSE* with *(v - 1)* and *(v - 1)(r - 1)* degrees of freedom. We may then be interested to either compare the treatments in pairs or evaluate special contrasts depending upon the objectives of the experiment. This is done as follows:

Let $\sum l_i t_i$ denote a treatment contrast, $\sum_i l_i = 0$. The BLUE of $\sum l_i t_i$ is $\sum_i l_i \hat{t}_i = \sum_i l_i \bar{y}_i$,

where $\bar{y}_i = T_i / r$, $Var(\sum_i l_i \hat{t}_i) = \frac{\sigma^2}{r} \sum_i l_i^2$, where σ^2 is estimated by the error mean squares, MSE. The sum of squares due to contrast $\sum_i l_i \hat{t}_i$ is $\left(\sum_i l_i \bar{y}_i \right)^2 / \left(\sum_i l_i^2 / r \right)$. The significance of the contrast can be tested as per procedure described in sections 2 and 3. The *100(1 - α)%* confidence interval for this contrast is

$$\sum l_i \bar{y}_i - t_{(v-1)(r-1),\alpha/2} \sqrt{MSE \sum l_i^2 / r} \le \sum l_i t_i \le \sum l_i \bar{y}_i + t_{(v-1)(r-1)-v,\alpha/2} \sqrt{MSE \sum l_i^2 / r}$$

As we know that the outcome of a hypothesis test can be deduced from the corresponding confidence interval in the following way. The null hypothesis $H_0 : \sum_i l_i t_i = 0$ will be rejected at significance level α in favor of the two-sided alternative hypothesis $H_1 : \sum_i l_i t_i \neq 0$ if the corresponding confidence interval for $\sum_i l_i t_i$ fails to contain *0*. The

interval fails to contain *0* if the absolute value of $\sum l_i \bar{y}_i$ is bigger than $t_{(v-1)(r-1),\alpha/2}\sqrt{MSE\sum_i l_i^2 / r}$. Therefore, all possible paired comparisons between treatment effects one may use the critical differences.

The critical difference for testing the significance of the difference of two treatment effects, say $t_i - t_j$ is $C.D. = t_{(v-1)(r-1),\alpha/2}\sqrt{2MSE/r}$, where $t_{(v-1)(r-1),\alpha/2}$ is the value of Student's *t* at the level of significance α and degree of freedom *(v - 1)(r - 1).* If the difference of any two-treatment means is greater than the C.D. value, the corresponding treatment effects are significantly different.

Example 4.1: An experiment was conducted to evaluate the efficacy of Londax 60 DF in transplanted rice as pre-emergent application as stand alone and as tank mix with grass partner against different weed flora. The weed counts were recorded. The details of the experiment are given below:

The weed Count in Rice

Treatment	Dose (gai/ha)	Replications		
		1	2	3
Londax 60 DF	30	72	60	59
Londax 60 DF	45	81	56	71
Londax 60 DF	60	66	49	56
Londax+ Butachlor	30+938	8	9	4
Londax + Butachlor	45+938	10	17	6
Londax+ Butachlor	60+938	4	8	3
Butachlor 50 EC	938	22	10	11
Pretilachlor 50 EC	625	4	8	10
Pyrazo.Eth.10 WP	100 g/acre	20	46	33
Untreated Control	-	79	68	84

Analyze the data and draw your conclusions.

Procedure and Calculations:

We compute the following totals:

Treatments totals ($y_{i.}$)	Treatment means ($\bar{y}_{i.} = y_{i.}/b$)
$y_{1.} = 72 + 60 + 59 = 191$	$\bar{y}_{1.} = 191/3 = 63.6667$
$y_{2.} = 81 + 56 + 71 = 208$	$\bar{y}_{2.} = 208/3 = 69.3333$
$y_{3.} = 66 + 49 + 56 = 171$	$\bar{y}_{3.} = 171/3 = 57.0000$
$y_{4.} = 8 + 9 + 4 = 21$	$\bar{y}_{4.} = 21/3 = 7.0000$
$y_{5.} = 10 + 17 + 6 = 33$	$\bar{y}_{5.} = 33/3 = 11.0000$
$y_{6.} = 4 + 8 + 3 = 15$	$\bar{y}_{6.} = 15/3 = 5.0000$
$y_{7.} = 22 + 10 + 11 = 43$	$\bar{y}_{7.} = 43/3 = 14.3333$
$y_{8.} = 4 + 8 + 10 = 22$	$\bar{y}_{8.} = 22/3 = 7.3333$
$y_{9.} = 20 + 46 + 33 = 99$	$\bar{y}_{9.} = 99/3 = 33.0000$
$y_{10.} = 79 + 68 + 84 = 231$	$\bar{y}_{10.} = 231/3 = 77.0000$

Replication (or Blocks) Totals ($y_{.j}$)	Replication Means ($\bar{y}_{.j} = y_{.j} / v$)
$y_{.1} = 72 + \ldots + 79 = 366$	$\bar{y}_{.1} =$ 366/10 = 36.6
$y_{.2} =$ 60 + … + 68 = 331	$\bar{y}_{.2} =$ 331/10 = 33.1
$y_{.3} =$ 59 + … + 84 = 337	$\bar{y}_{.3} =$ 337/10 = 33.7

Grand Total (of all the observations) = $\sum_i \sum_j y_{ij} = y_{..} = \sum_i y_{i.} = \sum_j y_{.j} = 1034.0000$.

Correction Factor = $(y_{..})^2 / vb = (1034)^2/30 =$ 35638.5333

Sum of Squares due to Trees = $\sum_i y_{i.}^2 / b - C.F.$

$= \left(191^2 + \cdots + 231^2\right)/3 - 35638.5333 = 23106.8$

Sum of Squares due to Replications = $\sum_j y_{.j}^2 / v - C.F.$

$= \left(366^2 + 331^2 + 337^2\right)/10 - 35638.5333 = 70.0667$.

Total Sum of Squares = $\sum_i \sum_j y_{ij}^2 - C.F.$

$= 72^2 + 81^2 + \cdots + 84^2 - C.F. = 24343.4667$.

Error Sum of Squares = Total Sum of Squares - Sum of Squares due to Trees - Sum of Squares due to Replications = *24343.4667 – 70.0667 – 23106.8000* = 1166.6000.

We now form the following Analysis of Variance Table:

ANOVA

Source	D.F.	S.S.	M.S.	F	Pr > F
Due to Treatments	9	23106.8000	2567.422	39.61	*0.000*
Due to Replications	2	70.0667	35.03335	0.54	*0.592*
Error	18	1166.6000	64.81111		
Total	29	24343.4667			

Critical Difference between any two tree means = $t_{\alpha, error\ d.f.} x \sqrt{2MSE / b}$

$= 2.101 \times \sqrt{(2 \times 64.81111)/3} = 13.810$

On the basis of the critical difference we prepare the following table giving the significance of the difference between two trees effects:

		Mean	Treatment No.
	A	77.0000	10
B	*A*	69.3330	2
B	*A*	63.6670	1

	B	57.0000	3
	C	33.0000	9
D		14.3330	7
D		11.0000	5
D		7.3330	8
D		7.0000	4
D		5.0000	6

Suppose now that treatment numbers 1, 2, 3 and treatment numbers 4, 5, 6 form two groups as the treatments in group 1 are with Londax only where as group2 comprises of treatments in which Butachlor is added along with Londax. Our interest is in comparing the two groups. We shall have the following contrast to be estimated and tested:

1. $t_1 + t_2 + t_3 - t_4 - t_5 - t_6$.

Similarly, suppose the other contrasts to be estimated and tested are:

2. $t_1 + t_2 + t_3 + t_4 + t_5 + t_6 + t_7 + t_8 + t_9 - 9t_{10}$
3. $t_4 + t_5 + t_6 - 3t_7$

We have the following table:

Sl. No.	D.F.	Contrast S.S.	M.S.	F	Pr > F
1	*1*	13944.5000	13944.5000	215.16	0.0001
2	*1*	6030.2815	6030.2815	93.04	0.0001
3	*1*	100.0000	100.0000	1.54	0.2301

Suppose now that the interest of the experimenter is to test certain hypothesis concerning the three treatments in the Group 1. The sum of squares for testing the equality of tree effects can be obtained by defining four mutually orthogonal contrasts as $t_1 - t_2$; $t_1 + t_2 - 2t_3$.

Using these sets of contrasts we get the following:

Sl. No.	D.F.	S.S.	M.S.	F	Pr > F
1	2	228.6667	114.3333	1.76	0.1997

Example 4.2: An initial varietal trial (Late Sown, irrigated) was conducted to study the performance of 20 new strains of mustard vis-a-vis four checks (Swarna Jyoti: ZC; Vardan: NC; Varuna: NC; and Kranti: NC) using a Randomized complete Block Design (RCB) design at Bhatinda with 3 replications. The seed yield in kg/ha was recorded. The details of the experiment are given below:

Yield in kg/ha

Strain	Code	Replications		
		1	2	3
RK-04-3	MCN-04-110	1539.69	1412.35	1319.73
RK-04-4	MCN-04-111	1261.85	1065.05	1111.36
RGN-124	MCN-04-112	1389.19	1516.54	1203.97
HYT-27	MCN-04-113	1192.39	1215.55	1157.66

PBR-275	MCN-04-114	1250.27	1203.97	1366.04
HUJM-03-03	MCN-04-115	1296.58	1273.43	1308.16
RGN-123	MCN-04-116	1227.12	1018.74	937.71
BIO-13-01	MCN-04-117	1273.43	1157.66	1088.20
RH-0115	MCN-04-118	1180.82	1203.97	1041.90
RH-0213	MCN-04-119	1296.58	1458.65	1250.27
NRCDR-05	MCN-04-120	1122.93	1065.05	1018.74
NRC-323-1	MCN-04-121	1250.27	926.13	1030.32
RRN-596	MCN-04-122	1180.82	1053.47	717.75
RRN-597	MCN-04-123	1146.09	1180.82	856.67
CS-234-2	**MCN-04-124**	1574.42	1412.35	1597.57
RM-109	MCN-04-125	914.55	972.44	659.87
BAUSM-2000	MCN-04-126	891.40	937.71	798.79
NPJ-99	MCN-04-127	1227.12	1203.97	1389.19
SWAN JYOTI (ZC)	**MCN-04-128**	1389.19	1180.82	1273.43
VARDAN (NC)	**MCN-04-129**	1331.31	1157.66	1180.82
PR-2003-27	MCN-04-130	1250.27	1250.27	1296.58
VARUNA (NC)	**MCN-04-131**	717.75	740.90	578.83
PR-2003-30	MCN-04-132	1169.24	1157.66	1111.36
KRANTI-(NC)	**MCN-04-133**	1203.97	1296.58	1250.27

Analyze the data and draw your conclusions.

Procedure and Calculations: We compute the following totals:

Treatment Total ($y_{i.}$)	**Treatment Mean ($\bar{y}_{i.} = y_{i.}/3$)**
$y_{1.} = 4271.77$	$\bar{y}_{1.} = 1423.92$
$y_{2.} = 3438.26$	$\bar{y}_{2.} = 1146.09$
$y_{3.} = 4109.70$	$\bar{y}_{3.} = 1369.90$
$y_{4.} = 3565.60$	$\bar{y}_{4.} = 1188.53$
$y_{5.} = 3820.28$	$\bar{y}_{5.} = 1273.43$
$y_{6.} = 3878.17$	$\bar{y}_{6.} = 1292.72$
$y_{7.} = 3183.57$	$\bar{y}_{7.} = 1061.19$
$y_{8.} = 3519.29$	$\bar{y}_{8.} = 1173.10$
$y_{9.} = 3426.68$	$\bar{y}_{9.} = 1142.23$
$y_{10.} = 4005.51$	$\bar{y}_{10.} = 1335.17$
$y_{11.} = 3206.72$	$\bar{y}_{11.} = 1068.91$
$y_{12.} = 3206.72$	$\bar{y}_{12.} = 1068.91$

Treatment Total ($y_{i.}$)	**Treatment Mean ($\bar{y}_{i.} = y_{i.}/3$)**
$y_{13.} = 2952.04$	$\bar{y}_{13.} = 984.01$
$y_{14.} = 3183.57$	$\bar{y}_{14.} = 1061.19$
$y_{15.} = 4584.34$	$\bar{y}_{15.} = 1528.11$
$y_{16.} = 2546.86$	$\bar{y}_{16.} = 848.95$
$y_{17.} = 2627.89$	$\bar{y}_{17.} = 875.96$
$y_{18.} = 3820.28$	$\bar{y}_{18.} = 1273.43$
$y_{19.} = 3843.44$	$\bar{y}_{19.} = 1281.15$
$y_{20.} = 3669.79$	$\bar{y}_{20.} = 1223.26$
$y_{21.} = 3797.13$	$\bar{y}_{21.} = 1265.71$
$y_{22.} = 2037.49$	$\bar{y}_{22.} = 679.16$
$y_{23.} = 3438.26$	$\bar{y}_{23.} = 1146.09$
$y_{24.} = 3750.82$	$\bar{y}_{24.} = 1250.27$

Replication (or Blocks) Totals ($y_{.j}$)	**Replication Means ($\bar{y}_{.j} = y_{.j}/v$)**
$y_{.1} = 29277.27$	$\bar{y}_{.1} = 29277.27/24 = 1219.89$
$y_{.2} = 28061.73$	$\bar{y}_{.2} = 28061.73/24 = 1169.24$
$y_{.3} = 26545.19$	$\bar{y}_{.3} = 26545.19/24 = 1106.05$

Grand Total (of all the observations) = $\sum_i \sum_j y_{ij} = y_{..} = \sum_i y_{i.} = \sum_j y_{.j} = 83884.19$.

Correction Factor = $(y_{..})^2 / vb = (83884.19)^2/72 = 97730396.53$

Sum of Squares due to treatments = $\sum_i y_{i.}^2 / b - C.F.$

$$= \left(4271.77^2 + \cdots + 3750.82^2\right)/3 - 97730396.53 = 2514143.05$$

Sum of Squares due to Replications = $\sum_j y_{.j}^2 / v - C.F.$

$$= \left(29277.27^2 + 28061.73^2 + 26545.19^2\right)/24 - 97730396.53$$
$$= 156139.3283$$

Total Sum of Squares = $\sum_i \sum_j y_{ij}^2 - C.F.$

$$= 1539.69^2 + \cdots + 1250.27^2 - C.F. = 3133406.13.$$

Error Sum of Squares = Total Sum of Squares - Sum of Squares due to treatments - Sum of Squares due to Replications = 3133406.13-2514143.05-156139.33=463123.75.

We now form the following Analysis of Variance Table:

ANOVA (Yield: Bhatinda)

Source	D.F.	S.S.	M.S.	F	Pr > F
Due to Treatments	23	2514143.05	109310.57	10.86	*<0.0001*
Due to Replications	2	156139.33	78069.66	7.75	*0.0013*
Error	46	463123.75	10067.91		
Total	71	3133406.13			

R-Square	CV	Root MSE	Mean Yield
0.852198	8.612337	100.3390	1165.06

2. Critical Difference between any two tree means = $t_{\alpha,error\ d.f.} x\sqrt{2MSE/b}$

$= 2.10290\times\sqrt{(2\times 10067.91)/3} = 164.91$

On the basis of the critical difference we prepare the following table giving the significance of the difference between two treatment effects:

Mean	Treatment No.		Mean	Treatment No.	
1528.11	15	A	1173.10	8	D E F
1423.93	1	A B	1146.10	23	E F G
1369.90	3	A B C	1146.10	2	E F G
1335.18	10	B C D	1142.22	9	E F G
1292.73	6	B C D E	1068.90	12	F G
1281.14	19	B C D E	1068.90	11	F G
1273.43	18	B C D E	1061.19	7	F G
1273.43	5	B C D E	1061.19	14	F G
1265.72	21	B C D E	984.02	13	G H
1250.27	24	C D E	875.97	17	H
1223.27	20	C D E F	848.96	16	H
1188.55	4	D E F	679.15	22	I

Suppose now that treatment numbers 19, 20, 22 and 24 are the checks and rest of the treatments are test entries. It is clear from the above Table that treatment 15 is significantly different from highest performing check. The above Table gives Our interest is in comparing the checks with new entries. We shall have the following contrast to be estimated and tested:

1. $4t_1 + 4t_2 + \cdots + 4t_{18} + 4t_{21} + 4t_{23} - 20t_{19} - 20t_{20} - 20t_{22} - 20t_{24}$.

We have the following table:

Sl. No.	D.F.	Contrast S.S.	M.S.	F	Pr > F
Checks vs Entries	*1*	46128.89	46128.89	4.58	0.0376

Suppose the experimenter can test any other hypothesis of interest.

Exercise 4.3: In order to select suitable tree species for Fuel, Fodder and Timber an experiment was conducted in a randomized complete block design with ten different trees

and four replications. The plant height was recorded in cms. The details of the experiment are given below:

Plant Height (Cms): Place – Kanpur

Name of Tree	Spacing	Replications			
		1	2	3	4
A. Indica	4x4	144.44	145.11	104.00	105.44
D. Sisso	4x2	113.50	118.61	118.61	123.00
A. Procer	4x2	60.88	90.94	80.33	92.00
A. Nilotic	4x2	163.44	158.55	158.88	153.11
T. Arjuna	4x2	110.11	116.00	119.66	103.22
L. Loucoc	4x1	260.05	102.27	256.22	217.80
M. Alba	4x2	114.00	115.16	114.88	106.33
C. Siamia	4x2	91.94	58.16	76.83	79.50
E. Hybrid	4x1	156.11	177.97	148.22	183.17
A. Catech	4x2	80.2	108.05	45.18	79.55

Analyze the data and draw your conclusions.

Procedure and Calculations: We compute the following totals:

Treatments totals ($y_{i.}$)	**Treatment means ($\bar{y}_{i.} = y_{i.} / b$)**
$y_{1.} = 144.44 + \ldots + 105.44 = 498.99$	$\bar{y}_{1.} = 498.99/4 = 124.7475$
$y_{2.} = 112.50 + \ldots + 123.00 = 473.72$	$\bar{y}_{2.} = 473.72/4 = 118.4300$
$y_{3.} = 60.88 + \ldots + 92.00 = 324.15$	$\bar{y}_{3.} = 324.15/4 = 81.0375$
$y_{4.} = 163.44 + \ldots + 153.11 = 633.98$	$\bar{y}_{4.} = 633.98/4 = 158.4950$
$y_{5.} = 110.11 + \ldots + 103.22 = 448.99$	$\bar{y}_{5.} = 448.99/4 = 112.2475$
$y_{6.} = 260.05 + \ldots + 217.8 = 836.34$	$\bar{y}_{6.} = 836.34/4 = 209.0850$
$y_{7.} = 114.00 + \ldots + 106.33 = 450.37$	$\bar{y}_{7.} = 450.37/4 = 112.5925$
$y_{8.} = 91.94 + \ldots + 79.50 = 306.43$	$\bar{y}_{8.} = 306.43/4 = 76.6075$
$y_{9.} = 156.11 + \ldots + 183.17 = 665.47$	$\bar{y}_{9.} = 665.47/4 = 166.3675$
$y_{10.} = 80.20 + \ldots + 79.55 = 312.98$	$\bar{y}_{10.} = 312.98/4 = 78.2450$

Replication (or Blocks) Totals ($y_{.j}$)	**Replication Means ($\bar{y}_{.j} = y_{.j} / v$)**
$y_{.1} = 144.44 + \ldots + 80.20 = 1294.67$	$\bar{y}_{.1} = 1294.67/10 = 129.4670$
$y_{.2} = 145.11 + \ldots + 108.05 = 1190.82$	$\bar{y}_{.2} = 1190.82/10 = 119.0820$
$y_{.3} = 104.00 + \ldots + 45.18 = 1222.81$	$\bar{y}_{.3} = 1222.81/10 = 122.2810$
$y_{.4} = 105.44 + \ldots + 79.55 = 1243.12$	$\bar{y}_{.4} = 1243.12/10 = 124.3120$

Grand Total (of all the observations) = $\sum_i \sum_j y_{ij} = y_{..} = \sum_i y_{i.} = \sum_j y_{.j} = 4951.42$.

Correction Factor = $(y_{..})^2 / vb = (4951.42)^2/40 = 612914.0004$

Sum of Squares due to Trees = $\sum_i y_{i.}^2 / b - C.F.$

$$= \left(498.99^2 + \cdots + 312.98^2\right)/4 - 612914.00 = 66836.35$$

Sum of Squares due to Replications = $\sum_j y_{.j}^2 / v - C.F.$

$$= \left(1294.67^2 + \cdots + 1243.12^2\right)/10 - 6121914.00 = 569.43 .$$

Total Sum of Squares = $\sum_i \sum_j y_{ij}^2 - C.F.$

$$= 144.44^2 + 145.12^2 + \cdots + 79.55^2 - C.F. = 89101.42 .$$

Error Sum of Squares = Total Sum of Squares - Sum of Squares due to Trees - Sum of

Squares due to Replications = *89101.42 - 66836.35 - 569.43 = 21695.26.*

We now form the following Analysis of Variance Table:

ANOVA

Source	**D.F.**	**S.S.**	**M.S.**	**F**	**Pr > F**
Due to Trees	*9*	*66836.35*	*7426.26*	*9.24*	*0.0001*
Due to Replications	*3*	*569.43*	*189.81*	*0.24*	*0.8703*
Error	*27*	*21695.26*	*803.53*		
Total	*39*	*89101.04*			

Critical Difference between any two tree means = $t_{\alpha,error\ d.f.} x\sqrt{2MSE / b}$

$$= 2.05x\sqrt{(2x803.53)/4} = 41.09$$

On the basis of the critical difference we prepare the following table giving the significance of the difference between two trees effects:

					Mean	**Tree No.**
				A	*209.085*	*6*
			B		*166.368*	*9*
		C	*B*		*158.500*	*4*
	D	*C*			*124.748*	*1*
E	*D*	*C*			*118.430*	*2*
F	*E*	*D*			*112.593*	*7*
F	*E*	*D*			*112.248*	*5*
F	*E*				*81.038*	*3*
F	*E*				*78.245*	*10*
F					*76.608*	*8*

Suppose now that tree numbers *1, 2, 3, 4, 10* and trees numbers *5, 6, 7, 8, 9* form two groups on the basis of some considerations. (The first group of trees is useful for fuel, fodder and timber while the second group of trees is useful for fuel and fodder only). Our interest is in comparing the two groups. We shall have the following contrast to be estimated and tested:

1. $t_1 + t_2 + t_3 + t_4 - t_5 - t_6 - t_7 - t_8 - t_9 + t_{10}$.

Similarly, suppose the other contrasts to be estimated and tested are:

2. $9t_1 - t_2 - t_3 - t_4 - t_5 - t_6 - t_7 - t_8 - t_9 - t_{10}$
3. $t_1 + t_2 + t_3 + t_4 - 4t_9$
4. $t_1 + t_2 + t_3 + t_4 - 4t_{10}$
5. $t_5 + t_6 + t_7 + t_8 - 4t_9$
6. $t_5 + t_6 + t_7 + t_8 - 4t_{10}$
7. $t_9 - t_{10}$

We have the following table:

Sl. No.	D.F.	Contrast S.S.	M.S.	F	Pr > F
1	*1*	*788.3285*	*788.3285*	*0.98*	*0.3307*
2	*1*	*4.1131*	*4.1131*	*0.01*	*0.9435*
3	*1*	*6680.2435*	*6680.2435*	*8.31*	*0.0076*
4	*1*	*5761.6546*	*5761.6546*	*7.17*	*0.0125*
5	*1*	*4801.1258*	*4801.1258*	*5.98*	*0.0213*
6	*1*	*7805.3981*	*7805.3981*	*9.71*	*0.0043*
7	*1*	*15531.1500*	*15531.1500*	*19.33*	*0.0002*

Suppose now that the interest of the experimenter is to test certain hypothesis concerning the five trees in the Group 1 (comprising of Trees Numbers *1, 2, 3, 4,* and *10*). The sum of squares for testing the equality of tree effects can be obtained by defining four mutually orthogonal contrasts as $t_1 - t_2;\ t_1 + t_2 - 2t_3;\ t_1 + t_2 + t_3 - 3t_3;\ t_1 + t_2 + t_3 + t_4 - 4t_{10}$. Using these sets of contrasts we get the following:

Sl. No.	D.F.	S.S.	M.S.	F	Pr > F
1	4	17854.0908	4463.5227	5.55	0.0021

5. Latin Square Design

Latin square designs are normally used in experiments where it is required to remove the heterogeneity of experimental material in two directions. These designs require that the number of replications equal the number of ***treatments*** or ***varieties***.

Definition 1. A Latin square arrangement is an arrangement of v symbols in v^2 cells arranged in v rows and v columns, such that every symbol occurs precisely once in each row and precisely once in each column. The term v is known as the **order** of the Latin square.

If the symbols are taken as *A, B, C, D,* a Latin square arrangement of order 4 is as follows:

A	*B*	*C*	*D*
B	*C*	*D*	*A*
C	*D*	*A*	*B*
D	*A*	*B*	*C*

A Latin square is said to be in the ***standard form*** if the symbols in the first row and first column are in natural order, and it is said to be in the ***semi-standard form*** if the symbols of

the first row are in natural order. Some authors denote both of these concepts by the term ***standard form***. However, there is a need to distinguish between these two concepts. The standard form is used for randomizing the Latin-square designs, and the semistandard form is needed for studying the properties of the orthogonal Latin squares.

Definition 2. If in two Latin squares of the same order, when superimposed on one another, every ordered pair of symbols occurs exactly once, the two Latin squares are said to be **orthogonal**. If the symbols of one Latin square are denoted by Latin letters and the symbols of the other are denoted by Greek letters, the pair of orthogonal Latin squares is also called a **graeco-latin square**.

Definition 3. If in a set of Latin squares every pair is orthogonal, the set is called a set of **mutually orthogonal latin squares (MOLS)**. It is also called a **hypergraeco latin square.**

The following is an example of graeco latin square:

A	B	C	D		α	γ	δ	β		$A\alpha$	$B\gamma$	$C\delta$	$D\beta$
B	A	D	C		β	δ	γ	α		$B\beta$	$A\delta$	$D\gamma$	$C\alpha$
C	D	A	B		γ	α	β	δ		$C\gamma$	$D\alpha$	$A\beta$	$B\delta$
D	C	B	A		δ	β	α	γ		$D\delta$	$C\beta$	$B\alpha$	$A\gamma$

We can verify that in the above arrangement every pair of ordered Latin and Greek symbols occurs exactly once, and hence the two latin squares under consideration constitute a graecolatin square.

It is well known that the maximum number of MOLS possible of order v is $v - 1$. A set of $v - 1$ MOLS is known as a complete set of MOLS. Complete sets of MOLS of order v exist when v is a ***prime or prime power.***

Randomization

According to the definition of a Latin square design, treatments can be allocated to the v^2 experimental units (may be animal or plots) in a number of ways. There are, therefore, a number of Latin squares of a given order. The purpose of randomization is to select one of these squares at random. The following is one of the methods of random selection of Latin squares.

Let a $v \times v$ Latin square arrangement be first written by denoting treatments by Latin letters *A, B, C, etc.* or by numbers *1, 2, 3, etc.* Such arrangements are readily available in the ***Tables for Statisticians and Biometricians*** (Fisher and Yates, 1974). One of these squares of any order can be written systematically as shown below for a 5×5 Latin square:

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

For the purpose of randomization rows and columns of the Latin square are rearranged randomly. There is no randomization possible within the rows and/or columns. For example, the following is a row randomized square of the above 5×5 Latin square;

$$\begin{array}{ccccc} A & B & C & D & E \\ B & C & D & E & A \\ E & A & B & C & D \\ D & E & A & B & C \\ C & D & E & A & B \end{array}$$

Next, the columns of the above row randomized square have been rearranged randomly to give the following random square:

$$\begin{array}{ccccc} E & B & C & A & D \\ A & C & D & B & E \\ D & A & B & E & C \\ C & E & A & D & B \\ B & D & E & C & A \end{array}$$

As a result of row and column randomization, but not the randomization of the individual units, the whole arrangement remains a Latin square.

Analysis of Latin Square Designs

In Latin square designs there are three factors. These are the factors *P*, *Q*, and treatments. The data collected from this design are, therefore, analyzed as a three-way classified data. Actually, there should have been v^3 observations as there are three factors each at *v* levels. But because of the particular allocation of treatments to the cells, there is only one observation per cell instead of *v* in the usual three way classified orthogonal data. As a result we can obtain only the sums of squares due to each of the three factors and error sum of squares. None of the interaction sums of squares of the factors can be obtained. Accordingly, we take the model

$$Y_{ijs} = \mu + r_i + c_j + t_s + e_{ijs}$$

where y_{ijs} denotes the observation in the i^{th} row, j^{th} column and under the s^{th} treatment; $\mu, r_i, c_j, t_s (i, j, s = 1,2,...,v)$ are fixed effects denoting in order the general mean, the row, the column and the treatment effects. The e_{ijs} is the error component, assumed to be independently and normally distributed with zero mean and a constant variance, σ^2.

The analysis is conducted by following a similar procedure as described for the analysis of two-way classified data. The different sums of squares are obtained as below: Let the data be arranged first in a *row* $\times$ *column* table such that y_{ij} denotes the observation of (*i, j*)th cell of table.

Let $R_i = \sum_j y_{ij} = i^{th}\ row\,total\ (i = 1,2,...,v)$, $C_j = \sum_i y_{ij} = j^{th}\ column\,total\ (j = 1,2,...,v)$,

$T_s =$ sum of those observations which come from s^{th} treatment *(s= 1,2,…,v),*

$G = \sum_i R_i = grand\ total$. Correction factor, $C.F. = \frac{G^2}{v^2}$. Treatment sum of squares = $\sum_s \frac{T_s^2}{v} - C.F.$, Row sum of squares = $\sum_i \frac{R_i^2}{v} - C.F.$, Column sum of squares = $\sum_j \frac{C_j^2}{v} - C.F.$

Analysis of Variance of $v \times v$ Latin Square Design

Sources of Variation	D.F.	S.S.	M.S.	F
Rows	v -1	$\sum_i \frac{R_i^2}{v} - C.F.$		
Columns	v - 1	$\sum_j \frac{C_j^2}{v} - C.F.$		
Treatments	v - 1	$\sum_s \frac{T_s^2}{v} - C.F.$	s_t^2	s_t^2 / s_e^2
Error	$(v - 1)(v - 2)$	By subtraction	s_e^2	
Total	v^2-1	$\sum_{ij} y_{ij}^2 - C.F.$		

The hypothesis of equal treatment effects is tested by F-test, where F is the ratio of treatment mean squares to error mean squares. If F is not significant, treatment effects do not differ significantly among themselves. If F is significant, further studies to test the significance of any treatment contrast can be made in exactly the same way as discussed for randomized block designs.

6. Illustrations for Combined Analysis of Data

Example 6.1: An initial varietal trial (Late Sown, irrigated) was conducted to study the performance of 20 new strains of mustard vis-a-vis four checks (Swarna Jyoti: ZC; Vardan: NC; Varuna: NC; and Kranti: NC) using a Randomized complete Block Design (RCB) design at four locations (Sriganganagar, Navgaon, Bhatinda and Hissar) with 2 replications at Sriganganagar and with 3 replications each at other 3 locations. The seed yield in kg/ha was recorded. The data pertaining to Bhatinda is given in Example 4.2. The data from the rest of 3 locations is given as below:

Yield in kg/ha

Strain No.	Sriganganagar		Navgaon			Hissar		
	Replications		Replications			Replications		
	1	2	1	2	3	1	2	3
1	778.00	667.00	533.28	488.84	799.92	945.68	1040.25	1040.25
2	556.00	444.00	444.40	488.84	466.62	567.41	945.68	803.83
3	556.00	444.00	977.68	888.80	799.92	1134.82	1182.10	1040.25

4	778.00	778.00	888.80	799.92	799.92	969.33	1229.39	1134.82
5	556.00	556.00	666.60	666.60	444.40	898.40	851.11	969.33
6	444.00	444.00	799.92	533.28	577.72	851.11	756.55	969.33
7	556.00	333.00	1066.56	1022.12	933.24	1134.82	1323.96	1040.25
8	556.00	444.00	1111.00	1066.56	1066.56	1229.39	1134.82	1134.82
9	444.00	556.00	666.60	888.80	844.36	1087.54	898.40	992.97
10	778.00	556.00	533.28	622.16	844.36	851.11	1134.82	945.68
11	667.00	778.00	1022.12	666.60	755.48	1040.25	1276.67	1229.39
12	444.00	444.00	799.92	666.60	622.16	803.83	945.68	992.97
13	333.00	556.00	799.92	666.60	688.82	992.97	1182.10	1323.96
14	444.00	333.00	888.80	933.24	666.60	1040.25	1134.82	1276.67
15	556.00	333.00	844.36	688.82	577.72	1182.10	1418.52	1229.39
16	333.00	333.00	711.04	622.16	622.16	1087.54	945.68	1040.25
17	556.00	333.00	799.92	577.72	533.28	969.33	1040.25	1040.25
18	333.00	333.00	1066.56	1111.00	999.90	969.33	1087.54	1040.25
19	444.00	444.00	933.24	711.04	711.04	1418.52	1040.25	945.68
20	444.00	444.00	755.48	799.92	733.26	1182.10	1134.82	1087.54
21	333.00	444.00	844.36	755.48	666.60	1087.54	1323.96	1040.25
22	444.00	333.00	666.60	533.28	488.84	992.97	803.83	992.97
23	556.00	333.00	755.48	799.92	1022.12	1134.82	992.97	1229.39
24	333.00	333.00	488.84	577.72	666.60	1040.25	992.97	1182.10

The data from each of the centers were analyzed separately using PROC GLM of SAS. The results for Bhatinda center are given in Example 1. The results of the other 3 locations are given in the sequel.

ANOVA (Yield: Hissar)

Source	D.F.	S.S.	M.S.	F	Pr > F
Due to Treatments	23	1007589.069	43808.220	3.06	*0.0006*
Due to Replications	2	37465.039	18732.519	1.31	*0.2795*

Error	46	657493.58	14293.33	
Total	71	1702547.68		

R-Square	CV	Root MSE	Mean Yield
0.613818	11.30376	119.5548	1057.65

Treatments are significantly different at 5% level of significance, where as replications are not significantly different. None of the entries gave significantly higher yield than best performing check (Swarna Jyoti).

ANOVA (Yield: Navgaon)

Source	D.F.	S.S.	M.S.	F	Pr > F
Due to Treatments	23	1685581.90	73286.19	6.51	*<0.0001*
Due to Replications	2	73332.38	36666.19	3.26	*0.0476*
Error	46	518154.24	11264.23		
Total	71	2277068.52			

R-Square	CV	Root MSE	Mean Yield
0.772447	14.15831	106.1330	749.6164

Both treatments and replications are significantly different at 5% level of significance. New entry at serial number 8 gave significantly higher yield than best performing check (Swarna Jyoti).

ANOVA (Yield: Sriganganagar)

Source	D.F.	S.S.	M.S.	F	Pr > F
Due to Treatments	23	699720.92	30422.65	4.03	*0.0007*
Due to Replications	1	31314.08	31314.08	4.15	*0.0533*
Error	23	173540.92	7545.26		
Total	47	904575.92			

R-Square	CV	Root MSE	Mean Yield
0.808152	17.95781	86.86344	483.7083

Both treatments and replications are significantly different at 5% level of significance, New entry at serial number 8 gave significantly higher yield than best performing check (Swarna Jyoti). Error mean squares and error degrees of freedom of the 4 locations are:

	Bhatinda	Hissar	Navgaon	Sriganganagar
Error degrees of freedom	46	46	46	23
Error Mean Square	10067.91	14293.33	11264.23	7545.26

In order to perform the combined analysis of the data for 4 locations (group of experiments), the mean square errors for the 4 locations were tested for the homogeneity of error variances using Bartlett's χ^2-test. The test is described in the sequel:

Let the experiment is conducted in *k* environments. The estimate of error variance for the i^{th} environment is s_i^2 (MSE for i^{th} environment) with f_i degrees of freedom (error degrees of freedom).

We are interested to test the null hypothesis $H_0 : \sigma_1^2 = \sigma_2^2 = ... = \sigma_k^2$ against the alternative hypothesis H_1 : at least two of the σ_i^2'*s* are not equal, where σ_i^2 is the error variance for treatment *i*. (σ_i^2 is the error variance for the i^{th} environment).

The procedure involves computing a statistic whose sampling distribution is closely approximated by the χ^2 distribution with *k - 1* degrees of freedom. The test statistic is

$$\chi_0^2 = 2.3026\frac{q}{c}$$

and null hypothesis is rejected when $\chi_0^2 > \chi_{\alpha,k-1}^2$, where $\chi_{\alpha,k-1}^2$ is the upper α percentage point of χ^2 distribution with *k - 1* degrees of freedom.

To compute χ_0^2, follow the steps:

Step 1: Compute mean and variance of all *v*-samples.

Step 2: Obtain pooled variance $S_p^2 = \dfrac{\sum_{i=1}^{k} f_i s_i^2}{\sum_{i=1}^{k} f_i}$

Step 3: Compute $q = \left(\sum_{i=1}^{k} f_i\right)\log_{10} S_p^2 - \sum_{i=1}^{k} f_i \log_{10} S_i^2$

Step 4: Compute $c = 1 + \dfrac{1}{3(k-1)}\left(\sum_{i=1}^{k} f_i^{-1} - \left(\sum_{i=1}^{k} f_i\right)^{-1}\right)$

Step 5: Compute χ_0^2.

For this example, the computed χ_0^2 was found to be 3.28. The tabulated value of $\chi_{3,0.05}^2 = 7.81$. Therefore, the null hypothesis is not rejected. Therefore, the error variances were found to be homogeneous. Now the combined analysis of data can be carried out using the following statements of SAS.

```
Data comb;
Input loc $ rep var yield;
Cards;
.
.
```

.
;

```
proc glm;
class loc rep trt;
model yield = loc rep(loc) trt trt*loc;
random loc rep(loc) trt*loc/test;
run;
```

The results obtained are:

Combined Analysis of Data Over 4 Locations of Rapeseed-Mustard Initial Varietal Trial

Source	DF	SS	Mean Square	F Value	Pr>F
loc	3	16794186.86	5598062.29	497.31	<.0001
Replications(loc)	7	298250.83	42607.26	3.79	0.0008
Treatments	23	2153545.49	93632.41	8.32	<.0001
loc*Treatment	69	3495630.98	50661.32	4.50	<.0001
Error	161	1812312.49	11256.60		
Total	263	24811785.12			

R-square	C.V.	Root MSE	Mean
0.93	11.81	106.10	898.59

Source	Type III Expected Mean Square
loc	Var(Error) + 2.7273 Var(loc*treatment) + 24 Var(rep(loc) + 65.455 Var(loc)
rep(loc)	Var(Error) + 24 Var(rep(loc)
treatment	Var(Error) + 2.6667 Var(loc*treatment) + Q(treatment)
loc*treatment	Var(Error) + 2.7273 Var(loc*treatment)

Tests of Hypotheses for Mixed Model Analysis of Variance

Source	DF	SS	MS	F-Value	Pr>F
loc	3	16794187	5598062	68.26	<.0001
*Error: MS(rep(loc)) + MS(loc*treatment) - MS(Error)*					
rep(loc)	7	298251	42607	3.79	0.0008
loc*treatment	69	3495631	50661	4.50	<.0001
Error: MS(Error)					
treatment	23	2153545	93632	1.88	0.0232
*Error: 0.9778*MS(loc*treatment)+0.0222*MS(Error)*					

Example 6.2: An experimenter was interested in comparing 49 treatments. The experiment was laid out in a lattice design with four replications. There were seven blocks per replication and seven treatments were allotted within each block. Observations were recorded on several characters but for illustration purposes only one data set (one character) is analyzed. The same design was repeated over two years. The layout of the design is given below:

Blocks	Replication - I						
1.	*1*	*2*	*3*	*4*	*5*	*6*	*7*
2.	*8*	*9*	*10*	*11*	*12*	*13*	*14*
3.	*15*	*16*	*17*	*18*	*19*	*20*	*21*
4.	*22*	*23*	*24*	*25*	*26*	*27*	*28*
5.	*29*	*30*	*31*	*32*	*33*	*34*	*35*
6.	*36*	*37*	*38*	*39*	*40*	*41*	*42*
7.	*43*	*44*	*45*	*46*	*47*	*48*	*49*
Blocks	**Replication - II**						
1.	*1*	*8*	*15*	*22*	*29*	*36*	*43*
2.	*2*	*9*	*16*	*23*	*30*	*37*	*44*
3.	*3*	*10*	*17*	*24*	*31*	*38*	*45*
4.	*4*	*11*	*18*	*25*	*32*	*39*	*46*
5.	*5*	*12*	*19*	*26*	*33*	*40*	*47*
6.	*6*	*13*	*20*	*27*	*34*	*41*	*48*
7.	*7*	*14*	*21*	*28*	*35*	*42*	*49*
Blocks	**Replication - III**						
1.	*1*	*9*	*17*	*25*	*33*	*41*	*49*
2.	*43*	*2*	*10*	*18*	*26*	*34*	*42*
3.	*36*	*44*	*3*	*11*	*19*	*27*	*35*
4.	*29*	*37*	*45*	*4*	*12*	*20*	*28*
5.	*22*	*30*	*38*	*46*	*5*	*13*	*21*
6.	*15*	*23*	*31*	*39*	*47*	*6*	*14*
7.	*8*	*16*	*24*	*32*	*40*	*48*	*7*
Blocks	**Replication - IV**						
1.	*1*	*37*	*24*	*11*	*47*	*34*	*21*
2.	*15*	*2*	*38*	*25*	*12*	*48*	*35*
3.	*29*	*16*	*3*	*39*	*26*	*13*	*49*
4.	*43*	*30*	*17*	*4*	*40*	*27*	*14*
5.	*8*	*44*	*31*	*18*	*5*	*41*	*28*
6.	*22*	*9*	*45*	*32*	*19*	*6*	*42*
7.	*36*	*23*	*10*	*46*	*33*	*20*	*7*

The analysis was carried out using **PROC GLM** of **SAS** and using the option of contrast for carrying out the contrast analysis. The results of the analysis of data for the first year are as given below:

RESULTS 1 (LATTICE DESIGN: FIRST YEAR)

Source	DF	SS	Mean Square	F Value	Pr>F
Replications	3	186.04	62.01	7.53	0.0001
Block(replication)	24	358.94	14.95	1.82	0.0192
Treatments	48	3442.14	71.71	8.70	0.0001
Error	120	988.70	8.23		
Total	195	6025.75			

R-square	C.V.	Root MSE	Mean
0.84	3.37	2.87	85.18

It may be noted that all sum of squares reported in the table are adjusted sums of squares and that the adjustments have been made for all the other remaining effects. The CV is

very small and, therefore, the design adopted is appropriate. The interesting feature of the design is that the blocks within replication sum of squares are significant and, therefore, formation of blocks within replications has been fruitful. Thus, the formation of incomplete blocks within replications has been very effective and the error mean square is quite small. The treatment effects are also highly significant. The *49* treatments tried in the experiment were formed into four groups on the basis of the nature of the treatments. The groups are - Group *1*: Treatments *1 - 15*; Group *2*: Treatments *16 - 30*; Group *3*: Treatments *31 - 46*; Group *4*: Treatments *47 - 49*. Contrast analysis was carried out to study the equality of the treatment effects within groups and desired between group comparisons. The results are as follows:

Contrast	DF	Contrast SS	Mean Square	F Value	Pr > F
gr1	14	985.53	70.39	8.54	0.0001
gr2	14	1004.60	71.75	8.71	0.0001
gr3	15	1373.17	91.54	11.11	0.0001
gr4	2	60.27	30.13	3.66	0.0287
gr1 vs gr4	1	47.29	47.29	5.74	0.0181
gr2 vs gr4	1	92.69	92.69	11.25	0.0011
gr3 vs gr4	1	41.74	41.74	5.07	0.0262
gr1 vs gr2	1	18.86	18.86	2.29	0.1329

It may be seen that the group *1* vs group *2* comparisons are not significantly different whereas all other comparisons are significantly different.

RESULT 2 (LATTICE DESIGN SECOND YEAR)

Source	DF	SS	Mean Square	F Value	Pr>F
Replications	3	176.404	58.79	11.81	0.0001
Block(replication)	24	556.49	23.18	4.66	0.0001
Treatments	48	3353.21	69.85	14.03	0.0001
Error	120	597.30	4.97		
Total	195	5413.92			

R-square	C.V.	Root MSE	Mean
0.89	2.50	2.23	89.31

It may be noted again that all sum of squares reported in the table are adjusted sums of squares and that the adjustments have been made for all other remaining effects. The CV is very small and therefore the design adopted is appropriate. The interesting feature of the design is that the blocks within replication sum of squares are highly significant and, therefore, formation of blocks within replications has been fruitful. Thus, the formation of incomplete blocks within replications has been very effective and the error mean square is quite small. The treatment effects are also highly significant.

In order to perform the combined analysis of the data for two years (group of experiments), the mean square errors for the two years were tested for the homogeneity of error variances. The value of *F* statistic was obtained as $F = MSE1 / MSE2 = 8.23 / 4.97 = 1.6559$ (significant at *5* % level of significance). Therefore, for performing the combined analysis weighted least squares was done, the weight being the reciprocals of the root mean square error. The weighted least squares analysis is carried out by defining a new variable *newres*

= *res/root mean square error*. The analysis of variance is then performed on the new variable. The following analysis is usually carried out for these situations.

RESULT 3 (LATTICE DESIGN COMBINED ANALYSIS FOR YEARS 1 & 2)

Source	DF	SS	Mean Square	F Value	Pr>F
Year	1	4911.42	4911.42	3672.45	0.0001
Replications	3	19.27	6.42	4.80	0.0028
Block(replication)	24	93.56	3.90	2.91	0.0001
Treatments	48	1142.94	23.81	17.80	0.0001
Year*Treatment	48	137.75	2.87	2.15	0.0001
Error	267	357.08	1.34		
Total	391	6780.42			

R-square	C.V.	Root MSE	Mean
0.95	3.34	1.16	34.66

The year*treatment interaction is highly significant. Therefore, treatment is tested against the year*treatment interaction mean square. The results obtained are given as:

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treatment	48	1142.94	23.81	8.30	<.0001

In the above analysis, the degrees of freedom for the replications and blocks (replications) are 3 and 24 respectively and are same as that of individual year analyses. Therefore, no distinction is made in the replications and blocks (replications) of the two years. Hence, this procedure is inappropriate.

The appropriate procedure, therefore, is to view the groups of experiments as a nested design with several factors nested within one another. The locations are treated as big blocks, with the experiments nested within these. The combined analysis of data, therefore, can be done as that of a nested design. An advantage of this analysis is that there is a further reduction in the error sum of squares because one more source of variability is taken out from the experimental error thus reducing the experimental error. This may also lead to the reduction in the value of CV. If we take the data for two years together there will be *56* blocks and hence the blocks will account for *55* degrees of freedom. The analysis of variance just described accounts for 28 degrees of freedom. The remaining *27* degrees of freedom go into the error. However, if we analyze the data as a nested design, we get *55* degrees of freedom for the blocks that can be split into various components. In the sequel, we present the appropriate analysis of groups of experiments. This enables us to further reduce the experimental error thus reducing the CV. The results obtained are reproduced below:

RESULT 4. (LATTICE DESIGN COMBINED ANALYSIS CONSIDERING NESTED CLASSIFICATIONS - REPLICATIONS NESTED WITHIN YEARS AND BLOCKS NESTED WITHIN REPLICATIONS AND YEARS ON THE TRANSFORMED DATA)

Source	DF	SS	Mean Square	F Value	Pr>F
Year	1	4911.42	4911.42	4344.23	<.0001
Replications(Year)	6	58.83	9.80	8.67	<.0001
Blocks(Year*replication)	48	139.74	2.91	2.58	<.0001
Treatments	48	968.42	20.18	17.85	<.0001
Year*Treatment	48	130.86	2.73	2.41	<.0001
Error	240	271.33	1.13		
Total	391	6780.42			

R-square	C.V.	Root MSE	Mean
0.96	3.07	1.06	34.66

It may be seen that the error sum of squares has a reduction of *27* degrees of freedom. The CV has also reduced from *3.34* to *3.07*. The sums of squares due to various components in the model are highly significant. The advantage of analyzing the data as a nested design is quite visible thus. The year*treatment interaction is highly significant. Therefore, treatment is tested against the year*treatment interaction mean square. The results obtained are given as:

Source	DF	Type III SS	Mean Square	F Value	Pr > F
treatment	48	968.42	20.18	7.40	<.0001

In the above analysis, the proper error terms can also be identified using PROC GLM of SAS along with random statement with TEST option. Using PROC GLM, the expected mean squares for different effects in the model are given as

Source	Type III Expected Mean Square
Year	Var(Error) + 4 Var(year*treatment) + 7 Var(block(year*rep)) + 49 Var(rep(year)) + 196 Var(year)
rep(year)	Var(Error) + 7 Var(block(year*rep)) + 49 Var(rep(year))
block(year*rep)	Var(Error) + 5.25 Var(block(year*rep))
treatment	Var(Error) + 3.5 Var(year*treatment) + Q(treatment)
year*treatment	Var(Error) + 3.5 Var(year*treatment)

Tests of Hypotheses for Mixed Model Analysis of Variance

Source	DF	SS	MS	F-Value	Pr>F
Year	1	4911.42	4911.42	422.37	<.0001
*Error: MS(rep(year)) + 1.1429*MS(year*treatment) - 1.1429*MS(Error)*					
rep(year)	6	58.83	9.80	2.80	0.0232
*Error: 1.3333*MS(block(year*rep)) - 0.3333*MS(Error)*					
block(year*rep)	48	139.74	2.91	2.58	<.0001
year*treatment	48	130.86	2.73	2.41	<.0001
Error: MS(Error)					
treatment	48	968.42	20.18	7.40	<.0001
*Error: MS(year*treatment)*					

It is observed that the year*treatment interaction is highly significant and the proper error term for testing the equality of treatment effects is year*treatment interaction mean square.

The above discussions refer to the combined analysis of experiments conducted at different locations or different times at the same location in general block designs with same treatments in each of the environments. There may arise situations where all the treatments are not common to the whole set. Only subsets of the treatments are common to the whole set. This may happen due to some location specific treatments that cannot be tried at all the locations. Different treatments at different locations do not give any problem in the combined analysis of data so long as there are some common treatments over all the locations.

7. Factorial Experiments

Example 7.1: An experiment was conducted at Ludhiana Centre of AICRP on Cropping Systems using a balanced confounded design for factorial experiments with three factors, *viz.*, Nitrogen (40, 80 and 120 kg/ha), Phosphorous (0, 40 and 80 kg/ha) and Potassium (0 and 40 kg/ha). These *18* treatment combinations were arranged in *3* blocks of size *6* each.

The analysis of the data was performed using PROC GLM of SAS. The SAS commands and the output are given in the sequel.

```
Options linesize=72;
data ludh98k;
input rep    block    N    P    K    trt    yield;
cards;
```

1	1	40	0	0	1	7.79
1	1	120	80	0	17	10.30
1	1	40	80	40	6	10.08
1	1	120	40	40	16	11.66
1	1	80	0	40	8	9.13
1	1	80	40	0	9	10.56
1	2	40	0	40	2	6.12
1	2	120	0	0	13	8.44
1	2	120	80	40	18	11.44
1	2	80	40	40	10	9.13
1	2	80	80	0	11	9.40
1	2	40	40	0	3	6.85
1	3	80	0	0	7	6.25
1	3	120	0	40	14	7.78
1	3	40	40	40	4	6.66
1	3	80	80	40	12	9.42
1	3	40	80	0	5	6.50
1	3	120	40	0	15	11.82
2	1	120	0	0	13	7.86
2	1	120	40	40	16	10.15
2	1	40	80	40	6	7.50
2	1	80	0	40	8	7.89
2	1	80	80	0	11	8.00
2	1	40	40	0	3	6.40
2	2	120	0	40	14	8.50

```
2 2 80 80 40 12 9.86
2 2 40 40 40 4 7.70
2 2 120 80 0 17 10.79
2 2 80 40 0 9 7.87
2 2 40 0 0 1 6.30
2 3 80 0 0 7 7.00
2 3 40 80 0 5 8.00
2 3 120 80 40 18 10.90
2 3 40 0 40 2 6.62
2 3 80 40 40 10 9.62
2 3 120 40 0 15 9.50
3 1 80 80 0 11 10.00
3 1 120 80 40 18 10.86
3 1 40 40 40 4 7.58
3 1 80 0 40 8 6.35
3 1 120 40 0 15 9.40
3 1 40 0 0 1 5.94
3 2 120 0 40 14 9.00
3 2 40 80 40 6 8.80
3 2 80 40 40 10 9.53
3 2 120 80 0 17 10.56
3 2 40 40 0 3 7.07
3 2 80 0 0 7 6.00
3 3 80 40 0 9 7.20
3 3 120 0 0 13 8.36
3 3 40 0 40 2 6.05
3 3 80 80 40 12 10.45
3 3 120 40 40 16 10.10
3 3 40 80 0 5 7.50
4 1 80 80 0 11 7.97
4 1 80 40 40 10 7.18
4 1 40 80 40 6 6.16
4 1 40 0 0 1 4.95
4 1 120 40 0 15 10.12
4 1 120 0 40 14 7.15
4 2 80 0 0 7 6.65
4 2 40 40 0 3 6.66
4 2 80 80 40 12 7.90
4 2 120 40 40 16 10.10
4 2 40 0 40 2 6.49
4 2 120 80 0 17 10.30
4 3 80 0 40 8 6.12
4 3 40 40 40 4 5.80
4 3 120 80 40 18 10.06
4 3 120 0 0 13 7.37
4 3 80 40 0 9 7.24
4 3 40 80 0 5 7.70
;
proc glm;
```

```
class rep block n p k;
model yield = rep block(rep)  n p k n*p n*k p*k n*p*k;
run;
```

The output is given as: Dependent Variable: yield

Source	DF	Sum of Squares	Mean Square	F-Vlaue	Pr>F
Model	28	185.8448	6.6373	13.56	<0.0001
Error	43	21.0427	0.4894		
Corrected Total	**71**	206.8876			

R-Square	Coeff Var	Root MSE	Yield Mean
0.8983	8.4444	0.699547	8.284

Source	DF	Type III SS	Mean Square	F-Vlaue	Pr>F
Rep	3	15.7187	5.2396	10.71	<.0001
Block(rep)	8	14.1946	1.7743	3.63	0.0027
N	2	89.1108	44.5554	91.05	<.0001
P	2	55.9270	27.9635	57.14	<.0001
K	1	3.2173	3.2173	6.57	0.0139
NP	4	4.2752	1.0688	2.18	0.0868
NK	2	0.7301	0.3650	0.75	0.4803
PK	2	0.1128	0.0564	0.12	0.8914
NPK	4	2.1958	0.5490	1.12	0.3588

From the above, it is clear that the blocks with in replication are significant indicating that the incomplete blocks have help in reducing mean square error. All the three main effects N, P and K are significant whereas none of the interaction is significant.

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DESIGNS FOR FACTORIAL EXPERIMENTS

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1. Introduction

Suppose that one wants to conduct an experiment to study the performance of a new crop or tree species on the basis of yield in an area where it has never been grown before. A sample of pertinent questions that arise for planning the experiment must be answered is given below:

1. What should be the best crop variety?
2. When should the crop be planted (Date of sowing)?
3. Should it be sown directly or transplanted? If sown directly, what would be the seeding rate and if transplanted, what would be the age of the seedlings?
4. Should the seed be drilled or broadcast?
5. Must we use fertilizer? If yes, how much of the major elements are needed?
6. Have we to add minor elements?
7. Is irrigation necessary?
8. What should be the plant-to-plant and line-to-line spacing?

This problem may be investigated by varying a single factor at a time using designs for single factor experiments (like completely randomized designs, randomized complete block designs, incomplete block designs, row-column designs, etc.). For example, an experiment may be conducted with varieties of the crop as treatments to pick the best variety. Using the best variety, another experiment may be conducted to obtain the date of sowing. Then using the best variety and the optimum date of sowing another experiment may be conducted to find the optimum level for the other factors one at a time. The soundness of this approach rests on the assumption that the response to different varieties is independent of amount of nitrogen given *i.e.* the factors act independent of each other. But then this is a big assumption and such situations are very rare.

To make the exposition simple, let us take two factors *viz*. irrigation and nitrogen fertilizer. It is known that for most of the crops, higher level of irrigation up to certain limit is required to secure an adequate response from a higher dose of manure. The two factors are not independent but interact with each other. Thus, ***interaction is the failure of the differences in response to changes in levels of one factor, to retain the same order and magnitude of performance through out all the levels of other factors*** *or* ***the factors are said to interact if the effect of one factor changes as the levels of the other factor(s) changes***.

In practice the experimenter deals with simultaneous variation in more than one factor. It may be required to find the combination of most suitable level of irrigation and the optimum dose of a nitrogenous fertilizer. Consider the results of a trial designed to measure the effects of nitrogen and irrigation, both alone and in combinations when applied to rice crop. The yield of rice crop in q/ha is given as

Rice yield in q/ha

Nitrogen → *Irrigation↓*	*0 kg N/ha* *(N_0)*	*60 kg N/ha* *(N_1)*	*Mean*
5 cm irrigation (I_0)	*$N_0 I_0$ 10.0*	*$N_1 I_0$ 30.0*	*20.0*
10 cm irrigation (I_1)	*$N_0 I_1$ 20.0*	*$N_1 I_1$ 40.0*	*30.0*
Mean	*15.0*	*35.0*	

Effect of nitrogen at I_0 level of irrigation = *30.0 - 10.0 = 20.0 q/ha*
Effect of nitrogen at I_1 level of irrigation = *40.0 - 20.0 = 20.0 q/ha*
Effect of irrigation at N_0 level of nitrogen = *20.0 - 10.0 = 10.0 q/ha*
Effect of irrigation at N_1 level of nitrogen = *40.0 - 30.0 = 10.0 q/ha*

As effect of nitrogen (irrigation) is same at all the levels of irrigation (nitrogen) hence, there is **no interaction** between nitrogen and irrigation. Consider the results of another trial designed to measure the effects of nitrogen and irrigation, both alone and in combinations when applied to rice crop. The yield of rice crop in q/ha is given as

Rice yield in q/ha

Nitrogen → *Irrigation↓*	*0 kg N/ha* *(N_0)*	*60 kg N/ha* *(N_1)*	*Mean*
5 cm irrigation (I_0)	*$N_0 I_0$ 10.0*	*$N_1 I_0$ 30.0*	*20.0*
10 cm irrigation (I_1)	*$N_0 I_1$ 20.0*	*$N_1 I_1$ 50.0*	*35.0*
Mean	*15.0*	*40.0*	

Effect of nitrogen at I_0 level of irrigation = *30.0 - 10.0 = 20.0 q/ha*
Effect of nitrogen at I_1 level of irrigation = *50.0 - 20.0 = 30.0 q/ha*
Effect of irrigation at N_0 level of nitrogen = *20.0 - 10.0 = 10.0 q/ha*
Effect of irrigation at N_1 level of nitrogen = *50.0 - 30.0 = 20.0 q/ha*

As effect of nitrogen (irrigation) is not same at all the levels of irrigation (nitrogen) hence, nitrogen and irrigation are **interacting**.

These effects as explained above are called as simple effects of the factors and average of these simple effects is called **main effect** of the factor. Thus,

Main effect of Nitrogen $= \frac{20.0 + 30.0}{2} = 25.0$ *q/ha*

Main effect of Irrigation $= \frac{10.0 + 20.0}{2} = 15.0$ *q/ha*

Interaction of Irrigation and Nitrogen is the difference between simple effects, *e.g.*, simple effect of Irrigation at N_1 level of Nitrogen minus the simple effect of Irrigation at N_0 level of Nitrogen = *20.0 - 10.0 = 10.0 q/ha*. It may also be defined as the simple effect of Nitrogen at I_1 level of Irrigation minus the simple effect of Nitrogen at I_0 level of Irrigation = *30.0 - 20.0 = 10.0 q/ha.*
If interactions exist, which is generally true, the experiments should be planned in such a way that these can be estimated and tested. It is now clear that it is not possible to estimate

interactions from the experiments in which levels of only one factor are studied at a time. For this purpose, we must use multi-level, multi-factor experiments.

2. What are factorial experiments?

Definition: A treatment arrangement in which the treatments consist of all combination of all levels of two or more factors. It is just an arrangement of treatments, not a design. One can use this approach with a variety of designs.

Also factorial experiments can be defined as experiments in which the effects (main effects and interactions) of more than one factor are studied together. In general if there are n factors, say, $F_1, F_2, \ldots, F_n$ and the i^{th} factor has s_i levels, $i=1,\ldots,n$, then the total number of treatment combinations is $\prod_{i=1}^{n} s_i$. Factorial experiments are of two types.

1. **Symmetrical Factorial Experiments**: In these experiments the number of levels of all factors is same *i.e*, $s_i = s \ \forall i = 1,\cdots,n$.
2. **Asymmetrical Factorial Experiments**: In these experiments the number of levels of all the factors are not same *i.e.* there are at least two factors for which the number of levels $s_i's$ are different.

Factorial experiments have many advantages over single factor experiments.
Advantages:

- More precision on each factor than with single factor experiments due to hidden replications.
- Provide an opportunity to study not only the individual effects of the factors but also their interactions.
- Good for exploratory work where we wish to find most important factor or the optimal level of factor or combination of levels of more than one factor.
- These experiments have the further advantage of economizing the experimental resources. When the experiments are conducted factor by factor a large number of experimental units are required for getting the same precision of estimation as one would have got when all the factors are experimented together in the same experiment, *i.e.,* factorial experiment. There is thus a considerable amount of saving of resources. Moreover, factorial experiments also enable us to study interactions which the experiments conducted factor by factor do not allow us to study.

Disadvantages:

- This approach is more complex than that of single factor experiments
- With a number of factors each at several levels, the experiment can become very large.

2.1 Symmetrical factorial experiments

The simplest symmetrical factorial experiments are 2^n factorial experiments in which all the n factors have 2 levels each. Consider the 2^2 factorial experiment with 2 factors say A and

B each at two levels, say *0* and *1*. There will be *4* treatment combinations that can be written as

00 = $a_0 b_0$ = *(1);* *A* and *B* both at first levels
10 = $a_1 b_0$ = *a ;* *A* at second level and *B* at first level
01 = $a_0 b_1$ = *b ;* *A* at first level and *B* at second level
11 = $a_1 b_1$ = *ab;* *A* and *B* both at second level.

We denote the treatment combinations by small letters *(1), a, b, ab* indicating the presence of low or high level of the factor and treatment totals by *[1], [a], [b], [ab]*. The following table gives the responses due to Factor *A* and Factor *B*.

FactorA→ *Factor B*↓	a_0 *or* *0*	a_1 *or* *1*	*Response due to A*
b_0 *or* *0*	*[1] or* $[a_0 b_0]$	*[a] or* $[a_1 b_0]$	*[a] - [1] or* $[a_1 b_0] - [a_0 b_0]$
b_1 *or* *1*	*[b] or* $[a_0 b_1]$	*[ab] or* $[a_1 b_1]$	*[ab] - [b] or* $[a_1 b_1] - [a_0 b_1]$
Response Due to B	*[b] - [1] or* $[a_0 b_1] - [a_0 b_0]$	*[ab] - [a] or* $[a_1 b_1] - [a_1 b_0]$	

The responses *[a] - [1]* and *[ab] - [b]* are called simple effects of the factor *A* at *0* and *1* levels, respectively of the factor *B*. If the factors *A* and *B* are independent, the responses *[a] - [1]* and *[ab] - [b]*, both provide the estimate of the response due to *A* (except for the experimental error). The average of these two simple effects is known as Main Effect of factor *A*. Thus the main effect of factor *A* is

$$A = \frac{1}{2}\{[a_1 b_1] - [a_0 b_1] + [a_1 b_0] - [a_0 b_0]\} \quad \text{or} \quad A = \frac{1}{2}\{[ab] - [b] + [a] - [1]\} \qquad (1)$$

This is simplified by writing it in the form $A = \frac{1}{2}(a - 1)(b + 1)$, where the right hand side is to be expanded algebraically and then the treatment combinations are to be replaced by corresponding treatment totals. From (1) we find that *A* is a linear function of the four treatments totals with the sum of the coefficients of the linear function equal to zero $(\frac{1}{2} - \frac{1}{2} + \frac{1}{2} - \frac{1}{2} = 0)$. Such a linear function among the treatment totals with sum of coefficients equal to zero is called a contrast (or a comparison) of the treatment totals. Similarly the main effect of factor *B* is

$$B = \frac{1}{2}\{[a_1 b_1] + [a_0 b_1] - [a_1 b_0] - [a_0 b_0]\} \quad \text{or} \quad B = \frac{1}{2}\{[ab] + [b] - [a] - [1]\} \qquad (2)$$

This is simplified by writing it in the form $B = \frac{1}{2}(a + 1)(b - 1)$ where the right hand side is to be expanded algebraically and then the treatment combinations are to be replaced by corresponding treatment totals. From (2), we find that *B* is a linear function of the four

treatments totals with the sum of the coefficients of the linear function equal to zero $(\frac{1}{2} + \frac{1}{2} - \frac{1}{2} - \frac{1}{2} = 0)$, hence a contrast.

Consider now the difference of two simple effects of *A*

$$= \{ [ab] - [b] - [a] + [1] \} \qquad (3)$$

Had the two factors been independent, then (3) would be zero. If not then this provides an estimate of interdependence of the two factors and it is called the interaction between *A* and *B*. The interaction between *A* and *B* is defined as

$$AB = \frac{1}{2} (a - 1)(b - 1)$$

where the expression on the right hand side is to be expanded algebraically and then the treatment combinations are to be replaced by the corresponding treatment totals. It is easy to verify that *AB* is a contrast of the treatment totals. The coefficients of the contrasts *A* and *AB* are such that the sum of the products of the corresponding coefficients of the contrasts *A* and *AB* is equal to zero *i.e.* $(\frac{1}{2})(\frac{1}{2}) + (-\frac{1}{2})(-\frac{1}{2}) + (\frac{1}{2})(-\frac{1}{2}) + (-\frac{1}{2})(\frac{1}{2}) = 0$. Thus the contrasts *A* and *AB* are orthogonal contrasts. It is easy to verify that the interaction of the factor *B* with factor *A*, *i.e.*, *BA* is the same as the interaction *AB* and hence the interaction does not depend on the order of the factors. It is also easy to verify that the main effect *B* is orthogonal to both *A* and *AB*.

The above three orthogonal contrasts defining the main effects and interaction can be easily obtained from the following table, which gives the signs with which to combine the treatment totals and also the divisor for obtaining the corresponding sum of squares. Main effects and interactions are expressed in terms of individual treatment totals.

Treatment Totals→ *Factorial Effect↓*	*[1]*	*[a]*	*[b]*	*[ab]*	*Divisor*
M	+	+	+	+	*4r*
A	-	+	-	+	*4r*
B	-	-	+	+	*4r*
AB	+	-	-	+	*4r*

Here *r* denotes the replication number. The rule to write down the signs of the main effect is to give a plus sign to the treatment combinations containing the corresponding small letter and a minus sign where the corresponding small letter is absent. The signs of interaction are obtained by multiplying the corresponding signs of the two main effects. The first line gives the general mean

$$M = \frac{1}{4} \{[ab] + [a] + [b] + [1]\}$$

Consider now the 2^3 factorial experiment with *3* factors *A*, *B*, and *C* each at two levels, say *0* and *1*. The *8* treatment combinations are written as

000 = $a_0\, b_0\, c_0$ = *(1);* *A, B* and *C,* all three at first level
100 = $a_1\, b_0\, c_0$ = *a ;* *A* at second level and *B* and *C* at first level
010 = $a_0\, b_1\, c_0$ = *b ;* *A* and *C* both at first level and *B* at second level
110 = $a_1\, b_1\, c_0$ = *ab;* *A* and *B* both at second level and *C* at first level
001 = $a_0\, b_0\, c_1$ = *c ;* *A* and *B* both at first level and *C* at second level.
101 = $a_1\, b_0\, c_1$ = *ac;* *A* and *C* both at second level and *B* at first level
011 = $a_0\, b_1\, c_1$ = *bc;* *A* at first level and *B* and *C* both at second level
111 = $a_1\, b_1\, c_1$ = *abc;* *A, B* and *C,* all three at second level

In a three factor experiment there are *3* main effects *A, B,* and *C*; *3* first order or two factor interactions *AB, AC,* and *BC*; and *one* second order or three factor interaction *ABC*. The main effects and interactions may be written as

$$A = \frac{1}{4}(a-1)(b+1)(c+1),\ B = \frac{1}{4}(a+1)(b-1)(c+1),\ C = \frac{1}{4}(a+1)(b+1)(c-1)$$

$$AB = \frac{1}{4}(a-1)(b-1)(c+1),\ AC = \frac{1}{4}(a-1)(b+1)(c-1),\ BC = \frac{1}{4}(a+1)(b-1)(c-1)$$

$$ABC = \frac{1}{4}(a-1)(b-1)(c-1).$$

These main effects and interactions are mutually orthogonal as may be verified from the following table of signs:

Treatment Totals→ Factorial Effect↓	*[1]*	*[a]*	*[b]*	*[ab]*	*[c]*	*[ac]*	*[bc]*	*[abc]*	*Divisor*
M	+	+	+	+	+	+	+	+	*8r*
A	-	+	-	+	-	+	-	+	*8r*
B	-	-	+	+	-	-	+	+	*8r*
AB	+	-	-	+	+	-	-	+	*8r*
C	-	-	-	-	+	+	+	+	*8r*
AC	+	-	+	-	-	+	-	+	*8r*
BC	+	+	-	-	-	-	+	+	*8r*
ABC	-	+	+	-	+	-	-	+	*8r*

The rule for obtaining the signs of main effects and two factor interactions is the same as that stated for a 2^2 experiment. The signs of *ABC* may be obtained by multiplying the signs of *AB* and *C* or *AC* and *B* or *BC* and *A* or *A, B* and *C*.

Incidentally, it may be remarked that the method of representing the main effects and interactions, which is due to Yates, is very useful and quite straightforward. For example, if the design is 2^4 then

$$A = \frac{1}{2^3}(a-1)(b+1)(c+1)(d+1),\ AB = \frac{1}{2^3}(a-1)(b-1)(c+1)(d+1),$$

$$ABC = \frac{1}{2^3}(a-1)(b-1)(c-1)(d+1), \text{ and } ABCD = \frac{1}{2^3}(a-1)(b-1)(c-1)(d-1)$$

By this rule the main effect or interaction of any design of the series 2^n can be written out directly without first obtaining the simple effects and then expressing the main effects or interactions. For example,

$$A = \frac{1}{2^{n-1}}(a-1)(b+1)(c+1)(d+1)(e+1)\ \ldots,\ AB = \frac{1}{2^{n-1}}(a-1)(b-1)(c+1)(d+1)(e+1)\ \ldots,$$

$$ABC = \frac{1}{2^{n-1}}(a-1)(b-1)(c-1)(d+1)(e+1)\ \ldots,$$

and $$ABCD = \frac{1}{2^{n-1}}(a-1)(b-1)(c-1)(d-1)(e+1)\ \ldots.$$

In case of a 2^n factorial experiment, there will be 2^n $(=v)$ treatment combinations. We shall have n main effects; $\binom{n}{2}$ first order or two factor interactions; $\binom{n}{3}$ second order or three factor interactions; $\binom{n}{4}$ third order or four factor interactions and so on, $\binom{n}{r}$, $(r\text{-}1)^{th}$ order or r factor interactions and $\binom{n}{n}$, $(n\text{-}1)^{th}$ order or n factor interaction. Using these v treatment combinations, the experiment may be laid out using any of the suitable experimental designs *viz.* completely randomized design or block designs or row-column designs, etc.

2.1.1 Steps of Analysis:

Step 1: Obtain the sum of squares (*S.S.*) due to treatments, *S.S.* due to replications (in case randomized block design is used), *S.S.* due to rows and columns (in case a row-column design is used), total *S.S.* and *S.S.* due to error as per established procedures. In case a completely randomized design is used, there will be no *S.S.* due to replications.

Step 2: In order to study the main effects and interactions, the treatment sum of squares is divided into different components *viz*. main effects and interactions each with single *d.f*. We can obtain the *S.S.* due to these factorial effects by dividing the squares of the factorial effect totals by $r.2^n$.

Step 3: Obtain mean squares (*M.S.*) by dividing each *S.S.* by corresponding respective degrees of freedom.

Step 4: After obtaining the different *S.S.*, the usual ANOVA table is prepared and the different effects are tested against error mean square and conclusions drawn.

Step 5: Obtain the standard errors (*S.E.*) for difference of means for all levels of single factor averaged over levels of all other factors and means for all level combinations of two factors averaged over levels of all other factors, using the following expressions.

S.E estimate of difference between means for all levels of single factor averaged over levels of all other factors = $\sqrt{\frac{2MSE}{r.2^{n-1}}}$

S.E estimate of difference between means for all level combinations of two factors averaged over levels of all other factors = $\sqrt{\frac{2MSE}{r.2^{n-2}}}$

In general, *S.E.* estimate for testing the difference between means for all level combinations of *p*- factors averaged over levels of all other factors

$$= \sqrt{\frac{2MSE}{r.2^{n-p}}} \quad \forall\ p=1,2,..,n.$$

The critical differences are obtained by multiplying the *S.E*. estimate by the student's *t* value at $\alpha\%$ level of significance and at error *d.f.*

Please note that when we say the critical difference for a factorial main effect, we actually mean to say that the critical difference for testing the pairwise difference between levels of that factor averaged over levels of other factors. Similarly, the critical difference for the interaction effect involving p factors means that the critical difference for testing the pairwise difference between the treatment combinations of levels of those factors averaged over levels of other factors.

The ANOVA for a 2^n factorial experiment with *r* replications conducted using a randomized complete block design will be

ANOVA

Source of variation	**Degrees of freedom**	**S.S.**	**M.S.**	**F-calculated**
Replications	*r-1*	*SSR*	*MSR = SSR/(r-1)*	*MSR/MSE*
Treatments	$2^n - 1$	*SST*	$MST = SST/(2^n - 1)$	*MST/MSE*
A	*1*	$SSA = [A]^2/r2^n$	*MSA = SSA*	*MSA/MSE*
B	*1*	$SSB = [B]^2/r2^n$	*MSB = SSB*	*MSB/MSE*
AB	*1*	$SSAB=[AB]^2/r2^n$	*MSAB = SSAB*	*MSAB/MSE*
C	*1*	$SSC = [C]^2/r2^n$	*MSC = SSC*	*MSC/MSE*
AC	*1*	$SSAC=[AB]^2/r2^n$	*MSAC = SSAC*	*MSAC/MSE*
	:	*:*	*:*	*:*
Error	$(r-1)(2^n-1)$	*SSE*	$MSE = SSE/(r-1)(2^n-1)$	
Total	$r.2^n-1$	*TSS*		

Example 1: Analyze the data of a 2^3 Factorial Experiment conducted using a RCBD with three replications. The three factors are the fertilizers *viz,* Nitrogen (*N*), Phosphorus (*P*) and Potassium (*K*). The purpose of the experiment is to determine the effect of different kinds of fertilizers on potato crop yield. The yields under *8* treatment combinations for each of the three randomized blocks are given below:

Block-I

npk	*(1)*	*K*	*np*	*p*	*n*	*nk*	*Pk*
450	*101*	*265*	*373*	*312*	*106*	*291*	*391*

Block-II

p	*nk*	*K*	*np*	*(1)*	*npk*	*pk*	*N*
324	*306*	*272*	*338*	*106*	*449*	*407*	*89*

Block-III

p	*npk*	*nk*	*(1)*	*n*	*k*	*pk*	*Np*

323	*471*	*334*	*87*	*128*	*279*	*423*	*324*

Analysis:

Step 1: To find the sum of squares due to blocks (replications), due to treatments and total S.S., arrange the data in the following table

Blocks↓	**Treatment Combinations→**								**Total**
	(1)	**n**	**p**	**np**	**k**	**nk**	**pk**	**npk**	
B_1	*101*	*106*	*312*	*373*	*265*	*291*	*391*	*450*	*2289* (B_1)
B_2	*106*	*89*	*324*	*338*	*272*	*306*	*407*	*449*	*2291* (B_2)
B_3	*87*	*128*	*323*	*324*	*279*	*334*	*423*	*471*	*2369* (B_3)
Total	*294*	*323*	*959*	*1035*	*816*	*931*	*1221*	*1370*	*6949* (G)
	(T_1)	(T_2)	(T_3)	(T_4)	(T_5)	(T_6)	(T_7)	(T_8)	

Grand Total, $G = 6949$; Number of observations (n) $=24 = (r.2^n)$

Correction Factor ($C.F.$) $= \frac{G^2}{n} = \frac{(6949)^2}{24} = 2012025.042$

Total *S.S. (TSS)* $= (101^2+106^2+...+449^2+471^2) - C.F.$
$= 352843.958$

Block (Replication) *S.S. (SSR)* $= \sum_{j=1}^{r} \frac{B_j^2}{2^3} - C.F$

$= \frac{\left[(2289)^2 + (2291)^2 + (2369)^2\right]}{8} - C.F$

$= 520.333$

Treatment *S.S. (SST)* $= \sum_{i=1}^{v} \frac{T_i^2}{r} - C.F$

$= \frac{(294)^2 + (323)^2+(959)^2 + (1035)^2 + (816)^2 + (931)^2 + (1221)^2 + (1370)^2}{3} - C.F$

$= \frac{7082029}{3} - 2012025.042 = 348651.2913$

Error *S.S. (SSE)* =Total *S.S.* - Block *S.S.* - Treatment *S.S.*
$=352843.958 - 520.333 - 348651.2913 = 3672.3337$

Step 2: Calculation of main effect totals and interactions totals is made by using the following contrasts

$N = [npk]- [pk] +[nk] - [k] +[np] - [p]+[n]- [1] = 369$
$P = [npk]+ [pk] - [nk] - [k] +[np] +[p] -[n]- [1] = 2221$
$K = [npk]+ [pk] +[nk] +[k] - [np] - [p] -[n]- [1] = 1727$
$NP = [npk] - [pk] - [nk] +[k] +[np] - [p] -[n]+[1] = 81$
$NK = [npk] - [pk] +[nk] - [k] - [np]+ [p] -[n]+[1] = 159$
$PK = [npk]+ [pk] - [nk] - [k] - [np]- [p]+[n]+[1] = -533$
$NPK = [npk] - [pk] - [nk] +[k] - [np]+ [p]+[n]- [1] =-13$

We now obtain factorial effects (main effects and interactions) and *S.S.* due to factorial effects

Factorial effects = $\frac{Factorial\ effect\ Total}{r.2^{n-1}(=12)}$.

Factorial effect SS = $\frac{(Factorial\ effect\ Total)^2}{r.2^{n}(=24)}$.

Factorial Effects:
N = 30.75, P = 185.083, K = 143.917, NP = 6.75, NK = 13.25, PK = -44.417, NPK = -1.083

SS due to Factorial effects
SS due to *N =5673.375*; SS due to *P = 205535.042*
SS due to *K =124272.0417;* SS due to *NP = 273.375*
SS due to *NK = 1053.375;* SS due to *PK = 11837.0417*
SS due to *NPK = 7.04166.*

Step 3: We now obtain *M.S.* by dividing *S.S.'s* by respective *d.f.*

Step 4: Construct ANOVA table as given below:

ANOVA

Source of Variation	Degrees of Freedom (d.f)	Sum of Squares (S.S)	Mean Squares (M.S.)	Variance Ratio F
Replications	*r-1 = 2*	*520.333*	*260.167*	*0.9918*
Treatments	2^3*-1=7*	*348651.291*	*49807.3273*	*189.8797**
N	*(s-1)=1*	*5673.375*	*5673.375*	*21.6285**
P	*1*	*205535.042*	*205535.042*	*783.5582**
K	*1*	*124272.042*	*124272.042*	*473.7606**
NP	*1*	*273.375*	*273.375*	*1.0422*
NK	*1*	*1053.375*	*1053.375*	*4.0158*
PK	*1*	*11837.041*	*11837.041*	*45.1262**
NPK	*1*	*7.0412*	*7.0412*	*0.02684*
Error	*(r-1) (*2^n*-1)=14*	*3672.337*	*262.3098*	
Total	$r.2^n$*-1=23*	*352843.958*		

(* indicates significance at *5*% level of significance).

Step 5: *S.E* estimate of difference between means of levels of single factor averaged over levels of all other factors = $\sqrt{\frac{MSE}{r.2^{n-2}}}$ *=6.612*

S.E estimate of difference between means for all level combinations of two factors averaged over levels of all other factors = $\sqrt{\frac{MSE}{r.2^{n-3}}}$ *= 9.351.*

$t_{0.05}$ at *14 d.f. =2.145*. Accordingly critical differences (*C.D.*) can be calculated.

2.2 Experiments with factors each at three levels

When factors are taken at three levels instead of two, the scope of an experiment increases. It becomes more informative. A study to investigate if the change is linear or quadratic is possible when the factors are at three levels. The more the number of levels the better, yet the number of the levels of the factors cannot be increased too much as the size of the experiment increases too rapidly with them. Let us begin with two factors *A* and *B*, each at three levels say *0, 1* and *2* (3^2-factorial experiment). The treatment combinations are

00	$= a_0b_0 = (1)$;	*A* and *B* both at first levels
10	$= a_1b_0 = a$;	*A* is at second level and *B* is at first level
20	$= a_2b_0 = a^2$;	*A* is at third level and *B* is at first level
01	$= a_0b_1 = b$;	*A* is at first level and *B* is at second level
11	$= a_1b_1 = ab$;	*A* and *B* both at second level
21	$= a_2b_1 = a^2b$;	*A* is at third level and *B* is at second level
02	$= a_0b_2 = b^2$;	*A* is at first level and *B* is at third level
12	$= a_1b_2 = ab^2$;	*A* is at second level and *B* is at third level
22	$= a_2b_2 = a^2b^2$;	*A* and *B* both at third level

Any standard design can be adopted for the experiment. The main effects *A*, *B* can respectively be divided into linear and quadratic components each with *1 d.f.* as A_L, A_Q, B_L and B_Q. Accordingly *AB* can be partitioned into four components as A_LB_L, A_LB_Q, A_QB_L, A_QB_Q, each with one *df.* The coefficients of the treatment combinations to obtain the above effects are given as

Treatment totals→ Factorial effects↓	*[1]*	*[a]*	*[a^2]*	*[b]*	*[ab]*	*[a^2b]*	*[b^2]*	*[ab^2]*	*[a^2b^2]*	*Divisor*
M	*+1*	*+1*	*+1*	*+1*	*+1*	*+1*	*+1*	*+1*	*+1*	*$9r=rx3^2$*
A_L	*-1*	*0*	*+1*	*-1*	*0*	*+1*	*-1*	*0*	*+1*	*6r=rx2x 3*
A_Q	*+1*	*-2*	*+1*	*+1*	*-2*	*+1*	*+1*	*-2*	*+1*	*18r=6x3*
B_L	*-1*	*-1*	*-1*	*0*	*0*	*0*	*+1*	*+1*	*+1*	*6r=rx2x3*
A_L B_L	*+1*	*0*	*-1*	*0*	*0*	*0*	*-1*	*0*	*+1*	*4r=rx2x2*
A_Q B_L	*-1*	*+2*	*-1*	*0*	*0*	*0*	*+1*	*-2*	*+1*	*12r=rx6x2*
B_Q	*+1*	*+1*	*+1*	*-2*	*-2*	*-2*	*+1*	*+1*	*+1*	*18r=rx3x6*
A_L B_Q	*-1*	*0*	*+1*	*+2*	*0*	*-2*	*-1*	*0*	*+1*	*12r=rx2x6*
A_Q B_Q	*+1*	*-2*	*+1*	*-2*	*+4*	*-2*	*+1*	*-2*	*+1*	*36r=rx6x6*

The rule to write down the coefficients of the linear (quadratic) main effects is to give a coefficient as *+1 (+1)* to those treatment combinations containing the third level of the corresponding factor, coefficient as *0(-2)* to the treatment combinations containing the second level of the corresponding factor and coefficient as *-1(+1)* to those treatment combinations containing the first level of the corresponding factor. The coefficients of the treatment combinations for two factor interactions are obtained by multiplying the corresponding coefficients of two main effects. The various factorial effect totals are given as

$[A_L] = +1[a^2b^2]+0[ab^2]\ -1[b^2]+1[a^2b]+0[ab]\ -1[b]+1[a^2]+0[a]\ -1[1]$

$[A_Q] = +1[a^2b^2]\ -2[ab^2]+1[b^2]+1[a^2b]\ -2[ab]+1[b]+1[a^2]\ -2[a]+1[1]$

$[B_L] = +1[a^2b^2]+1[ab^2]+1[b^2]+0[a^2b]+0[ab]+0[b]\ -1[a^2]\ -1[a]\ -1[1]$

$[A_L B_L] = +1[a^2b^2] + 0[ab^2] - 1[b^2] + 0[a^2b] + 0[ab] + 0[b] - 1[a^2] + 0[a] - 1[1]$
$[A_Q B_L] = +1[a^2b^2] - 2[ab^2] + 1[b^2] + 0[a^2b] + 0[ab] + 0[b] - 1[a^2] + 2[a] - 1[1]$
$[B_Q] = +1[a^2b^2] + 1[ab^2] + 1[b^2] - 2[a^2b] - 2[ab] - 2[b] - 1[a^2] - 1[a] - 1[1]$
$[A_L B_Q] = +1[a^2b^2] + 0[ab^2] - 1[b^2] - 2[a^2b] + 0[ab] + 2[b] + 1[a^2] + 0[a] - 1[1]$
$[A_Q B_Q] = +1[a^2b^2] - 2[ab^2] + 1[b^2] - 2[a^2b] + 4[ab] - 2[b] + 1[a^2] - 2[a] + 1[1]$

The sum of squares due to various factorial effects is given by

$SSA_L = \frac{[A_L]^2}{r.2.3}$; $SSA_q = \frac{[A_Q]^2}{r.6.3}$; $SSB_L = \frac{[B_L]^2}{r.3.2}$; $SSA_L B_L = \frac{[A_L B_L]^2}{r.2.2}$;

$SSA_Q B_L = \frac{[A_Q B_L]^2}{r.6.2}$; $SSB_Q = \frac{[B_Q]^2}{r.3.6}$; $SSA_L B_Q = \frac{[A_L B_Q]^2}{r.2.6}$; $SSA_Q B_Q = \frac{[A_Q B_Q]^2}{r.6.6}$;

If a randomized complete block design is used with *r*-replications then the outline of analysis of variance is

ANOVA

Source of Variation	D.F.	S.S.	M.S.
Replications	$r-1$	*SSR*	$MSR=SSR/(r-1)$
Treatments	$3^2-1=8$	*SST*	$MST=SST/8$
A	2	*SSA*	$MSA=SSA/2$
A_L	1	SSA_L	$MSA_L= SSA_L$
A_Q	1	SSA_Q	$MSA_Q=SSA_Q$
B	2	*SSB*	$MSB=SSB/2$
B_L	1	SSB_L	$MSB_L= SSB_L$
B_Q	1	SSB_Q	$MSB_Q=SSB_Q$
AB	4	*SSAB*	$MSAB=SSAB/2$
$A_L B_L$	1	$SSA_L B_L$	$MSA_L B_L=SSA_L B_L$
$A_Q B_L$	1	$SSA_Q B_L$	$MSA_Q B_L=SSA_Q B_L$
$A_L B_Q$	1	$SSA_L B_Q$	$MSA_L B_Q=SSA_L B_Q$
$A_Q B_Q$	1	$SSA_Q B_Q$	$MSA_Q B_Q=SSA_Q B_Q$
Error	$(r-1)(3^2-1)=8(r-1)$	*SSE*	$MSE=SSE/8(r-1)$
Total	$r.3^2-1=9r-1$	*TSS*	

In general, for *n* factors each at *3* levels, the sum of squares due to any linear (quadratic) main effect is obtained by dividing the square of the linear (quadratic) main effect total by $r.2.3^{n-1}$ $(r.6.3^{n-1})$. Sum of squares due to a *p*-factor interaction is given by taking the square of the total of the particular interaction component divided by $r.(a_1 a_2 \ldots a_p).3^{n-p}$, where a_1, a_2, ... , a_p are taken as *2* or *6* depending upon whether the effect of a particular factor is linear or quadratic.

Example 2: A 3^2 experiment was conducted to study the effects of the two factors, *viz.*, Nitrogen (*N*) and Phosphorus (*P*) each at three levels *0,1,2* on sugar beets. Two replications of nine plots each were used. The table shows the plan and the percentage of sugar (approximated to nearest whole number).

Plan and percentage of sugar of a 3^2 experiment

Replication	Treatment		% of sugar	Replication	Treatment		% of sugar
	N	P			N	P	
I	*0*	*1*	*14*	*II*	*1*	*2*	*20*
	2	*0*	*15*		*1*	*0*	*19*
	0	*0*	*16*		*1*	*1*	*17*
	2	*1*	*15*		*0*	*0*	*18*
	0	*2*	*16*		*2*	*1*	*19*
	1	*2*	*18*		*0*	*1*	*16*
	1	*1*	*17*		*0*	*2*	*16*
	1	*0*	*19*		*2*	*2*	*19*
	2	*2*	*17*		*2*	*0*	*16*

Analyze the data.

Analysis:

Step 1: In order to obtain the sum of squares due to replications, due to treatments and total sum of squares arrange the data in a Replication $\times$ Treatment combinations table as follows:

	Treatment Combinations									Total
Repl.	**1**	**n**	**n^2**	**p**	**np**	**n^2p**	**p^2**	**np^2**	**n^2p^2**	
	00	**10**	**20**	**01**	**11**	**21**	**02**	**12**	**22**	
1	*16*	*19*	*15*	*14*	*17*	*15*	*16*	*18*	*17*	*147* (R_1)
2	*18*	*19*	*16*	*16*	*17*	*19*	*16*	*20*	*19*	*160* (R_2)
Total	*34*	*38*	*31*	*30*	*34*	*34*	*32*	*38*	*36*	*307* (G)
	(T_1)	(T_2)	(T_3)	(T_4)	(T_5)	(T_6)	(T_7)	(T_8)	(T_9)	

Grand Total = *307*, Number of observations (n) = $r.3^2 = 18$.

Correction Factor ($C.F.$)= $\frac{(307)^2}{18} = 5236.0556$

Total *S.S. (TSS)* $= 16^2+18^2+...+17^2+19^2 - 5236.0556 = 48.9444$

Replication *SS (SSR)* $= \frac{R_1^2 + R_2^2}{9} - C.F.$

$= \frac{147^2 + 160^2}{9} - 5236.0556 = 9.3888$

Treatment *SS (SST)* $= \frac{Sum(treatment\ totals)^2}{r} - C.F.$

$= \frac{34^2 + 38^2 + ... + 38^2 + 36^2}{2} - 5236.0556 = 32.4444$

Error *SS* = Total *SS* - Replication *SS* - Treatment *SS* = *7.1112*

Step 2: Obtain various factorial effects totals

$[N_L] = +1[n^2p^2]+0[np^2] -1[p^2]+1[n^2p]+0[np] -1[p]+1[n^2]+0[n] -1[1] = 5$

$[N_Q] = +1[n^2p^2] -2[np^2]+1[p^2]+1[n^2p] -2[np]+1[p]+1[n^2] -2[n]+1[1] = -23$

$[P_L] = +1[n^2p^2]+1[np^2]+1[p^2]+0[n^2p]+0[np]+0[p] -1[n^2] -1[n] -1[1] = 3$

$[N_LP_L] = +1[n^2p^2]+0[np^2] -1[p^2]+0[n^2p]+0[np]+0[p] -1[n^2]+0[n]+1[1] = 7$

$[N_QP_L] = +1[n^2p^2] -2[np^2]+1[p^2]+0[n^2p]+0[np]+0[p] -1[n^2]+2[n] -1[1] = 3$

$[P_Q] = +1[n^2p^2]+1[np^2]+1[p^2] -2[n^2p] -2[np] -2[p] +1[n^2] +1[n] +1[1] = 13$

$[N_LP_Q] = +1[n^2p^2]+0[np^2] -1[p^2] -2[n^2p]+0[np]+2[p]+1[n^2]+0[n] -1[1] = -7$

$[N_QP_Q] = +1[n^2p^2] -2[np^2]+1[p^2] -2[n^2p]+4[np] -2[p]+1[n^2] -2[n]+1[1] = -11$

Step 3: Obtain the sum of squares due to various factorial effects

$$SSN_L = \frac{[N_L]^2}{r.2.3} = \frac{5^2}{12} = 2.0833; \qquad SSN_Q = \frac{[N_Q]^2}{r.6.3} = \frac{(-23)^2}{36} = 14.6944;$$

$$SSP_L = \frac{[P_L]^2}{r.3.2} = \frac{3^2}{12} = 0.7500; \qquad SSN_LP_L = \frac{[N_LP_L]^2}{r.2.2} = \frac{7^2}{8} = 6.1250;$$

$$SSN_QP_L = \frac{[N_QP_L]^2}{r.6.2} = \frac{3^2}{24} = 0.375; \qquad SSP_Q = \frac{[P_Q]^2}{r.3.6} = \frac{13^2}{36} = 4.6944;$$

$$SSN_LP_Q = \frac{[N_LP_Q]^2}{r.2.6} = \frac{(-7)^2}{24} = 2.0417; \qquad SSN_QP_Q = \frac{[N_QP_Q]^2}{r.6.6} = \frac{(-11)^2}{72} = 1.6806;$$

Step 4: Construct the ANOVA table as given above and test the significance of the various factorial effects:

ANOVA

Source of Variation	D.F.	S.S.	M.S.	F
Replications	*1*	*9.3888*	*9.3888*	*10.5623**
Treatments	*8*	*32.4444*	*4.0555*	*4.5624**
N	*2*	*16.7774*	*8.3887*	*9.4371**
N_L	*1*	*2.0833*	*2.0833*	*2.3437*
N_Q	*1*	*14.6944*	*14.6944*	*16.5310**
P	*2*	*5.4444*	*2.7222*	*3.0624*
P_L	*1*	*0.7500*	*0.7500*	*0.8437*
P_Q	*1*	*4.6944*	*4.6944*	*5.2811*
NP	*4*	*10.2223*	*2.5556*	*2.875*
N_LP_L	*1*	*6.1250*	*6.1250*	*6.8905**
N_QP_L	*1*	*0.3750*	*0.3750*	*0.4219*
N_LP_Q	*1*	*2.0417*	*2.0417*	*2.2968*
N_QP_Q	*1*	*1.6806*	*1.6806*	*1.8906*
Error	*8*	*7.1112*	*0.8889*	
Total	*17*	*48.9444*		

(* indicates the significance at 5% level of significance)

2.3 Yates Algorithm

We now describe below a general procedure of computing the factorial effects:

Step 1: Write the treatment combinations in the lexicographic order, *i.e.*, first vary the levels of the first factor from *0* to s_1 - *1* by keeping fixed the levels of other $n - 1$ factors at level *0*. Then vary the levels of the second factor from *1* to s_2 -*1* levels in each of the first s_1 treatment combinations by keeping the levels of factors *3* to *n* factors at *0* levels so as to get s_1xs_2 treatment combinations; then vary the levels of the third factor from *1* to s_3 - *1* by keeping the levels of factors *4* to *n* at *0* levels in the earlier s_1xs_2 treatment combinations and repeat the process till you get all the $\prod_{i=1}^{n} s_i$ treatment combinations. For example, if

there are three factors, first factor at *3* levels, second factor at *4* levels and third factor at 5 levels. Then *3x4x5 = 60* treatment combinations are:

000, 100, 200, 010, 110, 210, 020, 120, 220, 030, 130, 230, 001, 101, 201, 011, 111, 211, 021, 121, 221, 031, 131, 231, 002, 102, 202, 012, 112, 212, 022, 122, 222, 032, 132, 232, 003, 103, 203, 013, 113, 213, 023, 123, 223, 033, 133, 233, 004, 104, 204, 014, 114, 214, 024, 124, 224, 034, 134, 234.

Write all these treatment combinations in the first column and in the second column write the corresponding treatment totals.

Step 2: Divide the observations in the second column in groups such that each group has s_1 observations. Then we add the observations in each of these s_1 groups in the third column, then we repeat the process of linear component of the main effect of the first factor with these groups and append the third column, repeat the process for quadratic effects and so on till the polynomial upto the order of s_1-*1*. For example, if the factor is at two levels, then we make the groups of two observations each, and first half of the third column is filled with sum of observations in these groups and second half with the differences of the second observation and the first observation in each group. If the factor is at three levels, we make the groups of three observations each, and one third column is filled with the sum of observations in these groups, next one third by using the linear component, say *-1, 0, 1, i.e.*, by taking the difference of the third observation and first observation in each group and rest one third is filled by using the quadratic component *1, -2, 1, i.e.*, by adding the first and third observation in each group and subtracting the twice of the second observation from this sum. If the factor is at four levels, we make the groups of four observations each, the first quarter of the next column is filled by sum of these observations in each of the groups, next quarter is filled by using the linear component *-3, -1, 1, 3, i.e.*, by adding the third observation and *3* times the fourth observation from each group and then subtracting the sum of second observation and three times the first observation from this sum. Next quarter is filled using the quadratic component *1, -1, -1, 1, i.e.* first observation minus second observation minus third observation plus fourth observation of each of the groups and last quarter is filled by using the cubic component say *-1, 3, -3, 1, i.e.* [–(first observation) + *3* (second observation) - *3(*third observation) + fourth observation] from each group, and so on.

In the third column, divide the observations into groups such that each group contains s_2 observations and then use these groups to obtain the fourth column as in second column. In the fourth column divide the observations into groups of s_3 observations each and so on. Repeat the process for all the *n* factors.

If all the factors are at same levels, then perform same operation on all the *n* columns.

For illustration, the various factorial effect totals in the Example 2, where each of the three factors is at *2* levels each, can be obtained as follows:

Treatment combinations (1)	**Treatment totals (2)**	**Operation as per first factor (3)**	**Operation as per second factor (4)**	**Operation as per third factor (5)**

000 (1)	*294 =I*	*617=I+II*	*2611*	*6949=G*
100 n	*323=II*	*1994=III+IV*	*4338*	*369=[N]*
010 p	*959=III*	*1747=V+VI*	*105*	*2221=[P]*
110 np	*1035=IV*	*2591=VII+VIII*	*264*	*81=[NP]*
001 k	*816=V*	*29=II-I*	*1377*	*1727=[K]*
101 nk	*931=VI*	*76=IV-III*	*844*	*159=[NK]*
011 pk	*1221=VII*	*115=VI-V*	*47*	*-533=[PK]*
111 npk	*1370=VII*	*149=VIII-VII*	*34*	*-13=[NPK]*

For Example 2, the various factorial effects totals can be obtained as given in the following table

Treatment combinations (1)	**Treatment totals (2)**	**Operation as per first factor (3)**	**Operation as per second factor (4)**
00 (1)	*34=I*	*103=I+II+III*	*307=G*
10 (n)	*38=II*	*98=IV+V+VI*	*5=N_L*
20 (n^2)	*31=III*	*106=VII+VIII+IX*	*-23=N_Q*
01 (p)	*30=IV*	*-3=III-I*	*3=P_L*
11 (np)	*34=V*	*4=VI-IV*	*7=N_LP_L*
21 (n^2p)	*34=VI*	*4=IX-VII*	*3=N_QP_L*
02 (p^2)	*32=VII*	*-11=III-2II+I*	*13=P_Q*
12 (np^2)	*38=VII*	*-4=VI-2V+IV*	*-7=N_LP_Q*
22 (n^2p^2)	*36=IX*	*-8=IX-2VIII+VII*	*-11=N_QP_Q*

Remark: The analysis demonstrated so far is computationally feasible for the situation when large number of factors is experimented with smaller number of levels. However, usual tabular method of analysis can be employed for the situations when there are few factors with more number of levels.

3. Confounding in Factorial Experiments

When the number of factors and/or levels of the factors increase, the number of treatment combinations increase very rapidly and it is not possible to accommodate all these treatment combinations in a single homogeneous block. For example, a 2^5 factorial would have 32 treatment combinations and blocks of 32 plots are quite big to ensure homogeneity within them. In such a situation it is desirable to form blocks of size smaller than the total number of treatment combinations (incomplete blocks) and, therefore, have more than one block per replication. The treatment combinations are then allotted randomly to the blocks within the replication and the total number of treatment combinations is grouped into as many groups as the number of blocks per replication.

A consequence of such an arrangement is that the block contrasts become identical to some of the interaction component contrasts. For example, consider a *2^4* factorial experiment to be conducted in two blocks of size *8* each per replication. The two blocks in a single replication are the following:

Block – I	**Block - II**
treatment combination	**treatment combination**
A B C D	A B C D

0	0	0	0	(1)	1	0	0	0	a
1	1	0	0	Ab	0	1	0	0	b
1	0	1	0	Ac	0	0	1	0	c
1	0	0	1	Ad	0	0	0	1	d
0	1	1	0	Bc	1	1	1	0	abc
0	1	0	1	Bd	1	1	0	1	abd
0	0	1	1	Cd	1	0	1	1	acd
1	1	1	1	Abcd	0	1	1	1	bcd

It may easily be verified that the block contrast is identical with the contrast for the interaction ABCD, *i.e., 0000+1100+1010+1001+0110+0101+0011+1111-1000-0100-0010-0001-1110-1101-1011-0111*. Thus, the interaction ABCD gets confounded with block effects and it is not possible to separate out the two effects.

Evidently the interaction confounded has been lost but the other interactions and main effects can now be estimated with better precision because of reduced block size. This device of reducing the block size by taking one or more interactions contrasts identical with block contrasts is known as **confounding**. Preferably only higher order interactions with three or more factors are confounded, because these interactions are less important to the experimenter. As an experimenter is generally interested in main effects and two factor interactions, these should not be confounded as far as possible. The designs for such confounded factorials are incomplete block designs. However usual incomplete block designs for single factor experiments cannot be adopted, as the contrasts of interest in two kinds of experiments are different. The treatment groups are first allocated at random to the different blocks. The treatments allotted to a block are then distributed at random to its different units.

When there are two or more replications in the design and if the same set of interaction components is confounded in all the replications, then confounding is called **complete** and if different sets of interactions are confounded in different replications, confounding is called **partial**. In complete confounding all the information on confounded interactions is lost. However, in partial confounding, the information on confounded interactions can be recovered from those replications in which these are not confounded.

Advantages of Confounding

- It reduces the experimental error considerably by stratifying the experimental material into homogeneous subsets or subgroups. The removal of the variation among incomplete blocks (freed from treatments) within replications results in smaller error mean square as compared with a RCB design, thus making the comparisons among some treatment effects more precise.

Disadvantages of Confounding

- In the confounding scheme, the increased precision is obtained at the cost of sacrifice of information (partial or complete) on certain relatively unimportant interactions.
- The confounded contrasts are replicated fewer times than are the other contrasts and as such there is loss of information on them and these can be estimated with a lower degree of precision as the number of replications for them is reduced.

- An indiscriminate use of confounding may result in complete or partial loss of information on the contrasts or comparisons of greatest importance. As such the experimenter should confound only those treatment combinations or contrasts that are of relatively less or of no importance at all.
- The algebraic calculations are usually more difficult and the statistical analysis is complex, especially when some of the units (observations) are missing. In this package, the attempt has been made to ease this problem.

3.1 Confounding in 2^3 Experiment

To make the exposition simple, we consider a small factorial experiment 2^3. Let the three factors be A, B, C each at two levels.

Effects→	A	B	C	AB	AC	BC	ABC
Treat. Combinations↓							
(1)	-	-	-	+	+	+	-
(a)	+	-	-	-	-	+	+
(b)	-	+	-	-	+	-	-
(ab)	+	+	-	+	-	-	-
(c)	-	-	+	+	-	-	+
(ac)	+	-	+	-	+	-	-
(bc)	-	+	+	-	-	+	-
(abc)	+	+	+	+	+	+	+

The various effects are given by

A = (abc) + (ac) + (ab) + (a) - (bc) - (c) - (b) - (1)
B = (abc) + (bc) + (ab) + (b) - (ac) - (c) - (a) - (1)
C = (abc) + (bc) + (ac) + (c) - (ab) - (b) - (a) - (1)
AB = (abc) + (c) + (ab) + (1) - (bc) - (ac) - (b) - (a)
AC = (abc) + (ac) + (b) + (1) - (bc) - (c) - (ab) - (a)
BC = (abc) + (bc) + (a) + (1) - (ac) - (c) - (ab) - (b)
ABC = (abc) + (c) + (b) + (a) - (bc) - (ac) - (ab) - (1)

Suppose that the experimenter decides to use two blocks of *4* units (plots) per replication and that the highest order interaction ABC is confounded. Thus, in order to confound the interaction ABC with blocks all the treatment combinations with positive sign are allocated at random in one block and those with negative signs in the other block. Thus the following arrangement gives ABC confounded with blocks and hence the entire information is lost on ABC in this replication.

Replication I

Block 1:	(1)	(ab)	(ac)	(bc)
Block 2 :	(a)	(b)	(c)	(abc)

We observe that the contrast estimating ABC is identical to the contrast estimating block effects. If the same interaction ABC is confounded in all the other replications, then the interaction is said to be completely confounded and we cannot recover any information on the interaction ABC through such a design. For the other six factorial effects *viz.* A, B, C, AB, AC, BC there are two treatment combinations with a positive sign and two treatment combinations with a negative sign in each of the two blocks and hence these differences are not influenced among blocks and can thus be estimated and tested as usual without any difficulty.

Similarly if we want to confound AB, then the two blocks will consists of

Block 1	(abc)	(c)	(ab)	(1)
Block 2	(bc)	(ac)	(b)	(a)

Here interaction AB is confounded with block effects whereas all other effects A, B, C, AC, BC and ABC can be estimated orthogonally.

3.2 Partial confounding

When different interactions are confounded in different replications, the interactions are said to be partially confounded. Consider again the 2^3 factorial experiment with each replicate divided into two blocks of 4 units each. It is not necessary to confound the same interaction in all the replications and several factorial effects may be confounded in one single experiment. For example, the following plan confounds the interaction ABC, AB, BC and AC in replications I, II, III and IV respectively.

Rep. I		**Rep. II**		**Rep. III**		**Rep. IV**	
Block 1	**Block 2**	**Block 3**	**Block 4**	**Block 5**	**Block 6**	**Block 7**	**Block 8**
(abc)	(ab)	(abc)	(ac)	(abc)	(ab)	(abc)	(ab)
(a)	(ac)	(c)	(bc)	(bc)	(ac)	(ac)	(bc)
(b)	(bc)	(ab)	(a)	(a)	(b)	(b)	(a)
(c)	(1)	(1)	(b)	(1)	(c)	(1)	(c)

In the above arrangement, the main effects A, B and C are orthogonal to block contrasts. The interaction ABC is completely confounded with blocks in replication I, but in the other three replications the interaction ABC is orthogonal to blocks and consequently an estimate of ABC may be obtained from replicates II, III and IV. Similarly it is possible to recover information on the other confounded interactions AB (from replications I, III, IV), BC (from replications I, II, IV) and AC (from replications I, II, III). Since the partially confounded interactions are estimated from only a portion of the observations, they are determined with a lower degree of precision than the other effects.

3.3 Construction of a Confounded Factorial

Given a set of interactions confounded, the blocks of the design can be constructed and vice-versa *i.e.*, if the design is given the interactions confounded can be identified.

3.4 Given a set of interactions confounded, how to obtain the blocks?

The blocks of the design pertaining to the confounded interaction can be obtained by solving the equations obtained from confounded interaction. We illustrate this through an example.

Example 3: Construct a design for 2^5 factorial experiment in 2^3 plots per block confounding interactions ABD, ACE and BCDE.

Let x_1, x_2, x_3, x_4 and x_5 denote the levels (*0* or *1*) of each of the *5* factors A, B, C, D and E. Solving the following equations would result in different blocks of the design.

For interaction ABD: $x_1 + x_2 + x_4 = 0, 1$

For interaction ACE : $x_1 + x_3 + x_5 = 0, 1$

The interactions ABD and ACE are independent and BCDE is a generalized interaction. In other words, a solution of the above two equations will also satisfy the equation $x_1 + x_2 + x_3 + x_4 = 0,1$. Treatment combinations satisfying the following solutions of above equations will generate the required four blocks

(0, 0) (0, 1) (1, 0) (1, 1)

The solution (0, 0) will give the key block (A key block is one that contains one of the treatment combination of factors, each at lower level).

There will be $\frac{2^5}{2^3}$ = 4 blocks per replication. The key block is as obtained below

A	**B**	**C**	**D**	**E**	
1	1	1	0	0	abc
1	1	0	0	1	abe
1	0	1	1	0	acd
1	0	0	1	1	ade
0	1	1	1	1	bcde
0	1	0	1	0	bd
0	0	1	0	1	ce
0	0	0	0	0	(1)

Similarly we can write the other blocks by taking the solutions of above equations as (0, 1) (1, 0) and (1, 1).

3.5 Given a block, how to find the interactions confounded?

The first step in detecting the interactions confounded in blocking is to select the key block. If the key block is not given, it is not difficult to obtain it. Select any treatment combination in the given block; multiply all the treatment combinations in the block by that treatment combination and we get the key block. From the key block we know the number of factors as well as the block size. Let it be *n* and *k*. We know then that the given design belongs to the 2^n factorial in 2^r plots per block. The next step is to search out a unit matrix of order r. From these we can find the interaction confounded. We illustrate this through an example.

Example 4: Given the following block, find out the interactions confounded.

(acde), (bcd), (e), (abec), (ad), (bde), (ab), (c)

Since the given block is not the key block we first obtain the key block by multiplying every treatment combination of the given block by e. We get the following block:

(acd), (bcde), (1), (abc), (ade), (bd), (abe), (ce)

This is the key block as it includes (1). It is obvious that the factorial experiment involves five factors and has 2^3 (=8) plots per block. Hence, the given design is (2^5, 2^3).

	A	**B**	**C**	**D**	**E**
	1	0	1	1	0
	0	1	1	1	1
	0	0	0	0	0
	1	1	1	0	0
*	1	0	0	1	1

*	0	1	0	1	0
	1	1	0	0	1
*	0	0	1	0	1

***** indicates the rows of a unit matrix of order *3*.

A	**B**	**C**	**D**	**E**
1	0	0	1(=α_1)	1(=β_1)
0	1	0	1(=α_2)	0(=β_2)
0	0	1	0(=α_3)	1(=β_3)

The interaction confounded is $A^{\alpha_1}B^{\alpha_2}C^{\alpha_3}D$, $A^{\beta_1}B^{\beta_2}C^{\beta_3}E$. Here ABD and ACE are independent interactions confounded and BCDE is obtained as the product of these two and is known as generalized interaction.

3.6 General rule for confounding in 2^n series

Let the design be (2^n, 2^r) *i.e*. 2^n treatment combinations arranged in 2^r plots per block.
Number of treatment combinations = 2^n, Block size = 2^r, Number of blocks per replication =2^{n-r},
Total number of interactions confounded = $2^{n-r} - 1$, Number of independent interactions confounded = $n - r$, Generalized interactions confounded = $(2^{n-r} - 1) - (n - r)$.

3.7 Analysis

For carrying out the statistical analysis of a (2^n, 2^r) factorial experiment in p replications, the various factorial effects and their S.S. are estimated in the usual manner with the modification that for **completely confounded** interactions neither the S.S due to confounded interaction is computed nor it is included in the ANOVA table. The confounded component is contained in the ($p2^{n-r} - 1$) *d.f.* due to blocks. The splitting of the total degrees of freedom is as follows:

Source of Variation	**Degrees of Freedom**
Replication	$p - 1$
Blocks within replication	$p(2^{n-r} - 1)$
Treatments	$(2^n - 1) - (2^{n-r} - 1)$
Error	By subtraction
Total	$p2^n - 1$

The *d.f* due to treatment has been reduced by $2^{n-r}-1$ as this is the total *d.f* confounded per block.

3.8 Partial Confounding

In case of partial confounding, we can estimate the effects confounded in one replication from the other replications in which it is not confounded. In (2^n, 2^r) factorial experiment with p replications, following is the splitting of *d.f's*.

Source of Variation	**Degrees of Freedom**
Replication	$p- 1$
Blocks within replication	$p(2^{n-r} - 1)$
Treatments	$2^n - 1$

Error	By subtraction
Total	$p2^n - 1$

The S.S. for confounded effects are obtained from only those replications where the given effect is not confounded. From practical point of view, the S.S. for all the effects including the confounded effects is obtained as usual and then some adjustment factor (A.F) is applied to the confounded effects. The adjusting factor for any confounded effect is computed as follows:

(i) Note the replication in which the given effect is confounded

(ii) Note the sign of (1) in the corresponding algebraic expression of the effect to the confounded. If the sign is positive then

A.F = [Total of the block containing (1) of replicate in which the effect is confounded] - [Total of the block not containing (1) of the replicate in which the effect is confounded] $= T_1 - T_2$.

If the sign is negative, then A.F = $T_2 - T_1$.

This adjusting factor will be subtracted from the factorial effects totals of the confounded effects obtained.

Example 5: Analyze the following 2^3 factorial-experiment conducted in two blocks of *4* plots per replication, involving three fertilizers N, P, K, each at two levels:

Replication I		**Replication II**		**Replication III**	
Block 1	**Block 2**	**Block 3**	**Block 4**	**Block 5**	**Block 6**
(np)	(p)	(1)	(np)	(pk)	(n)
101	88	125	115	75	53
(npk)	(n)	(npk)	(k)	(nk)	(npk)
111	90	95	95	100	76
(1)	(pk)	(nk)	(pk)	(1)	(p)
75	115	80	90	55	65
(k)	(nk)	(p)	(n)	(np)	(k)
55	75	100	80	92	82

Step 1: Identify the interactions confounded in each replication. Here, each replication has been divided into two blocks and one effect has been confounded in each replication. The effects confounded are

Replication I $\rightarrow$ NP; Replicate II $\rightarrow$ NK; Replicate III $\rightarrow$ NPK

Step 2: Obtain the blocks S.S. and Total S.S.

S.S. due to Blocks = $\sum_{i=1}^{6} \frac{B_i^2}{4}$ - C.F = 2506

Total S.S. = $\sum (\text{Obs.})^2$ - C.F = 8658

Step 3: Obtain the sum of squares due to all the factorial effects other than the confounded effects.

Treatment Combinations	Total Yield	Factorial Effects	Sum of Squares (S.S) = [Effect]^2/ 2^3.r
(1)	255	G=0	
n	223	[N]=48	96 = S_N^2
p	253	[P]=158	1040.17 = S_P^2
np	308	[NP]=66	-
k	232	[K]=10	4.17 = S_K^2
nk	255	[NK]=2	-
pk	280	[PK]=-8	2.67 = S_{PK}^2
npk	282	[NPK]=-108	-

Total for the interaction NP is given by

[NP] = [npk] - [pk] - [nk] + [k] + [np] - [p] - [n] + [1]

Here the sign of (1) is positive. Hence the adjusting factor (A.F) for NP, which is to be obtained from replicate 1 is given by

A.F. for NP = (101 + 111 + 75 + 55) - (88 + 90 + 115 + 75) = -26

Adjusted effect total for NP becomes, [NP*] = [NP]- (-26) = 66 + 26 = 92.

It can easily be seen that the total of interaction NP using the above contrast from replications II and III also gives the same total *i.e.* 92.

Similarly A.F. for NK =20, A.F. for NPK = -46

Hence adjusted effect totals for NK and NPK are respectively [NK*] =-18 and [NPK*] = -62.

S_{NP}^2 = S.S. due to NP = $\frac{1}{16}$[NP*]^2 = 529; S_{NK}^2 = S.S. due to NK = $\frac{1}{16}$ [NK*] = 20.25

S_{NPK}^2 = S.S. due to NPK = $\frac{1}{16}$ [NPK*] = 240.25

Treatment S.S. = $S_N^2 + S_P^2 + S_K^2 + S_{NP}^2 + S_{NK}^2 + S_{PK}^2 + S_{NPK}^2$ = 1932.7501

ANOVA

Source	d.f.	Sum of Squares	M.S.	F
Blocks	5	2506	501	1.31
Treatments	7	1932.75	276.107	-
N	1	96.00	96.00	-
P	1	1040.16	1040.16	2.71
NP	1	529.00	529.00	1.3
K	1	4.41	4.41	-
NK	1	20.25	20.25	-
PK	1	2.66	2.66	-
NPK	1	240.25	240.25	-
Error	11	4219.24	383.57	
Total	**23**	**8658**		

'-' indicates that these ratios are less than one and hence these effects are non-significant.

From the above table it is seen that effects due to blocks, main effects due to factor N, P, and K or interactions are not significant.

4. Confounding in 3^n Series

The concept of confounding here also is the same as in 2^n series. We shall illustrate the principles of confounding in 3^n in 3^r plots per block with the help of a 3^3 experiments laid out in blocks of size $3^2(=9)$. Let the three factors be A, B and C and the confounded interaction be ABC^2. The three levels of each of the factor are denoted by *0, 1* and *2* and a particular treatment combination be $x_i\, x_j\, x_k$, *i, j, k = 0, 1, 2*.

Number of blocks per replication $=3^{n-r} = 3$; Block size $= 3^r = 9$; Degrees of freedom confounded per replication $= 3^{n-r} - 1 = 2$.

Number of interactions confounded per replicate $= \dfrac{3^{n-r}-1}{3-1} = 1$.

The treatment combinations in 3 blocks are determined by solving the following equations mod(*3*)

$x_1 + x_2 + 2x_3 = 0$; $x_1 + x_2 + 2x_3 = 1$; $x_1 + x_2 + 2x_3 = 2$

Block I			Block II			Block III		
A	**B**	**C**	**A**	**B**	**C**	**A**	**B**	**C**
1	0	1	1	0	0	1	0	2
0	1	1	0	1	0	0	1	2
1	1	2	1	1	1	1	1	0
2	0	2	2	0	1	2	0	0
0	2	2	0	2	1	0	2	0
2	1	0	2	1	2	2	1	1
1	2	0	1	2	2	1	2	1
2	2	1	2	2	0	2	2	2
0	0	0	0	0	2	0	0	1

5. Confounding in s^n Factorial Experiments in s^r experimental units per block

s^n Factorial Experiments in s^r experimental units per block are represented by (s^n, s^r) factorial experiments. For generation of (s^n, s^r), *s* should be a prime or prime power, i.e., $s = p^m$, where *p* is prime and *m* is a positive integer. For the factorial experiments of the type (s^n, s^r) there will be s^{n-r} blocks per replication with (s^r) experimental units per block. The total number of degrees of freedom confounded per replication is $s^{n-r} - 1$, while the total number of interaction components confounded per replication is $\dfrac{s^{n-r}-1}{s-1}$ as each interaction component has $(s-1)$ degrees of freedom. The total number of independent interaction components to be confounded is n-r and rest are generalized interaction components. For the $(n-r)$ independent interaction components confounded, we have the following set of $(n-r)$ equations as:

$$\sum_{j=1}^{n} p_{j1}x_j = 0,1,\ \alpha_2,\alpha_3,\ldots,\ \alpha_{s-1} \bmod s$$

$$\sum_{j=1}^{n} p_{j2}x_j = 0,1,\ \alpha_2,\alpha_3,\ldots,\ \alpha_{s-1} \bmod s$$

$$\vdots$$

$$\sum_{j=1}^{n} p_{jk}x_j = 0,1,\ \alpha_2,\alpha_3,\ldots,\ \alpha_{s-1} \bmod s$$

$$\vdots$$

$$\sum_{j=1}^{n} p_{j(n-r)}x_j = 0,1,\ \alpha_2,\alpha_3,\ldots,\ \alpha_{s-1} \bmod s$$

where p_{jk}'s and 0,1, $\alpha_2,\alpha_3,\ldots,\ \alpha_{s-1}$ are the elements of the Galois Field s and $x_1,x_2,\ldots,x_n$ are the variates corresponding to the *n*-factors and denote the levels of the corresponding factors in the different treatment combinations. If $m>1$, then mod s in the above equations should be replaced by mod {*p, p(x)*}, where *p(x)* is the minimal function for GF(p^m) and *x* is the primitive root of the GF(p^m). These equations result into s^{n-r} different sets. Solution of each set gives one block. For example, if one wants to generate a $(3^4,3^2)$ factorial experiment, then the number of independent interaction components to be confounded are 4-2=2. These two independent interactions are represented by:

$$p_{11}x_1 + p_{21}x_2 + p_{31}x_3 + p_{41}x_4 = 0,1,2 \bmod 3$$

$$p_{12}x_1 + p_{22}x_2 + p_{32}x_3 + p_{42}x_4 = 0,1,2 \bmod 3$$

These sets of equations give rise to 9 combinations viz. the left hand sides satisfying (0,0); (0,1); (0,2); (1,0); (1,1); (1,2); (2,0); (2,1) and (2,2). The treatment combinations in 9 blocks in one replication are those satisfy the above combinations. The block containing the treatment combinations satisfying

$p_{11}x_1 + p_{21}x_2 + p_{31}x_3 + p_{41}x_4 = 0 \bmod 3$ and

$p_{12}x_1 + p_{22}x_2 + p_{32}x_3 + p_{42}x_4 = 0 \bmod 3$ is the key block.

For the situations, where *s* is a prime power, we make use of the concept of minimal functions. For example, if one wants to generate a $(4^2,4)$-factorial experiment, then the number of levels for each of the two factors is a prime power, i.e. 4 = 2^2. The minimal function for GF(4) is *p(x)* = x^2+x+1 and the elements of the GF(4) are 0, 1, x , x+1. The total number of treatment combinations is 16 and are given by

A	0	0	0	0	1	1	1	1	x	x	x	x	x+1	x+1	x+1	x+1
B	0	1	x	x+1	0	1	x	x+1	0	1	x	x+1	0	1	x	x+1

Here *n* = 2 and *r* = 1, therefore, the number of blocks per replication is 4 and number of experimental units in each block is also 4. The number of independent interaction components to be confounded is *n - r = 1*. Let the experimenter is interested in confounding the interaction component AB. Therefore, the block contents can be obtained from the solution of

$$x_1 + x_2 = 0,1,x,x+1 \mod\{2, x^2 + x + 1)$$

The block contents obtained through the solution of the above equations are

Block - I		Block - II		Block - III		Block - IV	
A	B	A	B	A	B	A	B
0	0	0	1	0	x	0	x+1
1	1	1	0	x	0	1	x
x	x	X	x+1	1	x+1	x	1
x+1	x+1	x+1	x	x+1	1	x+1	0

Similarly, we can get the block contents, if the other interaction components are confounded.

The above discussion relates to the methods of construction of symmetrical factorial experiments with confounding. The loss of information on the confounded interaction components depends upon the number of replications in which these are confounded. The designs in which the loss of information is equally distributed over the different components of the interaction of given orders (order of an interaction is one less than the number of factors involved in the interaction) may be desirable. A design with the above characterization is a **balanced confounded design**. This design in case of symmetrical factorials is defined as:

6. Balanced Confounded Design

A partially confounded design is said to be balanced if all the interactions of a particular order are confounded in equal number of replications.

How to construct a Balanced confounded Factorial Design?

Let us take the example of a (2^5, 2^3)- factorial experiment. The interest is in constructing a design for a (2^5, 2^3)- factorial experiment achieving balance over three and four factor interactions. In this case, s = 2, n = 5 and r = 3. Therefore, the total number of treatment combinations is 32, the block size is 8 and the number of blocks per replicate is 32/8. The number of degrees of freedom confounded is $2^{5-3} - 1 = 3$. Each interaction component has 1 degree of freedom. Therefore, the number of interaction components to be confounded is 3. The number of independent interactions to be confounded is 5-3 = 2 and one is the generalized interaction component.

The number of 3 factor interactions = ($^5C_3$) = 10 viz. ABC, ABD, ABE, ACD, ACE, ADE, BCD, BCE, BDE, and CDE and number of four factor interactions = ($^5C_4$) = 5 viz. ABCD, ABCE, ABDE, ACDE, and BCDE. Therefore, to achieve balance total number f degrees of freedom to be confounded is 10+5=15. As each interaction component has 1 d.f., therefore, number of degrees of freedom to be confounded are 15. The number of degrees of freedom confounded in one replication is 3. Therefore, the number of replications required is 15/3=5. The balance can be achieved by confounding the following interactions in different replications:

Replication – I: ABD, ACE and BCDE
Replication – II: ACD, BCE and ABDE
Replication – III: ADE, BCD and ABCE
Replication – IV: ABE, CDE and ABCD
Replication – V: ABC, BDE and ACDE

The block contents may be obtained following the above procedure. The confounding in asymmetrical factorials is somewhat different from symmetrical factorials. When an interaction component is confounded in a replication in these designs, it is not necessary that it is completely confounded with the blocks in the sense that the block contrasts and the interaction contrasts become identical. These two sets of contrasts although not identical, yet are dependent so that the contrasts for obtaining a confounded interaction from the treatment totals are not free from block effects. Therefore, more than one replication is needed in obtaining balanced confounded designs for asymmetrical factorial experiment. A design is said to be Balanced confounded factorial experiment (BFE) if (i) any contrasts of a confounded interaction component is estimable independently of any other contrasts belonging to any other confounded interaction and (ii) the loss of information of each degrees of freedom of any confounded interaction is same. To be more specific, BFE may be defined as:

A factorial experiment will be called a balanced factorial experiment if

(i) Each treatment is replicated the same number of times.
(ii) Each of the blocks has the same number of plots.
(iii) Estimates of the contrasts belonging to different interactions are uncorrelated with each other.
(iv) Complete balance is achieved over each of the interactions, i.e., all the normalized contrasts belonging to the same interaction are estimated with the same variance.

Several methods of construction of designs for balanced factorial experiments are available in literature based on pseudo factors or pairwise balanced block designs. We shall not be presenting these methods here. The user may refer to standard textbooks for the same. Further, it is known that an *extended group divisible* (EGD) design, if existent, has orthogonal factorial structure with balance. In other words, an EGD design is a balanced confounded factorial experiment. Therefore, the vast literature on the methods of construction of extended group divisible designs may be used for the construction of BFE. The conditions of equal replications and equal block sizes may now be relaxed.

Generation of a design for factorial experiments is easy. But when the number of factors or the number of levels become large it becomes difficult to generate the layout of the design. To circumvent this problem, IASRI has developed a statistical package SPFE (Statistical Package for Factorial Experiments). This package is essentially for symmetrical factorial experiments. There is a provision of generation of designs as well as the randomized layout of the designs including totally and partially confounded designs. The design is generated once the independent interactions to be confounded are listed. One can give different number of independent interactions to be confounded in different replications (The package is also capable of generating the design for factorial experiments by simply giving the number of factors along with the number of levels and the block size. In this case the package will itself determine the number of blocks per replication and the layout by keeping the higher interactions confounded). Provision has also been made in this package for analyzing the data generated from the experiments using these designs. The data generated are analyzed as a general block design and the contrast analysis is carried out to obtain the sum of squares due to main effects and interactions. Separate modules have been developed for generating the probabilities using χ^2, F and t distributions for testing the levels of significance.

This package deals with only symmetrical factorial experiments. However, in practice an experimenter encounters situations where one has to use various factors with unequal number of levels. The generation of the design for asymmetrical factorial experiments is, however, a tedious job. We, therefore, give below a catalogue of designs commonly used. In this catalogue A, B, C, etc. denote the factors and a, b, c, etc. denote the blocks within replications.

Plan 1. Balanced group of sets for $3x2^2$ factorial, blocks of *6* units each

BC, ABC confounded

Replication I							Replication II							Replication III					
Block-1			Block-2				Block-1			Block-2				Block-1			Block-2		
0	0	1	0	0	0		0	0	0	0	0	1		0	0	0	0	0	1
0	1	0	0	1	1		0	1	1	0	1	0		0	1	1	0	1	0
1	0	0	1	0	1		1	0	1	1	0	0		1	0	0	1	0	1
1	1	1	1	1	0		1	1	0	1	1	1		1	1	1	1	1	0
2	0	0	2	0	1		2	0	0	2	0	1		2	0	1	2	0	0
2	1	1	2	1	0		2	1	1	2	1	0		2	1	0	2	1	1

Plan 2. Balanced group of sets for $3x2^3$ factorial, blocks of *6* units

BC, BD, CD

ABC, ABD, ACD confounded

Replication I																
Block-1				Block-2				Block-3				Block-4				
0	1	0	0	0	0	0	0	0	0	0	1	0	0	1	0	
0	0	1	1	0	1	1	1	0	1	1	0	0	1	0	1	
1	0	1	0	1	0	0	1	1	0	0	0	1	1	0	0	
1	1	0	1	1	1	1	0	1	1	1	1	1	0	1	1	
2	0	0	1	2	0	1	0	2	1	0	0	2	0	0	0	
2	1	1	0	2	1	0	1	2	0	1	1	2	1	1	1	

Replication II																
Block-1				Block-2				Block-3				Block-4				
0	0	1	0	0	0	0	1	0	0	0	0	0	1	0	0	
0	1	0	1	0	1	1	0	0	1	1	1	0	0	1	1	
1	0	0	1	1	0	1	0	1	1	0	0	1	0	0	0	
1	1	1	0	1	1	0	1	1	0	1	1	1	1	1	1	
2	1	0	0	2	0	0	0	2	0	0	1	2	0	1	0	
2	0	1	1	2	1	1	1	2	1	1	0	2	1	0	1	

Replication III																
Block-1				Block-2				Block-3				Block-4				
0	0	0	1	0	0	1	0	0	1	0	0	0	0	0	0	
0	1	1	0	0	1	0	1	0	0	1	1	0	1	1	1	
1	1	0	0	1	0	0	0	1	0	0	1	1	0	1	0	
1	0	1	1	1	1	1	1	1	1	1	0	1	1	0	1	
2	0	1	0	2	0	0	1	2	0	0	0	2	1	0	0	
2	1	0	1	2	1	1	0	2	1	1	1	2	0	1	1	

Plan 3. Balanced group of sets for 3^2 *x2* factorial, blocks of *6* units

AB, ABC Confounded

Replication I									Replication II								
Block-1			Block-2			Block-3			Block-1			Block-2			Block-3		
1	0	0	2	0	0	0	0	0	2	0	0	0	0	0	1	0	0
2	1	0	0	1	0	1	1	0	0	1	0	1	1	0	2	1	0
0	2	0	1	2	0	2	2	0	1	2	0	2	2	0	0	2	0
2	0	1	0	0	1	1	0	1	1	0	1	2	0	1	0	0	1
0	1	1	1	1	1	2	1	1	2	1	1	0	1	1	1	1	1
1	2	1	2	2	1	0	2	1	0	2	1	1	2	1	2	2	1

Replication III									Replication IV								
Block-1			Block-2			Block-3			Block-1			Block-2			Block-3		
1	0	0	2	0	0	0	0	0	2	0	0	0	0	0	1	0	0
0	1	0	1	1	0	2	1	0	1	1	0	2	1	0	0	1	0
2	2	0	0	2	0	1	2	0	0	2	0	1	2	0	2	2	0
2	0	1	0	0	1	1	0	1	1	0	1	2	0	1	0	0	1
1	1	1	2	1	1	0	1	1	0	1	1	1	1	1	2	1	1
0	2	1	1	2	1	2	2	1	2	2	1	0	2	1	1	2	1

Plan 4. Balanced group of sets for *4x2*^{2} factorial, blocks of *8* units

ABC Confounded

Replication I							Replication II							Replication III					
Block-1			Block-2				Block-1			Block-2				Block-1			Block-2		
0	0	0	0	0	1		0	0	0	0	0	1		0	0	0	0	0	1
0	1	1	0	1	0		0	1	1	0	1	0		0	1	1	0	1	0
1	0	0	1	0	1		1	0	1	1	0	0		1	0	1	1	0	0
1	1	1	1	1	0		1	1	0	1	1	1		1	1	0	1	1	1
2	0	1	2	0	0		2	0	1	2	0	0		2	0	0	2	0	1
2	1	0	2	1	1		2	1	0	2	1	1		2	1	1	2	1	0
3	0	1	3	0	0		3	0	0	3	0	1		3	0	1	3	0	0
3	1	0	3	1	1		3	1	1	3	1	0		3	1	0	3	1	1

Plan 5. Balanced group of sets for 4 x *3x2* factorial, blocks of *12* units

AC, ABC confounded

Replication I							Replication II							Replication III					
Block-1			Block-2				Block-1			Block-2				Block-1			Block-2		
0	0	0	0	0	1		0	0	1	0	0	0		0	0	1	0	0	0
0	1	1	0	1	0		0	1	0	0	1	1		0	1	1	0	1	0
0	2	1	0	2	0		0	2	1	0	2	0		0	2	0	0	2	1
1	0	0	1	0	1		1	0	1	1	0	0		1	0	1	1	0	0
1	1	1	1	1	0		1	1	0	1	1	1		1	1	1	1	1	0
1	2	1	1	2	0		1	2	1	1	2	0		1	2	0	1	2	1
2	0	1	2	0	0		2	0	0	2	0	1		2	0	0	2	0	1
2	1	0	2	1	1		2	1	1	2	1	0		2	1	0	2	1	1
2	2	0	2	2	1		2	2	0	2	2	1		2	2	1	2	2	0
3	0	1	3	0	0		3	0	0	3	0	1		3	0	0	3	0	1
3	1	0	3	1	1		3	1	1	3	1	0		3	1	0	3	1	1
3	2	0	3	2	1		3	2	0	3	2	1		3	2	1	3	2	0

A^2C, A^2BC confounded

Replication IV			Replication V			Replication VI	
Block-1	Block-2		Block-1	Block-2		Block-1	Block-2

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

A^3C, A^3BC confounded

Replication VII							Replication VIII							Replication IX					
Block-1			Block-2				Block-1			Block-2				Block-1			Block-2		
0	0	0	0	0	1		0	0	1	0	0	0		0	0	1	0	0	0
0	1	1	0	1	0		0	1	0	0	1	1		0	1	1	0	1	0
0	2	1	0	2	0		0	2	1	0	2	0		0	2	0	0	2	1
1	0	1	1	0	0		1	0	0	1	0	1		1	0	0	1	0	1
1	1	0	1	1	1		1	1	1	1	1	0		1	1	0	1	1	1
1	2	0	1	2	1		1	2	0	1	2	1		1	2	1	1	2	0
2	0	0	2	0	1		2	0	1	2	0	0		2	0	1	2	0	0
2	1	1	2	1	0		2	1	0	2	1	1		2	1	1	2	1	0
2	2	1	2	2	0		2	2	1	2	2	0		2	2	0	2	2	1
3	0	1	3	0	0		3	0	0	3	0	1		3	0	0	3	0	1
3	1	0	3	1	1		3	1	1	3	1	0		3	1	0	3	1	1
3	2	0	3	2	1		3	2	0	3	2	1		3	2	1	3	2	0

Plan 6. Balanced group of sets for $3x2^3$ factorial, blocks of *12* units

ABC, ABCD confounded

Replication I										Replication II									
Block-1					Block-1					Block-1					Block-1				
0	0	0	0		0	0	0	1		0	0	0	0		0	0	0	1	
0	0	1	1		0	0	1	0		0	0	1	1		0	0	1	0	
0	1	0	1		0	1	0	0		0	1	0	1		0	1	0	0	
0	1	1	0		0	1	1	1		0	1	1	0		0	1	1	1	
1	0	0	1		1	0	0	0		1	0	0	1		1	0	0	0	
1	0	1	0		1	0	1	1		1	0	1	0		1	0	1	1	
1	1	0	0		1	1	0	1		1	1	0	0		1	1	0	1	
1	1	1	1		1	1	1	0		1	1	1	1		1	1	1	0	
2	0	0	1		2	0	0	0		2	0	0	0		2	0	0	1	
2	0	1	0		2	0	1	1		2	0	1	1		2	0	1	0	
2	1	0	0		2	1	0	1		2	1	0	1		2	1	0	0	
2	1	1	1		2	1	1	0		2	1	1	0		2	1	1	1	

Replication III								
Block-1					Block-2			
0	0	0	0		0	0	0	1

0	0	1	1		0	0	1	0
0	1	0	1		0	1	0	0
0	1	1	0		0	1	1	1
1	0	0	0		1	0	0	1
1	0	1	1		1	0	1	0
1	1	0	1		1	1	0	0
1	1	1	0		1	1	1	1
2	0	0	1		2	0	0	0
2	0	1	0		2	0	1	1
2	1	0	0		2	1	0	1
2	1	1	1		2	1	1	0

RESPONSE SURFACE DESIGNS

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1. Introduction

The subject of Design of Experiments deals with the statistical methodology needed for making inferences about the treatment effects on the basis of responses (univariate or multivariate) collected through the planned experiments. To deal with the evolution and analysis of methods for probing into mechanism of a system of variables, the experiments involving several factors simultaneously are being conducted in agricultural, horticultural and allied sciences. Data from experiments with levels or level combinations of one or more factors as treatments are normally investigated to compare level effects of the factors and also their interactions. Though such investigations are useful to have objective assessment of the effects of levels actually tried in the experiment, this seems to have inadequate, especially when the factors are quantitative in nature. The above analysis cannot give any information regarding the possible effects of the intervening levels of the factors or their combinations, *i.e.*, one is not able to interpolate the responses at the treatment combinations not tried in the experiment. In such cases, it is more realistic and informative to carry out investigations with the twin purposes:

a) To determine and to quantify the relationship between the response and the settings of a group of experimental factors.
b) To find the settings of the experimental factors that produces the best value or the best set of values of the response(s).

If all the factors are quantitative in nature, it is natural to think the response as a function of the factor levels and data from quantitative factorial experiments can be used to fit the response surfaces over the region of interest. Response surfaces besides inferring about the twin purposes can provide information about the rate of change of a response variable. They can also indicate the interactions between the quantitative treatment factors. The special class of designed experiments for fitting response surfaces is called response surface designs. A good response surface design should possess the properties *viz.*, detectability of lack of fit, the ability to sequentially build up designs of increasing order and the use of a relatively modest, if not minimum, number of design points. Before formulating the problem mathematically, we shall give examples of some experimental situations, where response surface methodology can be usefully employed.

Example 1: The over-use of nitrogen (N) relative to Phosphorus (P) and Potassium (K) concerns both the agronomic and environmental perspective. Phosphatic and Potassic fertilizers have been in short supply and farmers have been more steadily adopting the use of nitrogenous fertilizers because of the impressive virtual response. There is evidence that soil P and K levels are declining. The technique of obtaining individual optimum doses for the N, P and K through separate response curves may also be responsible for unbalanced fertilizer use. Hence, determining the optimum and balanced dose of N, P and K for different crops has been an important issue. This optimum and balanced dose should be recommended to farmers in terms of doses from the different sources and not in terms of the values of N, P and K alone, as the optimum combination may vary from source to source.

However, in actual practice the values of N, P and K are given in terms of kg/ha rather than the combined doses alongwith the source of the fertilizers.

Example 2: For value addition to the agriculture produce, food-processing experiments are being conducted. In these experiments, the major objective of the experimenter is to obtain the optimum combination of levels of several factors that are required for the product. To be specific, suppose that an experiment related to osmotic dehydration of the banana slices is to be conducted to obtain the optimum combination of levels of concentration of sugar solution, solution to sample ratio and temperature of osmosis. The levels of the various factors are the following

	Factors	**Levels**
1.	Concentration of sugar solution	40%, 50%, 60%, 70% and 80%
2.	Solution to sample ratio	1:1, 3:1, 5:1, 7:1and 9:1
3.	Temperature of osmosis	25^0C, 35^0C, 45^0C, 55^0C and 65^0C

In this situation, response surface designs for 3 factors each at five equispaced levels can be used.

Example 3: Yardsticks (a measure of the average increase in production per unit input of a given improvement measure) of many fertilizers, manures, irrigation, pesticides for various crops are being obtained and used by planners and administrators in the formulation of policies relating to manufacture/import/subsidy of fertilizers, pesticides, development of irrigation projects etc.

The yardsticks have been obtained from the various factorial experiments. However, these will be more reliable and satisfy more statistical properties, if response surface designs for slope estimation are used.

In general response surface methodology is useful for all the factorial experiments in agricultural experimental programme that are under taken so as to determine the level at which each of these factors must be set in order to optimize the response in some sense and factors are quantitative in nature. To achieve this we postulate that the response is a function of input variables, *i.e.*

$$y_u = \varphi(x_{1u}, x_{2u}, ..., x_{vu}) + e_u \quad (1.1)$$

where $u = 1,2,..., N$ represents the N observations and x_{iu} is the level of the i^{th} factor in the u^{th} observation. The function φ describes the form in which the response and the input variables are related and e_u is the experimental error associated with the u^{th} observation such that E (e_u) = 0 and Var(e_u) = σ^2. Knowledge of function φ gives a complete summary of the results of the experiment and also enables us to predict the response for values of the x_{iu} that are not included in the experiment. If the function φ is known then using methods of calculus, one may obtain the values of $x_1, x_2, ..., x_v$ which give the optimum (say, maximum) response. In practice the mathematical form of φ is not known; we, therefore, often approximate it, within the experimental region, by a polynomial of suitable degree in variables x_{iu}. The adequacy of the fitted polynomial is tested through the usual analysis of variance. Polynomials which adequately represent the true dose-response relationship are

called **Response Surfaces** and the designs that allow the fitting of response surfaces and provide a measure for testing their adequacy are called **response surface designs**. If the function φ in (1.1) is of degree one in $x_{iu}'s$ *i.e.*

$$y_u = \beta_0 + \beta_1 x_{1u} + \beta_2 x_{2u} + ... + \beta_v x_{vu} + e_u \tag{1.2}$$

we call it a first-order response surface in $x_1, x_2, ..., x_v$. If (1.1) takes the form

$$y_u = \beta_0 + \sum_{i=1}^{v} \beta_i x_{iu} + \sum_{i=1}^{v} \beta_{ii} x_{iu}^2 + \sum_{i=1}^{v-1} \sum_{i'=i+1}^{v} \beta_{ii'} x_{iu} x_{i'u} + e_u \tag{1.3}$$

We call it a second-order (quadratic) response surface. Henceforth, we shall concentrate on the second order response surface which is more useful in agricultural experiments.

2. The Quadratic Response Surface

The general form of a second-degree (quadratic) surface is

$$y_u = \beta_0 + \beta_1 x_{1u} + \beta_2 x_{2u} + ... + \beta_v x_{vu} + \beta_{11} x_{1u}^2 + \beta_{22} x_{2u}^2 + ... + \beta_{vv} x_{vu}^2 + \beta_{12} x_{1u} x_{2u} + \beta_{13} x_{1u} x_{3u} + ... + \beta_{v-1,v} x_{v-1,u} x_{vu} + e_u$$

Let us assume that $x_{iu}'s$ satisfy the following conditions:

(A) $\sum_{u=1}^{N} \left\{ \prod_{u=1}^{v} x_{iu}^{\alpha_i} \right\} = 0$, if any α_i is odd, for $\alpha_i = 0,1,2$ *or* 3 and $\sum \alpha_i \le 4$.

(B) $\sum_{u=1}^{N} x_{iu}^2 = \text{constant (for all } i) = N\lambda_2$ (say)

(C) $\sum_{u=1}^{N} x_{iu}^4 = \text{constant (for all } i) = CN\lambda_4$ (say) (2.1)

(D) $\sum_{u=1}^{N} x_{iu}^2 x_{i'u}^2 = \text{constant} = N\lambda_4$ (say), for all $i \neq i'$

We shall estimate the parameters $\beta_i's$ through the method of least squares. Let $b_0, b_i's, b_{ii}'s, b_{ii'}'s$ denote the best linear unbiased estimate of $\beta_0, \beta_i's, \beta_{ii}'s, \beta_{ii'}'s$ respectively. Under the above restrictions on $x_{iu}'s$, the normal equations are found to be:

$$\sum_{u=1}^{N} y_u = Nb_0 + N\lambda_2 \sum_{i=1}^{v} b_{ii}$$

$$\sum_{u=1}^{N} x_{iu} y_u = N\lambda_2 b_i$$

$$\sum_{u=1}^{N} x_{iu} x_{i'u} y_u = N\lambda_4 b_{ii'}$$

(2.2)

$$\sum_{u=1}^{N} x_{iu}^2 y_u = N\lambda_2 b_0 + CN\lambda_4 b_{ii} + N\lambda_4 \sum_{i' \neq i} b_{i'i'}$$

$$= N\lambda_2 b_0 + (C-1)N\lambda_4 b_{ii} + N\lambda_4 \sum_{i=1}^{v} b_{ii}$$

Solving the above normal equations, we obtain the estimates b_i's as

$$b_0 = \left[\lambda_4 (C+v-1) \sum_{u=1}^{N} y_u - \lambda_2 \sum_{i=1}^{v} \sum_{u=1}^{N} x_{iu}^2 y_u \right] \Big/ N\Delta$$

$$b_i = \sum_{u=1}^{N} x_{iu} y_u \Big/ N\lambda_2$$

$$b_{ii'} = \sum_{u=1}^{N} x_{iu} x_{i'u} y_u \Big/ N\lambda_4 \qquad (2.3)$$

$$b_{ii} = \left[\sum_{u=1}^{N} x_{iu}^2 y_u - \left\{ \left(\lambda_2^2 - \lambda_4\right) \sum_{i=1}^{v} \sum_{u=1}^{N} x_{iu}^2 y_u - (C-1)\lambda_2 \lambda_4 \sum_{u=1}^{N} y_u \right\} \Big/ \Delta \right] \Big/ \left[(C-1)N\lambda_4 \right]$$

where $\Delta = (C+v-1)\lambda_4 - v\lambda_2^2$.

The variances of and covariances between the estimated parameters are as follows:

$$V(b_0) = \lambda_4 (C+v-1)\sigma^2 / N\Delta$$

$$V(b_i) = \sigma^2 / N\lambda_2$$

$$V(b_{ii'}) = \sigma^2 / N\lambda_4$$

$$V(b_{ii}) = \sigma^2 \left[1 + \left(\lambda_2^2 - \lambda_4\right) \right] / \left[(C-1)N\lambda_4 \right]$$

$$Cov(b_0, b_{ii}) = -\lambda_2 \sigma^2 / N\Delta$$

$$Cov(b_{ii}, b_{i'i'}) = \left(\lambda_2^2 - \lambda_4\right) \sigma^2 / \left[(C-1)N\lambda_4 \Delta \right] \qquad (2.4)$$

Other covariances are zero. From the above expressions it is clear that a necessary condition for the design to exist is that $\Delta > 0$. Thus, a necessary condition for a Second Order Design to exist is that

(E) $\lambda_4 / \lambda_2^2 > v/(C+v-1)$ (2.5)

If $\hat{y}$ is the estimated response at any given experimental point $(x_{10}, x_{20}, ..., x_{v0})$, then the variance of $\hat{y}$ is given by

$$V(\hat{y}) = V(b_0) + V(b_i) \left(\sum_{i=1}^{v} x_{i0}^2 \right) + V(b_{ii}) \left(\sum_{i=1}^{v} x_{i0}^4 \right) + V(b_{ii'}) \left(\sum_{i=1}^{v-1} \sum_{i'=i+1}^{v} x_{i0}^2 x_{i'0}^2 \right)$$

$$+ 2\,Cov(b_0, b_{ii}) \left(\sum_{i=1}^{v} x_{i0}^2 \right) + 2\,Cov(b_{ii}, b_{i'i'}) \left(\sum_{i=1}^{v-1} \sum_{i'=1+1}^{v} x_{i0}^2 x_{i'0}^2 \right) \qquad (2.6)$$

If $\sum_{i=1}^{v} x_{i0}^2 = d^2$, where d is the distance of the point $(x_{10}, x_{20},..., x_{v0})$ from the origin, then we may write

$$V(\hat{y}) = V(b_0) + d^2[V(b_i) + 2Cov(b_0, b_{ii})] + d^4 V(b_{ii}) + \sum_{i=1}^{v-1} \sum_{i'=i+1}^{v} x_{i0}^2 x_{i'0}^2 [V(b_{ii'}) + 2Cov(b_{ii}, b_{i'i'}) - 2V(b_{ii})]$$

(2.7)

From the above expression, it is clear that if the coefficient of $\sum_{i=1}^{v-1} \sum_{i'=i+1}^{v} x_{i0}^2 x_{i'0}^2$ is made equal to zero, the variance of the estimated response at $(x_{10}, x_{20},..., x_{v0})$ will be a function of d, the distance of the point $(x_{10}, x_{20},..., x_{v0})$ from the origin. Now, the coefficient of $\sum_{i=1}^{v-1} \sum_{i'=i+1}^{v} x_{i0}^2 x_{i'0}^2$ is

$$V(b_{ii'}) + 2\,Cov(b_{ii}, b_{i'i'}) - 2V(b_{ii})$$

$$= \frac{\sigma^2}{N\lambda_4}\left[1 + \frac{2(\lambda_2^2 - \lambda_4)}{\Delta(C-1)} - \frac{2}{(C-1)}\left\{1 + \frac{\lambda_2^2 - \lambda_4}{\Delta}\right\}\right] \qquad (2.8)$$

$$= \frac{\sigma^2}{N\lambda_4}\left[1 - \frac{2}{(C-1)}\right]$$

Obviously, this is zero, if and only if $C = 3$. Thus, when $C = 3$, the variance of the estimated response at a given point, the response being estimated through a design satisfying (A), (B), (C), (D), (E) becomes a function of the distance of that point from the origin. Such designs are called as Second Order Rotatable Designs (SORD). We may now formally define a SORD:

Let us consider N treatment combinations (points) $\{x_{iu}\}$, $i = 1,2,..., v, u = 1,2,..., N$ to form a design in v factors, through which a Second-degree surface can be fitted. This design is said to be a SORD if the variance of the estimated response at any given point is a function of the distance of that point from the origin. The necessary and sufficient conditions for a set of points $\{x_{iu}\}$, $i = 1,2,..., v, u = 1,2,..., N$ to form a SORD are

(A') $\sum_{u=1}^{N}\left\{\prod_{u=1}^{v} x_{iu}^{\alpha_i}\right\} = 0$, if any α_i is odd, for $\alpha_i = 0,1,2$ *or* 3 and $\sum \alpha_i \le 4$.

(B') $\sum_{u=1}^{N} x_{iu}^2 = N\lambda_2$

(C') $\sum_{u} x_{iu}^4 =$ constant $= 3N\lambda_4$ $\qquad i = 1,2,..., v$

(D') $\sum_u x_{iu}^2 x_{i'u}^2 = N\lambda_4$; $i \neq i'$

(E') $\lambda_4 \big/ \lambda_2^2 > v/(v+2)$

(2.9)

The conditions (A'), (B') and (D') are same as conditions (A), (B) and (D) in (2.1).

We now prove the following.

Lemma: If a set of points $\{x_{iu},\ i = 1,2,...,v, u = 1,2,...,N\}$, satisfying (A'), (B'), (C') and (D') are such that every point is equidistant from the origin, then

$$\lambda_4 \big/ \lambda_2^2 = v/(v+2) \tag{2.10}$$

Proof: Let d be the distance of any point from the origin. Then, since all the points are equidistant from the origin, we have

$$d^2 = \frac{1}{N}\sum_{u=1}^{N}\left(\sum_{i=1}^{v} x_{iu}^2\right) = v\lambda_2$$

$$d^4 = \frac{1}{N}\sum_{u=1}^{N}\left(\sum_{i=1}^{v} x_{iu}^2\right)^2$$

and
$$= \frac{1}{N}\sum_{u=1}^{N}\left[\sum_{i=1}^{v} x_{iu}^4 + 2\sum_{i=1}^{v}\sum_{i'=i+1}^{v} x_{iu}^2 x_{i'u}^2\right]$$

$$= 3v\lambda_4 + v(v-1)\lambda_4$$

Thus, $v^2\lambda_2^2 = 3v\lambda_4 + v(v-1)\lambda_4$

or, $\lambda_4(v+2) - v\lambda_2^2 = 0$

An arrangement of points satisfying (A'), (B'), (C') and (D') but not (E') is called a Second Order Rotatable Arrangement (SORA). A SORA can always be converted to an SORD by adding at least one central point.

A near stationary region is defined as a region where the surface slopes along the v variable axes are small compared to the estimate of experimental error. The stationary point of a near stationary region is the point at which the slope of the response surface is zero when taken in all the directions. The coordinates of the stationary point $\mathbf{x_0} = (x_{10}, x_{20}, ..., x_{v0})'$ are obtained by differentiating the following estimated response equation with respect to each x_i and equating the derivatives to zero and solving the resulting equations

$$\hat{Y}(x) = b_0 + \sum_{i=1}^{v} b_i x_i + \sum_{i=1}^{v} b_{ii} x_i^2 + \sum_{i=1}^{v-1}\sum_{i'=i+1}^{v} b_{ii'} x_i x_{i'} \tag{2.11}$$

In matrix notation (2.11) can be written as

$$\hat{Y}(x) = b_0 + \mathbf{x}'\mathbf{b} + \mathbf{x}'\mathbf{B}\mathbf{x} \quad (2.12)$$

where $\mathbf{x} = (x_1, x_2, ..., x_v)'$, $\mathbf{b} = (b_1, b_2, ..., b_v)'$

and

$$\mathbf{B} = \begin{bmatrix} b_{11} & b_{12}/2 & \dots & b_{1v}/2 \\ b_{12}/2 & b_{22} & \dots & b_{2v}/2 \\ \dots & \dots & \dots & \dots \\ b_{1v}/2 & b_{2v}/2 & \dots & b_{vv} \end{bmatrix}.$$

From equation (2.12)

$$\frac{\partial \hat{Y}(x)}{\partial x} = \mathbf{b} + 2\mathbf{B}\mathbf{x} \quad (2.13)$$

The stationary point $\mathbf{x_0}$ is obtained by equating (2.13) to zero and solving for $\mathbf{x}$, *i.e.*

$$\mathbf{x_0} = -\frac{1}{2}\mathbf{B}^{-1}\mathbf{b} \quad (2.14)$$

To find the nature of the surface at the stationary point we examine the second derivative of $\hat{Y}(x)$. From (2.13)

$$\frac{\partial^2 \hat{Y}(x)}{\partial x^2} = 2\mathbf{B} \qquad \text{(since } \mathbf{B} \text{ is symmetric).}$$

The stationary point is a maximum, minimum or a saddle point according as **B** is negative definite, positive definite or indefinite matrix. If $\lambda_1, \lambda_2, ..., \lambda_v$ represent the *v* eigenvalues of **B**. Then it is easy to see that if $\lambda_1, \lambda_2, ..., \lambda_v$ are

(i) All negative, then at $\mathbf{x}_0$ the surface is a maximum

(ii) All positive, then at $\mathbf{x}_0$ the surface is a minimum

(iii) of mixed signs, i.e. some are positive and others are negative, then x_0 is a saddle point of the fitted surface.

Furthermore, if λ_i is zero (or very close to zero), then the response does not change in value in the direction of the axis associated with x_i variable. The magnitude of λ_i indicates how quickly the response changes in the direction of axis associated with x_i variable.

The conditions in (2.1) and (2.9) help in fitting of the response surfaces and define some statistical properties of the design like rotatability. However, these conditions need not necessary be satisfied before fitting a response surface. This can be achieved by using the software packages like the Statistical Analysis System (SAS). PROC RSREG fits a second order response surface design and locates the coordinates of the stationary point, predict the response at the stationary point and give the eigenvalues $\lambda_1, \lambda_2, ..., \lambda_v$ and the corresponding

eigen vectors. It also helps in determining whether the stationery point is a point of maxima, minima or is a saddle point. The lack of fit of a second order response surface can also be tested using LACKFIT option under model statement in PROC RSREG. The lack of fit is tested using the statistic

$$F = \frac{SS_{LOF}/(N'-p)}{SS_{PE}/(N-N')} \tag{2.15}$$

where N is the total number of observations, N' is the number of distinct treatments and p is the number of terms included in the model. SS_{PE} (sum of squares due to pure error) has been calculated in the following manner: denote the l^{th} observation at the u^{th} design point by y_{lu}, where $l = 1, \ldots, r_u (\geq 1), u = 1, \ldots, N'$. Define $\bar{y}_u$ to be average of r_u observations at the u^{th} design point. Then, the sum of squares for pure error is

$$SS_{PE} = \sum_{u=1}^{N'} \sum_{l=1}^{r_u} (y_{lu} - \bar{y}_u)^2 \tag{2.16}$$

Then sum of squares due to lack of fit (SS_{LOF}) = sum of squares due to error - SS_{PE}

The analysis of variance table for a second order response surface design is given below.

Table 1. Analysis of variance for second order response surface

Source	d.f.	S.S.
Due to regression coefficients	$2v + \binom{v}{2}$	$\hat{b}_0 \sum_{u=1}^{N} y_u + \sum_i \hat{b}_i \left(\sum_{u=1}^{N} x_{iu} y_u \right) + \sum_i \hat{b}_{ii} \left(\sum_{u=1}^{N} x_{iu}^2 y_u \right) + \sum_{i \neq i'} \sum \hat{b}_{ii'} \left(\sum_{u=1}^{N} x_{iu} x_{i'u} y_u \right) - CF$
Error	$N - 2v - \binom{v}{2} - 1$	*By subtraction = SSE*
Total	$N - 1$	$\sum_{u=1}^{N} y_u^2 - CF$

In the above table CF = correction factor = $\frac{(Grand\ Total)^2}{N}$. For testing the lack of fit the sum of squares is obtained using (2.16) and then sum of squares is obtained by subtracting the sum of squares due to pure error from sum of squares due to error. The sum of squares due to lack of fit and sum of squares due to pure error are based on $N' - 2v - \binom{v}{2} - 1$ and $N - N'$ degrees of freedom respectively.

It is suggested that in the experiments conducted to find a optimum combination of levels of several quantitative input factors, at least one level of each of the factors should be higher than the expected optimum. It is also suggested that the optimum combination should be determined from response surface fitting rather than response curve fitting, if the experiment involves two or more than two factors.

3. Construction of Second Order Rotatable designs

A second order response surface design is at least resolution V fractional factorial design. Here

3.1 Central Composite Rotatable Designs

Let there be *v* factors in the design. A class of SORD for *v* factors can be constructed in the following manner. Construct a factorial *v*-factors with levels $\pm\alpha$ containing 2^p combinations, where 2^p is the smallest fraction of 2^v without confounding any interaction of third order or less. Next, another $2v$ points of the following type are considered: $(\pm\beta\ 0\ 0\ \ldots\ 0), (0\ \pm\beta\ 0\ \ldots\ 0), (0\ 0\ \ldots\ \pm\beta)$. These $N = 2^p + 2v$ points, give rise to a SORD in *v* factors with levels $\pm\alpha, \pm\beta, 0$. We have for this design,

$$\sum_{u=1}^{N} x_{iu}^2 = 2^p\alpha^2 + 2\beta^2$$

$$\sum_{u=1}^{N} x_{iu}^4 = 2^p\alpha^4 + 2\beta^4$$

$$\sum_{u=1}^{N} x_{i0}^2 x_{i'0}^2 = 2^p\alpha^4.$$

On applying the condition of rotatability, we have

$$3.2^p\alpha^4 = 2^p\alpha^4 + 2\beta^4$$

$$\Rightarrow \beta^4 = \alpha^4 2^p$$

$$or\ \beta^2/\alpha^2 = 2^{p/2}.$$

This equation gives a relationship between β and α. For determining α and β uniquely, we either fix $\alpha = 1$ or $\lambda_2 = 1$. For $\alpha = 1, \Rightarrow \beta^2 = 2^{p/2}$.

Example. Let $v = 4$. Then the points of the SORD are

$-\alpha$	$-\alpha$	$-\alpha$	$-\alpha$
$-\alpha$	$-\alpha$	$-\alpha$	α
$-\alpha$	$-\alpha$	α	$-\alpha$
$-\alpha$	$-\alpha$	α	α
$-\alpha$	α	$-\alpha$	$-\alpha$
$-\alpha$	α	$-\alpha$	α
$-\alpha$	α	α	$-\alpha$
$-\alpha$	α	α	α
α	$-\alpha$	$-\alpha$	$-\alpha$
α	$-\alpha$	$-\alpha$	α
α	$-\alpha$	α	$-\alpha$
α	$-\alpha$	α	α
α	α	$-\alpha$	$-\alpha$
α	α	$-\alpha$	α
α	α	α	$-\alpha$
α	α	α	α

$$
\begin{array}{cccc}
\beta & 0 & 0 & 0 \\
-\beta & 0 & 0 & 0 \\
0 & \beta & 0 & 0 \\
0 & -\beta & 0 & 0 \\
0 & 0 & \beta & 0 \\
0 & 0 & -\beta & 0 \\
0 & 0 & 0 & \beta \\
0 & 0 & 0 & -\beta \\
0 & 0 & 0 & 0
\end{array}
$$

There are *25* points – a central point has been added because, all the non-central points are equidistant from the origin, as $\beta = 2\alpha$, here.

3.2 Construction of SORD using BIB Designs

If there exists a BIB design D with parameters $v^*, b^*, r^*, k^*, \lambda^*$ **such that** $r^* = 3\lambda^*$**, then a SORD with each factor at *3* levels can be constructed.**

Let $\mathbf{N}^*$ be the $v^* \times b^*$ incidence matrix of D. Then $\mathbf{N}^{*\prime}$ is a matrix of order $b^* \times v^*$, every row of which contains exactly k^* unities and every column contains exactly r^* unities, rest positions being filled up by zeros. In $\mathbf{N}^{*\prime}$, replace the unity by α. Then, we get b^* combinations involving α and zero. Next, each of these combinations are 'multiplied' with those of a 2^{k^*} factorial with levels ± 1 where, the term 'multiplication' means the multiplication of the corresponding entries in the two combinations, zero entries remaining unaltered. Thus, if $(\alpha \ \alpha \ 0)$ is multiplied by $(-1 \ -1)$ we get $(-\alpha \ \ -\alpha \ \ 0)$. The procedure of multiplication gives rise to $b^* 2^{k^*}$ points each of v^*-dimension. These points evidently satisfy all the conditions (A'), (B'), (C') and (D'); however, since each point in the arrangement is at the same distance from the origin, we have to take at least one central point to get a SORD in $v = v^*$ factors. The levels of the factors are $\pm\alpha, 0$. The value of α can be determined by fixing $\lambda_2 = 1$.

SORD's can be constructed using BIB designs, even when $r^* \neq 3\lambda^*$. In the case, where $r^* < 3\lambda^*$ the set of $b^* 2^{k^*}$ points obtained using $\mathbf{N}^{*\prime}$ is to be augmented with further $2v^*$ points of the type

$$(\pm\beta \ 0 \ 0 \ \ldots \ 0), (0 \ \pm\beta \ 0 \ \ldots \ 0), (0 \ 0 \ \ldots \pm\beta)$$

For the *N* points ($N = b^* 2^{k^*} + 2v^*$), we have

$$\sum_u x_{iu}^4 = r^* 2^{k^*} \alpha^4 + 2\beta^4$$

$$\sum_u x_{iu}^2 x_{i'u}^2 = \lambda^* 2^{k^*} \alpha^4.$$

Thus $2\beta^4 + r^* 2^{k^*} \alpha^4 = 3\lambda^* 2^{k^*} \alpha^4$

or, $\beta^2/\alpha^2 = (3\lambda^* - r^*)^{1/2}.2^{\frac{k^*-1}{2}}$

When $r^* > 3\lambda^*$, the points augmented are of type $(\pm\beta \pm\beta \ldots\ldots \pm\beta)$ and 2^p in number, where 2^p is the smallest fraction of 2^{v^*} factorial with levels $\pm\beta$, such that no interaction of order three or less is confounded. In this case,

$$\sum_u x_{iu}^4 = r^* 2^{k^*}\alpha^4 + 2^p\beta^4$$

$$\sum_u x_{iu}^2 x_{i'u}^2 = \lambda^* 2^{k^*}\alpha^4 + 2^p\beta^4.$$

Thus, $3\lambda^* 2^{k^*}\alpha^4 + 3.2^p\beta^4 = r^* 2^{k^*}\alpha^4 + 2^p\beta^4$

or, $2^{p+1}\beta^4 = (r^* - 3\lambda^*)2^{k^*}\alpha^4$,

which gives $\beta^2/\alpha^2 = (r^* - 3\lambda^*)^{1/2}.2^{(k^*-p-1)/2}$.

In both the cases, we get v^*-factor SORD with each factor at five levels

4. Practical Exercise

Exercise 1: Consider an experiment that was conducted to investigate the effects of three fertilizer ingredients on the yield of a crop under fields conditions using a second order rotatable design. The fertilizer ingredients and actual amount applied were nitrogen (N), from 0.89 to 2.83 kg/plot; phosphoric acid (P_2O_5) from 0.265 to 1.336 kg/plot; and potash (K_2O), from 0.27 to 1.89 kg/plot. The response of interest is the average yield in kg per plot. The levels of nitrogen, phosphoric acid and potash are coded, and the coded variables are defined as

X_1=(N-1.629)/0.716, X_2=(P_2O_5-0.796)/0.311, X_3=(K_2O -1.089)/0.482

The values 1.629, 0.796 and 1.089 kg/plot represent the centres of the values for nitrogen, phosphoric acid and potash, respectively. Five levels of each variable are used in the experimental design. The coded and measured levels for the variables are listed as

	Levels of x_I				
	-1.682	**-1.000**	**0.000**	**+1.000**	**+1.682**
N	0.425	0.913	1.629	2.345	2.833
P_2O_5	0.266	0.481	0.796	1.111	1.326
K_2O	0.278	0.607	1.089	1.571	1.899

Six center point replications were run in order to obtain an estimate of the experimental error variance. The complete second order model to be fitted to yield values is

$$Y = \beta_0 + \sum_{i=1}^{3}\beta_i x_i + \sum_{i=1}^{3}\beta_{ii}x_i^2 + \sum_{i=1}^{2}\sum_{i'=2}^{3}\beta_{ii'}x_i x_{i'} + e$$

The following table list the design settings of x_1, x_2 and x_3 and the observed values at 15 design points N, P_2O_5, K_2O and yield are in kg.

Table 2: Central Composite Rotatable Design Settings in the Coded Variables x_1, x_2 and x_3, the original variables N, P_2O_5, K_2O and the Average Yield of a Crop at Each Setting

x_1	x_2	x_3	N	P_2O_5	K_2O	Yield
-1	-1	-1	0.913	0.481	0.607	5.076
1	-1	-1	2.345	0.481	0.607	3.798
-1	1	-1	0.913	1.111	0.607	3.798
1	1	-1	2.345	1.111	0.607	3.469
-1	-1	1	0.913	0.481	1.571	4.023
1	-1	1	2.345	0.481	1.571	4.905
-1	1	1	0.913	1.111	1.571	5.287
1	1	1	2.345	1.111	1.571	4.963
-1.682	0	0	0.425	0.796	1.089	3.541
1.682	0	0	2.833	0.796	1.089	3.541
0	-1.682	0	1.629	0.266	1.089	5.436
0	1.682	0	1.629	1.326	1.089	4.977
0	0	-1.682	1.629	0.796	0.278	3.591
0	0	1.682	1.629	0.796	1.899	4.693
0	0	0	1.629	0.796	1.089	4.563
0	0	0	1.629	0.796	1.089	4.599
0	0	0	1.629	0.796	1.089	4.599
0	0	0	1.629	0.796	1.089	4.275
0	0	0	1.629	0.796	1.089	5.188
0	0	0	1.629	0.796	1.089	4.959

```
OPTIONS LINESIZE = 72;
DATA RP;
INPUT N P K YIELD;
CARDS;
….
….
….
;

PROC RSREG;
MODEL YIELD = N P K /LACKFIT NOCODE;
RUN;
```

Response Surface for Variable YIELD

Response Mean	4.464050
Root MSE	0.356424
R-Square	0.8440
Coef. of Variation	7.9843

Regression	d.f.	Sum of Squares	R-Square	F-Ratio	Prob > F
Linear	3	1.914067	0.2350	5.022	0.0223
Quadratic	3	3.293541	0.4044	8.642	0.0040

Crossproduct	3	1.666539	0.2046	4.373	0.0327
Total Regression	9	6.874147	0.8440	6.012	0.0049

Regression	**d.f.**	**Sum of Squares**	**R-Square**	**F-Ratio**	**Prob > F**
Lack of Fit	5	0.745407	0.149081	1.420	0.3549
Pure Error	5	0.524973	0.104995		
Total Error	10	1.270380	0.127038		

Parameter	**d.f**	**Estimate**	**Std Error**	**T-ratio**	**Prob > |T|**
INTERCEPT	1	6.084180	1.543975	3.941	0.0028
N	1	1.558870	0.854546	1.824	0.0981
P	1	-6.009301	2.001253	-3.003	0.0133
K	1	-0.897830	1.266909	-0.709	0.4947
N*N	1	-0.738716	0.183184	-4.033	0.0024
P*N	1	-0.142436	0.558725	-0.255	0.8039
P*P	1	2.116594	0.945550	2.238	0.0491
K*N	1	0.784166	0.365142	2.148	0.0573
K*P	1	2.411414	0.829973	2.905	0.0157
K*K	1	-0.714584	0.404233	-1.768	0.1075

Factor	**d.f.**	**Sum of Squares**	**Mean Squares**	**F-Ratio**	**Prob > F**
N	4	2.740664	0.685166	5.393	0.0141
P	4	1.799019	0.449755	3.540	0.0477
K	4	3.807069	0.951767	7.492	0.0047

Canonical Analysis of Response Surface

Factor	**Critical Value**
N	1.758160
P	0.656278
K	1.443790

Predicted value at stationary point **4.834526 kg**

	Eigenvectors		
Eigenvalues	**N**	**P**	**K**
2.561918	0.021051	0.937448	0.347487
-0.504592	0.857206	-0.195800	0.476298
-1.394032	-0.514543	-0.287842	0.807708

Stationary point is a saddle point.

The eigenvalues obtained are λ_1, λ_2 and λ_3 as 2.561918, -0.504592, -1.394032. As λ_2 and λ_3 are negative, therefore, take $W_2 = W_3 = 0$. Let

M = {0.021051 0.857206 -0.514543,
0.937448 -0.195800 -0.287842,
0.34787 0.476298 0.807708};

denotes the matrix of eigenvectors. The estimated response at the stationary points be 4.834526 kg/plot. Let the desired response be Y_{des}=5.0 kg/plot. Therefore, let W_1, obtained from the equation is sqrt (difference/2.561918)=AX1, say. To obtain various different sets of many values of W_1, generate a random variable, u, which follows uniform distribution and multiply this value with $2u-1$ such that W_1 lies within the interval, (-AX1, AX1). Now to get a combination of $x_i's$ that produces the desired response obtain $\mathbf{x} = \mathbf{M} * \mathbf{W} + \mathbf{x_0}$.

```
PROC IML;
W=J(3,1,0);
Ydes=5.0;
W2=0;
W3=0;
Dif=Ydes - 4.834526;
Ax1=Sqrt(dif/2.561918);
u= uniform(0);
W1= ax1*(2*u-1); print w1;
w[1,] = w1;
w[2,] = 0;
w[3,] = 0;
m = {0.021051  0.857206     -0.514543,
      0.937448   -0.195800    -0.287842,
      0.34787      0.476298    0.807708};
xest = {1.758160, 0.656278, 1.443790};
x = m*W+xest;
print x;
run;
```

Combinations of N, P, K estimated to produce 5.0 kg/plot of Beans.

Y	N	P	K
5.0	1.760	0.730	1.471
	1.762	0.815	1.503
	1.754	0.460	1.371

One can select a practically feasible combination of N, P and K.

5. Response Surface Designs for Slope Estimation

The above discussion relates to the response surface designs for response optimization. In many practical situations, however, the experimenter is interested in estimation of the rate of change of response for given value of independent variable(s) rather than optimization of response. This problem is frequently encountered *e.g.*, in estimating rates of reaction in chemical experiments; rates of growth of biological populations; rates of changes in response of a human being or an animal to a drug dosage, rate of change of yield per unit of fertilizer dose. Efforts have been made in the literature for obtaining efficient designs for the estimation of differences in responses *i.e.*, for estimating the slope of a response surface.

Many researchers with different approaches have taken up the problem of designs for estimating the slope of a response surface. We confine ourselves to two main approaches, namely

- Slope Rotatability
- Minimax Designs

The designs possessing the property that the estimate of derivative is equal for all points equidistant from the origin are known as **slope rotatable designs**. For a second order response surface, the rate of change of response due to i^{th} independent variable is given by

$$\frac{\partial \hat{y}(x)}{\partial x_i} = b_i + 2b_{ii}x_i + \sum_{i' \neq i}^{v} b_{ii'}x_{i'}$$

For second order design satisfying (2.1) we have

$$Cov(b_i, b_{ii}) = Cov(b_i, b_{ij}) = Cov(b_{ii}, b_{ii'})$$

Thus variance of $\frac{\partial \hat{y}(x)}{\partial x_i}$ is given by

$$Var\left(\frac{\partial \hat{y}(x)}{\partial x_i}\right) = Var(b_i) + \rho^2 Var(b_{ii}) + x_i^2\left[4Var(b_{ii}) - Var(b_{ii'})\right]$$

Thus in order to obtain slope rotatable design, the design must satisfy

- Conditions of symmetry (2.1)
- $\frac{\lambda_4}{\lambda_2^2} > v/(c+v-1)$
- $4Var(b_{ii}) = Var(b_{ii'})$.

It is important to note here that no rotatable design can be slope rotatable.

A minimax design is one that minimizes the variance of the estimated slope maximized over all points in the design.

6. Web Resources on Response Surface designs

Response surface fitting can also be done using SAS and SPSS steps given on the link Response Surface Designs at Design Resources Server using the link http://www.iasri.res.in/design/Analysis%20of%20data/response_surface.html. Response surface fitting can also be performed from IP Authenticated Indian NARS Statistical Computing Portal (http://stat.iasri.res.in/sscnarsportal) using the link response surface designs.

Some Useful References

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DESIGNS FOR BIOASSAYS

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1. Introduction

Bioassay (commonly used for **biological assay**), or **biological standardization** is a type of scientific experiment. Bioassays are typically conducted to measure the effects of a substance on a living organism and are essential in the development of new drugs and in monitoring environmental pollutants. Both are procedures by which the potency (pharmacology) or the nature of a substance is estimated by studying its effects on living matter. We are generally interested in the relative potency of a new drug to a standard drug. Normally, two preparations of the stimulus-one of known strength (standard preparation) and another of unknown strength (test preparation), both with quantitative doses, are applied to a set of living organisms. The general objective of bioassay is to draw statistically valid conclusions on the relative potency of the test preparation with respect to the standard preparation. Bioassays are procedures that can determine the concentration of purity or biological activity of a substance such as vitamin, hormone, and plant growth factor. While measuring the effect on an organism, tissue cells, enzymes or the receptor is preparing to be compared to a standard preparation. Bioassays may be qualitative or quantitative. Qualitative bioassays are used for assessing the physical effects of a substance that may not be quantified, such as abnormal development or deformity. Quantitative bioassays involve estimation of the concentration or potency of a substance by measurement of the biological response that it produces. Quantitative bioassays are typically analyzed using the methods of biostatistics. Statistical methods have been effectively and gainfully employed in problems relating to biological assays or bioassays. Bioassay is a method for the quantitative estimation of the effects that result in a biological system after its exposure to a substance. This is done by comparing the activity of living organisms and/or their parts under standardized conditions versus the conditions under investigation. Bioassays allow for a quantitative measurement of the effect of a substance on a biological system. The biological material in which the effect is measured can range from sub cellular components and microorganisms to groups of animals.

Definition of bioassay: To sum up a typical bioassay involves (i) *stimulus*, (ii) *subject*, and (iii) *response*, the change produced on the subject due to application of stimulus (such as an analytical value like blood sugar content or bone ash percentage, occurrence or non-occurrence of a certain muscular contraction, recovery from symptoms of a dietary deficiency, or death, etc).

Normally, two preparations of the stimulus, one of known strength (*standard preparation*) and another of unknown strength (*test preparation*), both with quantitative doses, are applied to a set of living organisms. The general objective of the bioassays is to draw statistically valid conclusions on the *relative potency* of *test preparation* with respect to *standard* one. If d_s and d_t denote the doses of the standard and the test preparations respectively such that each of them produces a pre-assigned response in some living organism, then the ratio $\rho = d_s / d_t$ is called the *relative potency* of the test preparation. If

ρ is greater than unity, it shows that a smaller dose of the *test preparation* produces as much response as relatively larger dose of *standard preparation* and therefore the potency of the *test preparation* is greater than that of *standard preparation.* Similarly, when ρ less than unity, the potency of the standard preparation is greater than that of the *test preparation*. Naturally, such statistical procedures may depend on the nature of the stimulus and response as well as on other extraneous experimental (biological or therapeutically) considerations.

As is clear from the above discussion, the biological assay is an experiment in which the interest lies in comparing the potencies of the treatments on an agreed scale. Biological assays are therefore, different from traditional comparative experiments where the interest lies in comparing the magnitude of effects of treatments. The experimental technique may be same, but the difference in purpose affects the designing and the statistical analysis of the experimental data. Thus, an investigation into the effects of different samples of insulin on blood sugar of rabbits is not necessarily a biological assay; it becomes one if the interest lies not simply in the change in blood sugar levels, but in their use for the estimation of the samples on a scale of standard units of insulin. Another kind of bioassay is used to test the effects of compounds being considered for use in drugs or skin care products. A field trial of the responses of the potatoes to various phosphates fertilizers would not generally be regarded as an assay; but if the yields of potatoes are to be used in assessing the potency of a natural rock phosphate relative to a standard super phosphate, and perhaps even in estimating the availability of phosphorus in the rock phosphate, then the experiment is an assay.

2. Types of Bioassays

Bioassays can broadly be classified into *direct* and *indirect* assays. Finney (1978) characterized bioassays as either direct or indirect.

Direct Assays are those assays where dose needed to produce a pre-assigned/ specified response is directly measurable for both the preparations. In this case the response is certain while the dose is a non-negative random variable that defines the tolerance distribution. These assays are practical only when it is possible to administer the dose in such a manner that the minimal amount of dose to produce a specified response can be measured directly. In these assays, the potency of an unknown preparation is determined as the ratio of exposure dose of the unknown and a standard preparation where each elicits the same specified response (e. g. if 10µl of an unknown preparation produces the same biological response as 1µl of a standard preparation then the potency of the unknown is 1/10 = 0.1 of the standard). This methodology is mostly seen in the older pharmaceutical literature. Following example given by Finney (1978) makes the ideas clear.

Example 1: (Burn, Finney and Goodwin 1950; Hatcher and Brody 1910). This is a typical example of direct assay 'the cat method' for the assay of digitalis. The standard or test preparation is infused at a fixed rate, into the blood stream of a cat until the heart stops beating. The total time of infusion multiplied by rate is termed as dose. This is repeated on several cats for each preparation and the mean doses are compared i.e., if $\bar{x}_s$ and $\bar{x}_t$ denote average of tolerances for standard and test preparations respectively then an estimate of relative potency is given by $R = \bar{x}_s / \bar{x}_t$. Three groups of cats were infused with two tinctures of Strophantus using the procedure described above. The tinctures were having same effective ingredients. The doses were recorded as quantities per kg body weight of cat.

Table 1 shows the fatal doses or *tolerances* of three groups of cats for two tinctures of strophanthus. The doses were recorded as quantities per kg body weight of cat, and unfortunately the total doses are not available: the tolerance is thus assumed to vary in proportion to body weight, or at least to show an approximately proportion variation rather than independence of body weight. Provided that the cats have been assigned at random to the different preparations, either form of expression of dose gives a valid method of estimating potency, but neither necessarily makes the best possible use of information on body weight.

Table 1: Tolerances of cats for tinctures of strophanthus and ouabain

Preparation	**Strophanthus A (In 0.01 c.c per kg.)**	**Strophanthus B (In 0.01 c.c per kg.)**
Tolerances ...	1.55	2.42
	1.58	1.85
	1.71	2.00
	1.44	2.27
	1.24	1.70
	1.89	1.47
	2.34	2.20
	--	--
	--	--
Mean …	1.68	1.99

Suppose that Strophanthus tincture B is to be regarded as the standard preparation, and A is to be compared with it as a test preparation. From the means in Table 1, 0.0168 c.c. of A is estimated to produce the same results as 0.0199 c.c. of B, either being just sufficient, on an average, to kill a cat. Hence the relative potency is estimated to be

$$R = \hat{\rho} = \frac{0.0199}{0.0168} = 1.18;$$

Thus, 1 c.c. of tincture A is estimated to be equivalent to 1.18 c.c. of tincture B.

Having obtained the estimate of relative potency ρ as above, a natural question is that of its precision. We now work out the precision of estimate. Let $R = \bar{x}_B / \bar{x}_A$, then variance of R is given by

$$\mathrm{V}(R) = \frac{1}{\bar{x}_A^2}\left[\mathrm{V}(\bar{x}_B) + R^2\,\mathrm{V}(\bar{x}_A)\right]$$

Now $\sum (x_i - \bar{x})^2 = 0.7587$ for A and 0.6815 for B. It can be seen that both the sums of squares are based on 6 degrees of freedom and, therefore, estimate of common variance is given by

$s^2 = \dfrac{0.7587 + 0.6815}{12} = 0.1200$ and $\mathrm{V}(\bar{x}_A) = \mathrm{V}(\bar{x}_B) = \dfrac{s^2}{7}$ (since both $\bar{x}_A, \bar{x}_B$ are based on 7 observations) Here, $R = 1.18$ and therefore $\mathrm{V}(R) = \dfrac{s^2}{\bar{x}_A^2}\left[\dfrac{1}{7} + \dfrac{R^2}{7}\right] = 0.0145$.

Hence, $R = 1.18 \pm 0.120$ since $\sqrt{0.0145} = 0.120$.

An analysis in terms of log doses may be more satisfactory than one in the absolute units. In direct assays, the assumption of a normal distribution of log tolerances, if admissible, has many advantages. All variance estimates may be pooled in order to give the best possible estimate of the population variance. The relative potency is estimated as the antilogarithm of the difference of two means, instead of as a ratio of two means.

Indirect Assays: In most of the bioassays, response is not directly measurable and therefore indirect methods are used to estimate the dose corresponding to a given response via a dose-response relationship. These kinds of assays are known as indirect assays. In these assays the dose is administered at some prefixed (usually non-stochastic) levels, and at each level the response is observed for subjects included in the study. Thus, the dose is generally non-stochastic and the responses are stochastic in nature. The stochastic response provides information about the tolerance distribution of a particular preparation. If the response is a quantitative variable (magnitude of some property like survival time, weight, etc.), then we have quantitative assay. On the other hand if the response is quantal (i.e., all or nothing), we have quantal assay. Both these assays are commonly adopted in statistical practice. Within this framework, the nature of dose-response regression may call for suitable transformation on the dose variable (called the dosage or dose-metameter) and/or response variable called the response metameter. The objective of these transformations is to achieve a linear dose-response regression that may induce simplification in statistical modeling and analytical techniques. If z represents the dose in the original scale, then the two transformations that have been found useful in bioassays are (i) $x = log_e(z)$ and (ii) $x = z^{\lambda}$, where $\lambda>0$ is a known constant. The first of these gives rise to parallel line assays and the second to slope ratio assays. These assays generally fall in the category of quantitative indirect assays. Transformation of response variable is generally not needed in such bioassays.

3. Parallel Line Assays

In a bioassay, doses for both the standard and test preparations are considered as treatments, i.e. there are the two groups of treatments, one for standard preparation and another for test preparation. Often, within each group, the treatment effects are represented as a polynomial in the logarithm of the doses. Particularly, when the polynomial is of degree one and both the groups are sharing the same slope; the assay is known as *parallel line assay*. If the number of doses of both the preparations are same, then the parallel line assay is called *symmetric*, otherwise, *asymmetric*. In parallel line (PL) assay, there are three major contrast of interest are (i) *preparation contrasts* ψ_p (ii) *combined regression contrast* ψ_1, and (iii) *parallelism contrast* ψ_1'. The first two provides an estimate of relative potency while the third contrast is important for making validity tests i.e. to test whether the regression lines for the two preparations are parallel. Hence it is desired that all of these contrasts are to be estimated with full efficiency.

Consider an assay in which two stimuli A and B, each administered at $m(\geq 2)$ prefixed levels (doses) $d_1, d_2, ..., d_m$. Let $Y_{s_i}(Y_{t_i})$ be the response variable of standard (test) preparations. It is not necessary to have the same number of doses for both the preparation, but the modifications are straight forward and hence we assume this congruence. We first

assume that both Y_{s_i} and Y_{t_i} are continuous (and possibly non-negative) random variables. Suppose that there exist some dosage $x_i = \xi_i(d_i), \mathrm{i} = 1,2,...,m$ and response metameter $Y^* = g(X)$ for some strictly monotone $g(.)$, such that the two dosage-response regressions may be taken as linear, namely

$$Y_{t_i}^* = \alpha_t + \beta_t x_{t_i} + e_{t_i}$$
$$Y_{s_i}^* = \alpha_s + \beta_s x_{s_i} + e_{s_i} \quad (1)$$

where, for statistical inferential purposes, certain distributional assumptions are needed for the error components e_{t_i} and e_{s_i}, $i = 1, 2, \ldots, m$. For linearizing transformation, $x_i = \log(dose)$, $i = 1, 2, \ldots, m$, let $E(Y_{s_i}^*) = \alpha_s + \beta x_{s_i}$ (2)

denote the relation between the expected response and x_S where $x_S = \log(d_S)$ and d_S denotes the dose of the standard preparation. Denoting by d_t a dose equipotent to d_S, we have $\rho = d_S / d_t$, that is

$$\log \rho = \log d_s - \log d_t = x_s - x_t .$$

That is $x_S = \log \rho + x_t$. Substituting for x_S in the relation of the standard preparation (2), we get the relation for the test preparation as

$$E(Y_{s_i}^*) = \alpha_s + \beta(\log \rho + x_{t_i})$$

that is, $E(Y_{s_i}^*) = \alpha_t + \beta x_{t_i} = E(Y_{t_i}^*)$, (3)

where $\alpha_t = \alpha_s + \beta \log \rho$. Hence, the relationship for the test preparation is also linear like that of the standard preparation for the same transformation. An examination of the two equations for the two preparations shows that the lines have the same slope and are, therefore, parallel.

In this setup we then have $\beta_s = \beta_t = \beta$ (unknown), while, $\alpha_t = \alpha_s + \beta \log \rho$, where ρ is the relative potency of the test preparation with respect to the standard one. This leads to the basic estimating function

$$\log \rho = (\alpha_t - \alpha_s)/\beta .$$

(4)

So that if the natural parameters β, α_s and α_t are estimated from the acquired bioassay data set, statistical inferences on $\log \rho$ (and hence ρ) can be drawn in a standard fashion. If in an assay *m* doses are taken for each of the two preparations and $\bar{x}_s$ and $\bar{x}_t$ denote the averages of the dose metameters and $\bar{y}_s$ and $\bar{y}_t$ are the average responses for the preparations, then it is known that

$$\alpha_s = \bar{y}_s - \beta \bar{x}_s$$

and

$$\alpha_t = \bar{y}_t - \beta \bar{x}_t .$$

(5)

Substituting these values in $\log \rho = (\alpha_t - \alpha_s)/\beta$, we get an estimate R of ρ from

$$\log R = \bar{x}_s - \bar{x}_t - \{\bar{y}_s - \bar{y}_t\}/\beta .$$

(6)

From equations (2) and (3) it is seen that the two lines for the two preparations should be parallel when the dose metameter is log (dose). The assays corresponding to this transformation are, therefore, called parallel line assays. Thus, in a parallel-line assay, the two dose-response regression lines (1) are taken to be parallel and, further that the errors e_{t_i} and e_{s_i} have the same distribution (often taken as normal). For normally distributed errors, the whole set of observations pertains to a conventional linear model with a constraint on the two slopes, β_s and β_t, so that the classical maximum likelihood estimators and allied *likelihood ratio tests* can be incorporated for drawing statistical conclusion on the relative potency or the fundamental assumption of parallelism of the two regression lines. The estimator of log ρ involves the ratio of two normally distributed statistics, and, therefore, it may be biased; moreover, generally the classical *Fieller's theorem* (see Finney, 1978) is incorporated for constructing a confidence interval for log ρ (and hence, ρ). Because of this difference in setups (with that of the classical linear model), design aspects for such parallel-line assays need a more careful appraisal.

Gupta and Mukerjee (1996) gave a general introduction for the derivation of the contrasts for symmetric and asymmetric parallel line assay. Considering an indirect assay with quantitative responses, let s and t denote the typical doses of the standard and test preparations and, with $x = \log_e s$, $z = \log_e t$. Let the quantitative doses of the standard and test preparations included in the assay are m_1 and m_2 respectively, where $m_1, m_2 \geq 2$ and $s_1, \ldots, s_{m_1}$ and $t_1, \ldots, t_{m_2}$ are prespecified doses for two preparations. These doses should adequately cover the ranges of s and t. The $v = m_1 + m_2$ doses $s_1, \ldots, s_{m_1}$ and $t_1, \ldots, t_{m_2}$ represent the treatments in the present context. For $(1 \leq i \leq m_1)$, let τ_i be the effect of the dose s_i of the standard preparation and for $(1 \leq i \leq m_2)$, let τ_{m_1+1} be the effect of the dose t_i of the test preparation. Then, using the notation introduced above, the contrasts are given as

$$\psi_p = m_1^{-1} \sum_{i=1}^{m_1} \tau_i - m_2^{-1} \sum_{i=1}^{m_2} \tau_{m_1+i} \tag{7}$$

$$\psi 1 = \frac{6}{\log h}\left[\frac{1}{m_1(m_1^2-1)}\sum_{i=1}^{m_1}\left\{i - \frac{1}{2}(m_1+1)\right\}\tau i \; + \frac{1}{m_2(m_2^2-1)}\sum_{i=1}^{m_2}\left\{i - \frac{1}{2}(m_2+1)\right\}\tau_{m_i+i}\right] \tag{8}$$

$$\psi_1^1 = \frac{6}{\log h}\left[\frac{1}{m_1(m_1^2-1)}\sum_{i=1}^{m_1}\left\{i - \frac{1}{2}(m_1+1)\right\}\tau i - \frac{1}{m_2(m_2^2-1)}\sum_{i=1}^{m_2}\left\{i - \frac{1}{2}(m_2+1)\right\}\tau_{m_i+i}\right]$$

(9)

The above treatment contrasts have natural interpretation. ψ_p is the contrast between preparations while ψ_p and ψ_1^1 are combined regression and parallelism contrasts respectively. The two straight lines are parallel if and only if $\psi_1^1 = 0$ in which the common

slope is given by ψ_1. Put $m_1 = m_2 = m$ in (7), (8), and (9) and we get the three major contrasts of interest for symmetric parallel line assays.

4. Slope Ratio Assays

For linearizing transformation, $x_i = (\text{dose})^{\lambda} \quad i = 1, 2, \ldots, m$, let

$$E(Y_{s_i}^*) = \alpha_s + \beta_s x_{s_i} \tag{10}$$

denote the relation between the expected response and x_s, where $x_S = (d_S)^{\lambda}$ and d_S denotes the dose of the standard preparation. Denoting by d_t a dose equipotent to d_s, we have $\rho = d_s / d_t$, or $\rho^{\lambda} = (d_s / d_t)^{\lambda}$ that is $\rho^{\lambda} = \dfrac{x_s}{x_t}$.

(11)

That is, $x_S = x_t \rho^{\lambda}$. Substituting for x_S in the relation of the standard preparation (11), we get the relation for the test preparation as

$$E(Y_{s_i}^*) = \alpha_s + \beta_s x_t \rho^{\lambda}$$

$$\text{or, } E(Y_{s_i}^*) = \alpha_s + \beta_t x_{t_i} = E(Y_{t_i}^*), \tag{12}$$

where $\beta_t = \beta_S \rho^{\lambda}$, i.e., $\rho^{\lambda} = \beta_t / \beta_S$ and $\rho = \{\beta_t / \beta_s\}^{1/\lambda}$. (13)

Since the relative potency is estimated from the ratio of the slopes of the two preparations, the assays, corresponding to the transformation z^{λ} are called slope ratio assays.

The relative potency is typically a non-linear function of the two shapes β_t and β_s, and presumes knowledge of λ. In such a case the two error components may not have the same distribution even if they are normal. This results in heteroscedastic linear model (unless ρ =1), where the conventional linear estimators or allied tests may no longer possess validity and efficiency properties.

Since ρ^{λ} is a ratio of two slopes, its conventional estimator based on the usual estimators of the two slopes is of the ratio type. For such ratio-type estimators, again the well-known Fieller theorem is usually adopted to attach a confidence set to ρ or to test a suitable null hypothesis. Again, the design aspects for such slope ratio assays need careful study and Finney (1978) contains a detailed account of this study. Because of the common intercept, usually a $2m+1$ point design is advocated here. In slope ratio assay, it is assumed that the two regression lines intersect at the same point on the response axis, *i.e.*, the lines have the same intercept. Since the dose takes value zero at response axis, it is logical to include a *blank dose* in the assay for validity test. If there are m_1 (m_2) doses in standard (test) preparation then a slope ratio assay contains $m_1 + m_2 + 1$ doses.

Inclusion of blank dose in the assay raises a question "does the linearity of the relation holds up to zero doses?" It is therefore, necessary to test this relation and corresponding contrast is known as *blank contrast*. The next question is "Whether the two lines intersect on the

response axis?" For this the two lines are fitted individually ignoring the blank dose and then their intercepts on the response axis are compared. This provides another validity test. The corresponding contrast is called *intersection contrast*. There are therefore, two major contrasts of interest in slope ratio assays viz., *blank contrast* and *intersection contrast*.

For slope ratio assay with $(m_1 + m_2 + 1)$ doses, the *blank* and *intersection* contrasts are given by

Blank contrast: $L_B = (g, \boldsymbol{\alpha}_1', \boldsymbol{\alpha}_2')\boldsymbol{\tau}$,

Intersection Contrast: $L_I = (0, -\boldsymbol{\alpha}_1', \boldsymbol{\alpha}_2')\boldsymbol{\tau}$,

(14)

Where $\boldsymbol{\alpha}_i = (2-2m_i, 5-2m_i, ..., m_i - 1)', i = 1,2$, $g = \frac{1}{2}\sum_{i=1}^{2} h_i$ and $h_i = m_i(m_i - 1)$; i=1, 2.

5. Designs for Bio-assays

The main purpose of a bioassay is the estimation of the relative potency of test preparation and standard preparation. It is desired that when a block design is used for the assay, these contrasts of interest are estimated free from block effects and with full efficiency. If the number of homogeneous experimental units is same as the number of doses, then the experiment can be conducted using randomized complete block (RCB) design. All treatment contrasts are estimated free from block effects in a RCB design. For large number of doses, however, it may not generally be possible to get the same number of homogeneous experimental units as the number of doses. In such situations recourse is made for using incomplete block designs. In an incomplete block design, all treatment contrast cannot be estimated free from block effects. Therefore, the problem is to choose an incomplete block design that estimates the contrasts of interest free from block effects. Das and Kulkarni (1966) obtained incomplete block designs for symmetric parallel line assays that estimate *preparation* and *combined regression* contrasts free from block effects. Kyi Win and Dey (1980) proposed block designs both for symmetric as well as asymmetric parallel line assays and these designs estimate all the three contrasts free from block effects. Nigam and Boopathy (1985) utilized the simple partially efficiency balanced block designs for generating designs for parallel line assays and showed that these designs are capable of estimating the three contrasts of interest free from block effects. They have presented two series of block designs each for even and odd number of doses. Other notable contributions in this area are by Puri and Gupta (1989) and Das and Saha (1986). The studies on optimality aspects of block designs for parallel line assays were initiated by Mukerjee and Gupta (1995). They presented A-optimal/efficient designs for the estimation of the three contrasts of interest namely *preparation*, *combined regression* and *parallelism* in the context of symmetric parallel line assays. Mukerjee (1997), however, studied the optimality of block designs for parallel line assays relevant to D criterion. Chai, Das and Dey (2001) considered the problem of obtaining A-optimal block designs for the estimation of two major contrasts namely, *preparation* and *combined regression*, in the context of both symmetric and asymmetric assays.

Das and Kulkarni (1966) obtained block designs for symmetric slope ratio assays using similar method as they did for parallel line assays. Kulshrestha (1972) obtained block designs for slope ratio assays by augmenting each block of a block design for parallel line assays with a blank dose. Dey, Subramanian and Gupta (1999) proposed a general technique for the construction of block designs for symmetrical slope ratio assays that

permit the estimation of 'blank' and 'intersection' contrasts free from block effects. The work on optimality aspects of block designs for slope ratio assays was initiated by Das, Dey and Gupta (2000).

6. Analysis of Bio-assays

6.1 Analysis of parallel line assays

The analysis of parallel line assays consists of two parts, (i) computation of the analysis of variance, including sum of squares due to the various contrasts as defined above, providing validity tests and the error mean squares, (ii) estimation of the relative potency and its variance and limits. The sum of square for any contrast unaffected by block differences is calculated by

$$\left[\sum_{i=1}^{k} l_i S_i - \sum_{i=1}^{k} l_i T_i\right]^2 \Big/ r\left(2\sum_{i=1}^{k} l_i^2\right) \tag{15}$$

where $l_1, l_2, \ldots$ are coefficients of the contrast and r is the number of observations (or replicates) on which S_i or T_i is based.

Consider the model

$$y_{mj} = \mu + \beta_j + \delta_m + e_{mj}, \tag{16}$$

where y_{mj} is the response to dose m in the jth block, μ is the general mean and β_j the jth block effect, δ_m denotes s_m or t_m, and the error e_{mj} is assumed to be normally distributed with mean zero and variance σ^2. The s.s. due to the other contrasts can be calculated from the least squares estimates $s_1, s_2, \ldots, s_k$ and $t_1, t_2, \ldots, t_k$ of the effects of various doses of standard and test preparations using the above model. The error s.s. is found in general as the difference between the total s.s. and (unadjusted block s.s + adjusted dose s.s.). The adjusted totals can be obtained by

$$Q_i^S = S_i - \sum_{j(i)} \bar{y}_i \qquad \text{and} \qquad Q_i^T = T_i - \sum_{j(i)} \bar{y}_i \tag{17}$$

where $\bar{y}_i$ ($j = 1, 2, \ldots, b$) is the jth block average, and summation is over all blocks containing dose i of the preparations. All the required contrasts are functions of these Q's. Those unaffected by block differences are functions of the differences $Q^S_i - Q^T_i = (= S_i - T_i)$: sum of squares due to them are most conveniently obtained, as described earlier, directly from the totals and remaining contrasts are functions of $Q_i = Q^S_i - Q^T_i$. The s.s due to any affected contrast, $\sum_{i=1}^{k} l_i Q_i$, is then

$$\left[\sum_{i=1}^{k} l_i Q_i\right]^2 \Big/ rE\left(2\sum_{i=1}^{k} l_i^2\right), \tag{18}$$

where $E = (rk' - r + \lambda)/rk'$, k' being the block size of the starting design and λ the number of blocks in each of which any two given doses of the standard preparation occur together.

The variance of the estimate of an affected contrast is $(2\sum_i l_i^2 / rE)\sigma^2_{2k'}$, where $\sigma^2_{2k'}$, is the error variance in the incomplete blocks of size $2k'$. If a randomized (complete) block design is used, the corresponding variance is $(2\sum_i l_i^2 / r)\sigma^2_{2k}$ and if a BIB with $2k$ doses, blocks of

size k_1, and r replications is used, the variance is $(2\sum_i l_i^2 / rE')\sigma_{k1}^2$, where E' is the efficiency factor of this BIB design. If the experiment is conducted using a block design with $n = \sum_{i=1}^{v} r_i$ experimental units arranged in b blocks with same or different sizes, then an outline of analysis of variance table for a block design is

Table 2: Analysis of Variance in $m_1 + m_2$ point assays for validity tests: Block Design

Source of Variation	d.f.	SS	MS	F
Between Blocks	b-1	SSB		
Doses (adjusted)	v-1 = ($m_1 + m_2 - 1$)	SST		
Preparation(Ψ_p)	1	SSLP		
Combined regression (Ψ_1)	1	SSCR		
Parallelism (Ψ_1')	1	SSP	$s_b^2 = SSP/1$	s_b^2 / s^2
Deviation from regression	$v - 4$	SSDR: by subtraction	$s_d^2 = SSDR/(v-4)$	s_d^2 / s^2
Within doses(error)	$\sum_{i=1}^{v} r_i - v$	SSE	$s^2 = SSE/(\sum_{i=1}^{v} r_i - v)$	
Total	$\sum_{i=1}^{v} r_i - 1$	TSS		

For testing the linearity of regression, the mean squares for the deviations from regression is tested by the *F*-test using the within squares as error. For testing parallelism, the "parallelism" component is tested. If both these are not significant, then the relative potency can be estimated.

Example 2: (Finney, 1978): This is an example related to the assay of a test preparation of the testosterone propionate against a standard, using three doses of each. Each of the six doses was injected into five capons, and the birds responded by showing a growth of comb. The experiment was conducted using a completely randomized design. The response used for bioassay is the increase in the sum of the length and height of the comb. The data obtained is given below:

Doses→ Responses↓	Standard Preparation			Test Preparation		
	20µg	40µg	80µg	20µg	40µg	80µg
	20.2^0	20.2^1	20.2^2	20.2^0	20.2^1	20.2^2
1	6	12	19	6	12	16
2	6	11	14	6	11	18
3	5	12	14	6	12	19
4	6	10	15	7	12	16
5	7	7	14	4	10	15

For the 6-point assay, the contrasts are

Contrast	s_1 **20.2^0**	s_2 **20.2^1**	s_3 **20.2^2**	t_1 **20.2^0**	t_2 **20.2^1**	t_3 **20.2^2**	**Coefficient Divisor**
Preparation (Ψ_p)	1	1	1	-1	-1	-1	3
Combined Regression (Ψ_1)	-1	0	1	-1	0	1	$6/(\theta \log h) = 6/(3*(3^2-1)* \log_{10}2) = 1.204$
Parallelism contrast (Ψ_1')	-1	0	1	1	0	-1	$12/(\theta \log h) = 12/(3*(3^2-1) * \log_{10}2) = 0.602$

The ANOVA table for the above is

Source of variation	**d.f.**	**SS**	**MS**	**F**	**Prob>F**
Doses	5	519.067	103.813	43.86	0.0001
Preparation (ψ_p)	1	4.800	4.800	2.03	0.1673
Combined regression (ψ_1)	1	510.050	510.050	215.51	0.0001
Parallelism (ψ_1')	1	4.050	4.050	1.71	0.2032
Deviation from regression	2	0.167	0.835	<1	NS
Within doses (error)	24	56.800	2.367		
Total	29	575.867			

We can see that both assumptions of linearity of regression and parallelism hold. Therefore, one has to obtain the estimates of the preparation and combined regression contrasts. The estimated values of these contrasts are

Contrast	**Estimate**	**SE of estimate**	**T for H_0: Contrast =0**	**Prob >T**
Preparation	-0.800	0.562	-1.42	0.1673
Combined Regression	16.777	1.143	14.68	0.0001
Parallelism	-2.990	2.286	-1.31	0.2032

Therefore, the estimate of relative potency is

$$\hat{\rho} = \exp\left(-\Psi_p / \Psi_1\right) = \frac{20}{20}\exp(0.800/16.777) = 1.048\,.$$

Example 3:(Das and Giri, 1986): Here, the data obtained from a 6 point symmetrical parallel line assay collected on a vitamin D assay by Coward and Kassner (1941) has been used with some modifications. The design used is a randomized complete block designs with liters as blocks (12 litters). To ensure comparability of the estimate of relative potency all observation were used, but were fitted into an incomplete block design of the present series by omitting two observations from each of the original block (litters), as shown by blanks in Table 4, and forming 6 additional blocks (13-18) from the 24 observations

omitted, ignoring litter differences, but retaining the dose-observation relations. The design assumed is that for 6 treatments in blocks of size 4. The data and the assumed design are shown in the following table 2.4

Table 4: Data with the assumed design

Blocks	Standard Preparation			Test Preparation			Block Totals
	s_1 2.5	s_2 5	s_3 10	t_1 2.5	t_2 5	t_3 10	
1	2	8	-	-	9	7	26
2	6	-	9	3	-	8	26
3	-	6	12	4	6	-	28
4	9	11	-	-	14	13	47
5	10	-	17	8	-	10	45
6	-	7	5	-	6	9	27
7	4	10	-	11	13	-	38
8	11	-	9	3	-	15	38
9	-	9	14	5	8	-	36
10	4	7	-	10	10	-	31
11	12	-	9	15	-	15	51
12	-	8	11	-	7	8	34
13	4	4	-	-	5	9	22
14	7	-	8	3	-	9	27
15	-	15	10	6	8	-	39
16	2	4	-	-	6	6	18
17	4	-	13	5	-	12	34
18	-	10	13	4	18	-	45
Dose Total	75	99	130	69	112	127	612

The ANOVA table for this example is given by

Nature of Variation	d.f.	s.s	m.s.	F	Prob.>F
Between Blocks (unadjusted)	17	358.00	21.06	-	-
Doses (adjusted)	5	302.333	60.47	8.83	<0.0001
Between Blocks (adjusted)	17	382.333	22.49	3.28	0.0006
Preparation	1	0.222	0.22	0.03	0.8578
Regression	1	266.02	266.02	38.83	<0.0001
Parallelism	1	7.563	7.563	1.10	0.2986
Deviation from regression	2	28.428	14.214	2.075	0.1370
Error (by subtraction)	49	335.667	6.850		
Total	71	996.00			

We can see that both assumptions of linearity of regression and parallelism hold.

One has to obtain the estimates of the preparation and combined regression contrasts. The estimated values of these contrasts is

Contrast	Estimate	SE of Estimate	T for H_0: Contrast=0	Prob.>T
Preparation	-0.111	0.617	-0.18	0.8578
Combined regression	7.821	1.255	6.23	<0.0001
Parallelism	-3.045	2.898	-1.05	0.2986

Therefore, the estimate of relative potency is $\hat{\rho} = (c_1/c_2)\exp(-\Psi_p/\Psi_1) = \frac{2.5}{2.5}\exp(0.111/7.821)$ **.**

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Design Resources Server
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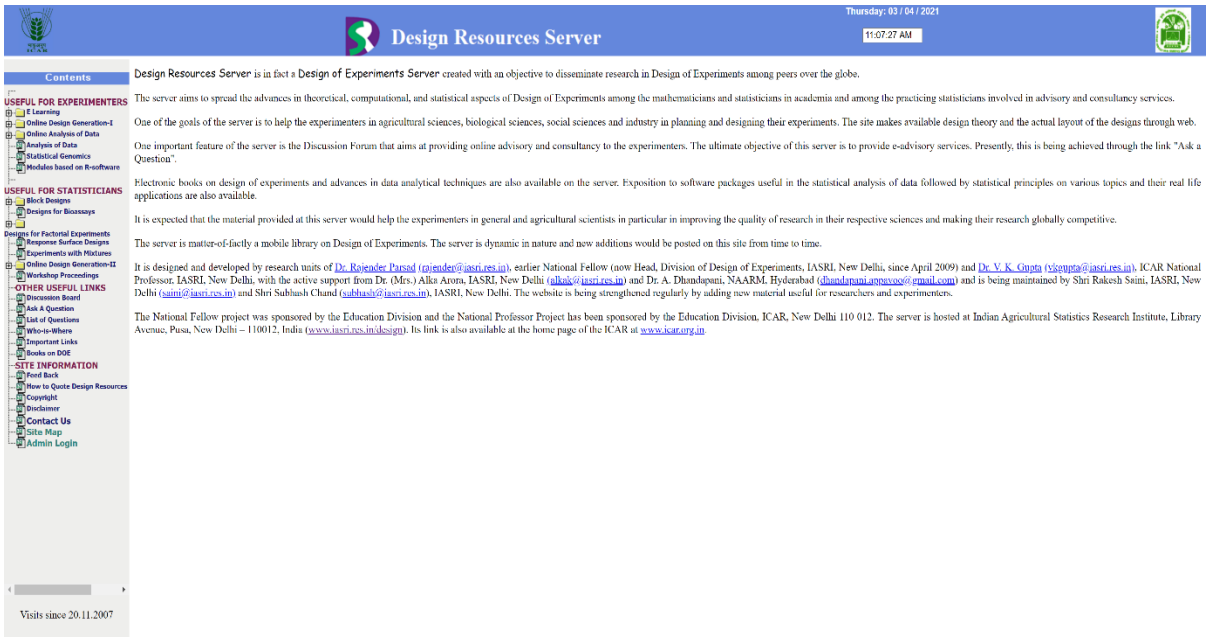
1. Introduction

Design Resources Server is developed to popularize and disseminate the research in Design of Experiments among the scientists of National Agricultural Research System (NARS) in particular and researchers all over the globe in general and is hosted at **http://drs.icar.gov.in** The home page of the server is



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- **Other Useful Links:** Discussion Board, Ask a Question, Who-is-where, important links.
- **Site Information:** Feedback, How to Quote Design Resources Server, Copyright, disclaimer, contact us and site map.

The major components are Useful for Experimenters and Research Statisticians. The scientists, however, can use either of the parts or parts of their choice. A brief description of all the above four components is given in the sequel.

2. Useful for Experimenters

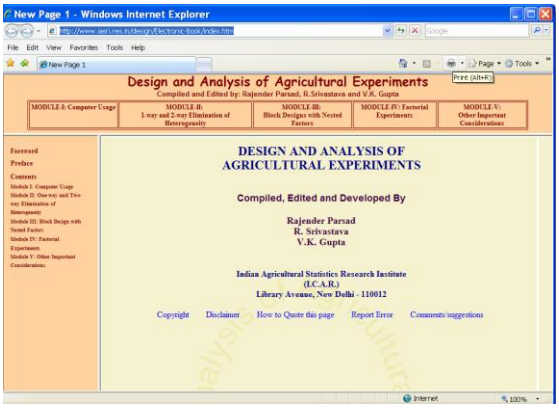
This link has been designed essentially to meet the requirements of the experimenters whose prime interest is in designing the experiment and then subsequently analyzing the data generated so as to draw statistically valid inferences. To meet this end, the link contains the following sub-links:

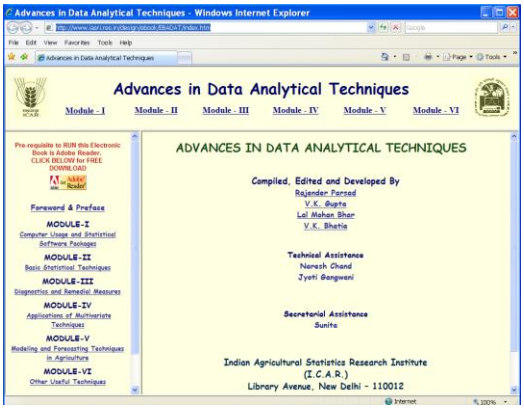
2.1 E-Learning

This is an important link that provides useful and important reading material on use of some statistical software packages, designing experiments, statistical analysis of data and other useful topics in statistics in the form of two electronic books viz.

1. Design and Analysis of Agricultural Experiments
   www.iasri.res.in/design/Electronic-Book/index.htm
2. Advances in Data Analytical Techniques
   www.iasri.res.in/design/ebook/EBADAT/index.htm

The screen shots of cover pages of these books are shown below:





The coverage of topics in these electronic books is very wide and almost all the aspects of designing an experiment and analysis of data are covered. The chapters are decorated with solved examples giving the steps of analysis. The users can have online access to these electronic books. This provides good theoretical support and also reading material to the users.

2.2 Online Design Generation-I

This link is very useful for experimenters because it helps in generation of randomized layout of the following designs:

Basic Designs: Generates of randomized layout of completely randomized design and randomized complete block design both for single factor and multifactor experiments and Latin square designs for single factor experiments. The field book can be created as a .csv file or a text file. This is available at

www.iasri.res.in/design/Basic Designs/generate_designs.htm.

Augmented Designs: A large number of germplasm evaluation trials are conducted using augmented designs. The experimenters generally compromise with the randomization of treatments in the design. Further, experimenters also need to know the optimum replication number of controls in each block so as to maximize the efficiency per observation. Online software for generation of randomized layout of an augmented randomized complete block design for given number of test treatments, control treatments and number of blocks with given block sizes, not necessarily equal, is developed and is available at

www.iasri.res.in/design/Augmented Designs/home.htm.

The design can be generated with optimum replication of control treatments in each block so as to maximize efficiency per observation.

Resolvable Block Designs: Resolvable block designs are an important class of incomplete block designs wherein the blocks can be formed together into sets with the blocks within each set constituting a complete replication. In the class of resolvable block designs, square lattice designs are very popular among experimenters. One can generate square lattice designs with three replications using

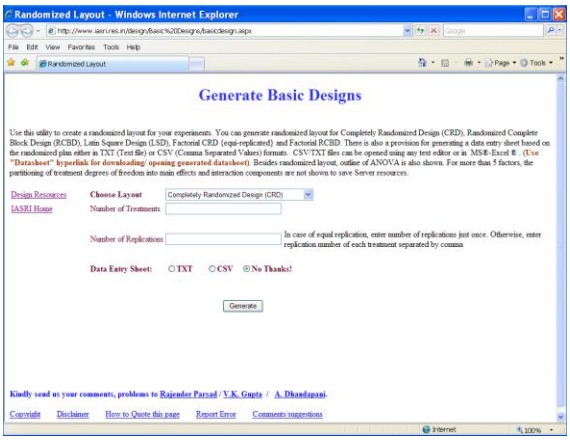
www.iasri.res.in/WebHadamard/square lattice.htm.

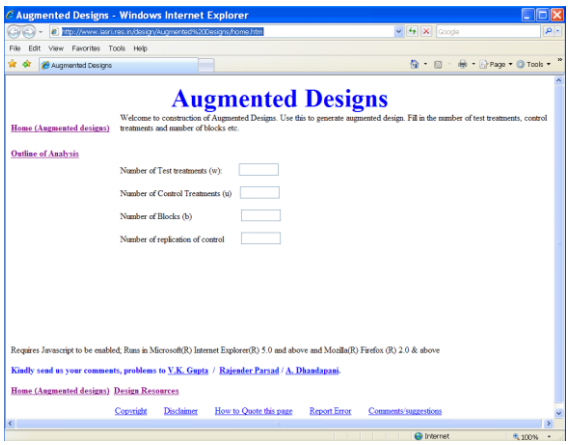
Another important class of resolvable block designs is the alpha designs. These designs are available when the number of treatments is a composite number. Literature on alpha designs is available at

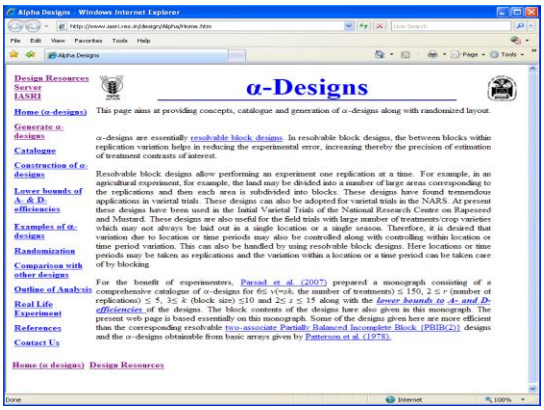
http://drs.icar.gov.in /Alpha/Home.htm.

This link also provides randomized layout of alpha designs for $6 \leq v$ (=sk, the number of treatments) ≤ 150, $2 \leq r$ (number of replications) ≤ 5, $3 \leq k$ (block size) ≤ 10 and $2 \leq s \leq 15$ along with the lower bounds to A- and D- efficiencies of the designs.

The screen shots for generation of randomized layout of basic designs, augmented designs, square lattice designs and alpha designs are

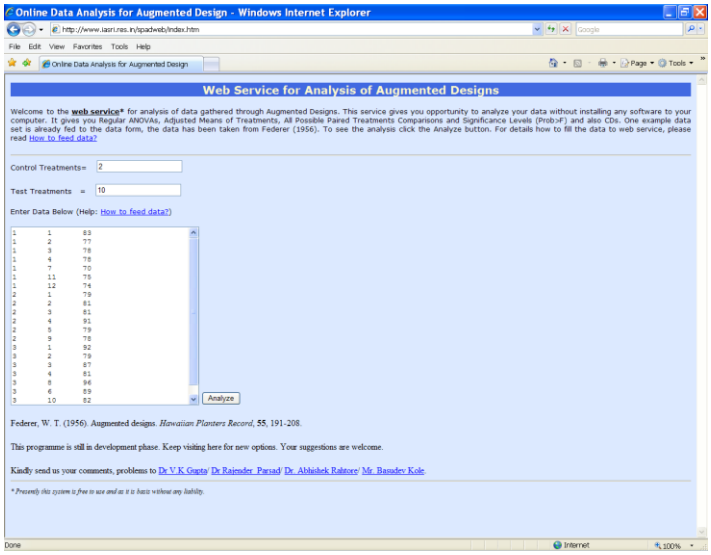






2.3 Online Analysis of Data

This link together with Analysis of Data forms the backbone of the Design Resources Server. This particular link targets at providing online analysis of data generated to the experimenter. At present an experimenter can perform online analysis of data generated from augmented randomized block designs. This is available at http://drs.icar.gov.in /spadweb/index.htm.



2.4 Analysis of Data

This is the most important link of the server because it targets at providing steps of analysis of data generated from designed experiments using several statistical packages like SAS, SPSS, GenStat, MINITAB, SYSTAT, SPAD, SPFE, SPAR 2.0, MS-Excel, etc. Some real life examples of experiments are given and the questions to be answered are listed. Steps for preparation of data files, the commands and macros to be used for analysis of data and the treatment contrasts to be used for answering specific questions, etc. are given, which the user can use without any difficulty. The data files and result files can also be downloaded. This is available at

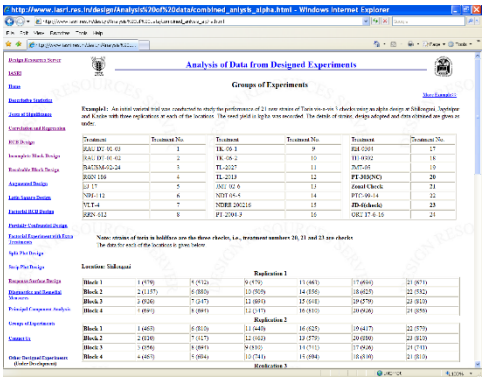
www.iasri.res.in/design/Analysis of data/Analysis of Data.html.

The following analysis can be performed using this link:

- Analysis of data generated from completely randomized designs, randomized complete block design; incomplete block design; resolvable incomplete block design; Latin square design; factorial experiments both without and with confounding; factorial experiments with extra treatments; split and strip plot designs; cross over designs using SAS and SPSS; steps of analysis of augmented design using SAS, SPSS and SPAD
- Response surface design using SAS and SPSS
- SAS code for analysis of groups of experiments conducted in different environments (locations or season / year), each experiment conducted as a complete block or an incomplete block design. Using this code, one can analyze the data for each of the environments separately, test the homogeneity of error variances using Bartlett's χ^2-test, perform combined analysis of data considering both environment effects as fixed and environment effects as random (both through PROC GLM and PROC MIXED) and prepare site regression or GGE biplots
- SAS Macro for performing diagnostics (normality and homogeneity of errors) in experimental data generated through randomized complete block designs and then applying remedial measures such as Box-Cox transformation and applying the non-parametric tests if the errors remain non-normal and / or heterogeneous even after transformation
- SAS codes are also available for obtaining descriptive statistics, generating discrete frequency distribution, grouped frequency distribution, histogram, testing the normality of a given variable (overall groups or for each of the groups separately)
- correlation and regression using SAS and SPSS

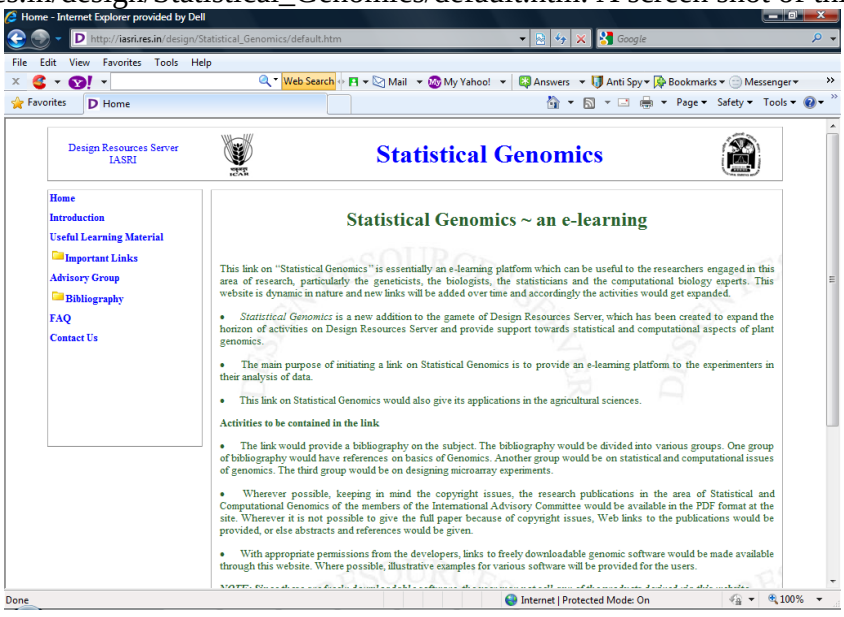
- Tests of significance based on Student's *t*-distribution using SAS, SPSS and MS-EXCEL
- SAS and SPSS codes for performing principal component analysis, cluster analysis and analysis of covariance
- SAS and SPSS codes for fitting non-linear models

The screens shots for analysis of data appear like



2.6 Statistical Genomics

A link on Statistical Genomics has been initiated essentially as an e-learning platform which can be useful to the researchers particularly the geneticists, the biologists, the statisticians and the computational biology experts. It contains the information on some public domain software's that can be downloaded free of cost. A bibliography on design and analysis of microarray experiments is also given. These are hosted at http://iasri.res.in/design/Statistical_Genomics/default.htm. A screen shot of this link is



3. Useful for Research Statisticians

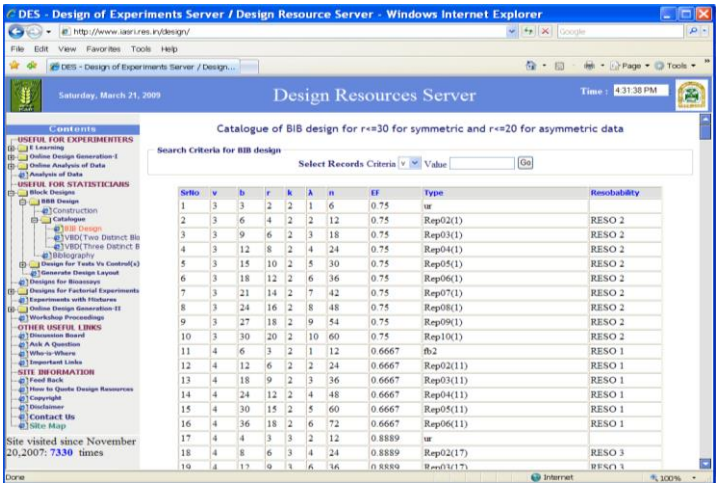
This link is useful for researchers engaged in conducting research in design of experiments and can be used for class room teaching also. The material on this link is divided into the following sub-links:

3.1 Block Designs

This link provides some theoretical considerations of balanced incomplete block (BIB) designs, binary variance balanced block (BBB) designs with 2 and 3 distinct block sizes, partially balanced incomplete block (PBIB) designs, designs for test treatments-control treatment(s) comparisons, etc. for research statisticians. The link also gives a catalogue of designs and a bibliography on the subject for use of researchers. At present the following material is available on this link:

- General method of construction of BBB designs; general methods of construction of block designs for making test treatments - control treatment(s) comparisons; bibliography
- Catalogue of BIB designs for number of replications $r \leq 30$ for symmetric BIB designs and $r \leq 20$ for asymmetric BIB designs
- Catalogue of BBB designs with 2 and 3 distinct block sizes for number of replications $r \leq 30$. The catalogue also gives the resolvability status of the designs along with the efficiency factor of the designs
- 6574 block designs for making all possible pair wise treatment comparisons for $v \leq 35$ (number of treatments), $b \leq 64$ (number of blocks), $k \leq 34$ (block size)

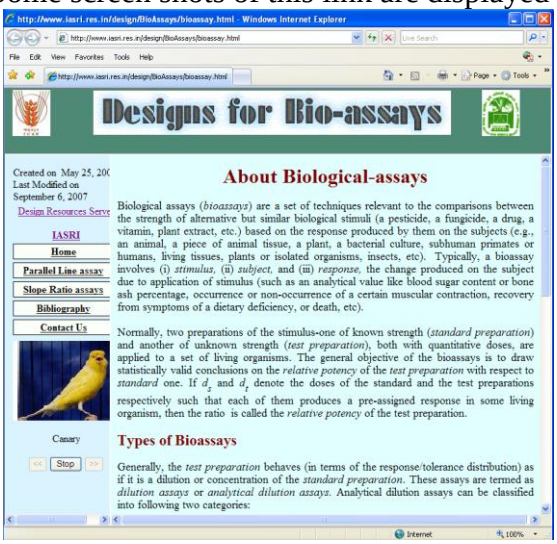
Some screen shots on block designs are given below:

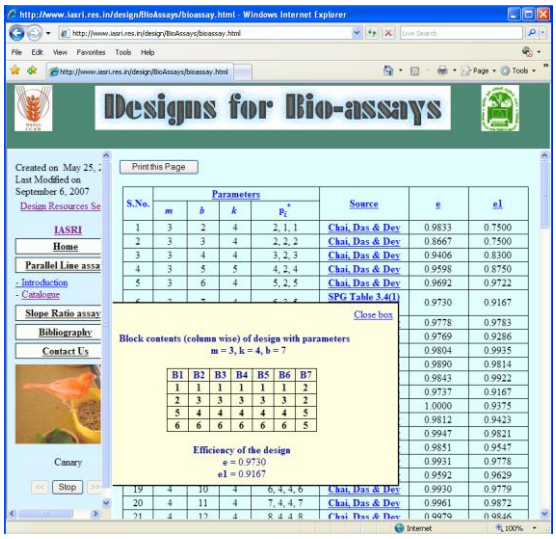


3.2 Designs for Bioassays

Designs for biological assays help in estimation of relative potency of the test preparation with respect to the standard one. The material uploaded includes contrasts of interest in parallel line assays and slope ratio assays. This link provides some theoretical considerations of designs for bioassays along with a catalogue of designs and a bibliography on the subject for use of researchers. Literature on bioassays is available at http://drs.icar.gov.in /BioAssays/bioassay.html.

Some screen shots of this link are displayed below:





3.3 Designs for Factorial Experiments

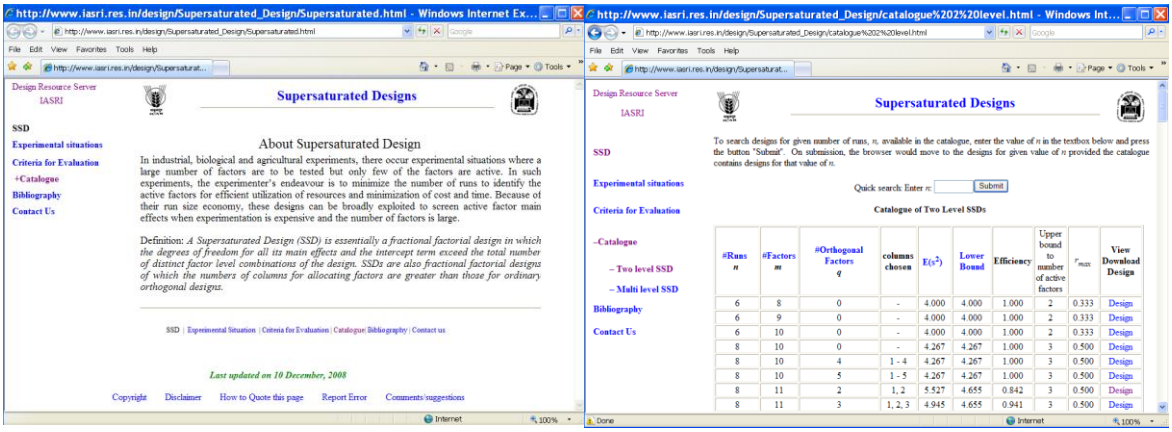
Factorial experiments are most popular among agricultural scientists. To begin with, material on block designs with factorial treatment structure and supersaturated designs is available on this link.

- **Supersaturated Designs**

Supersaturated designs are fractional factorial designs in which the degrees of freedom for all its main effects and the intercept term exceed the total number of distinct factor level combinations of the design. These designs are useful when the experimenter is interested in identifying the active factors through the experiment and experimental resources are scarce. Definition of supersaturated designs, experimental situations in which supersaturated designs are useful, efficiency criteria for evaluation of supersaturated designs, catalogue of supersaturated designs for 2-level factorial experiments and asymmetrical factorial experiments and bibliography on supersaturated designs has been uploaded on the Server. The complete details of the runs can be obtained by clicking on the required design in the catalogue.

http://drs.icar.gov.in /Supersaturated_Design/Supersaturated.html.

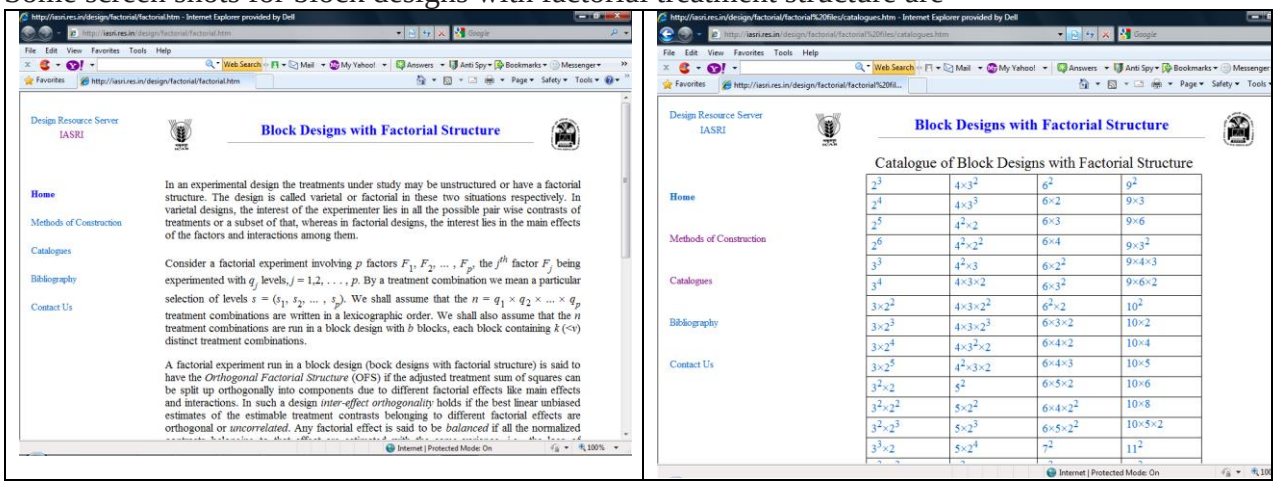
Some screen shots of supersaturated designs are



➢ **Block Designs with Factorial Treatment Structure**

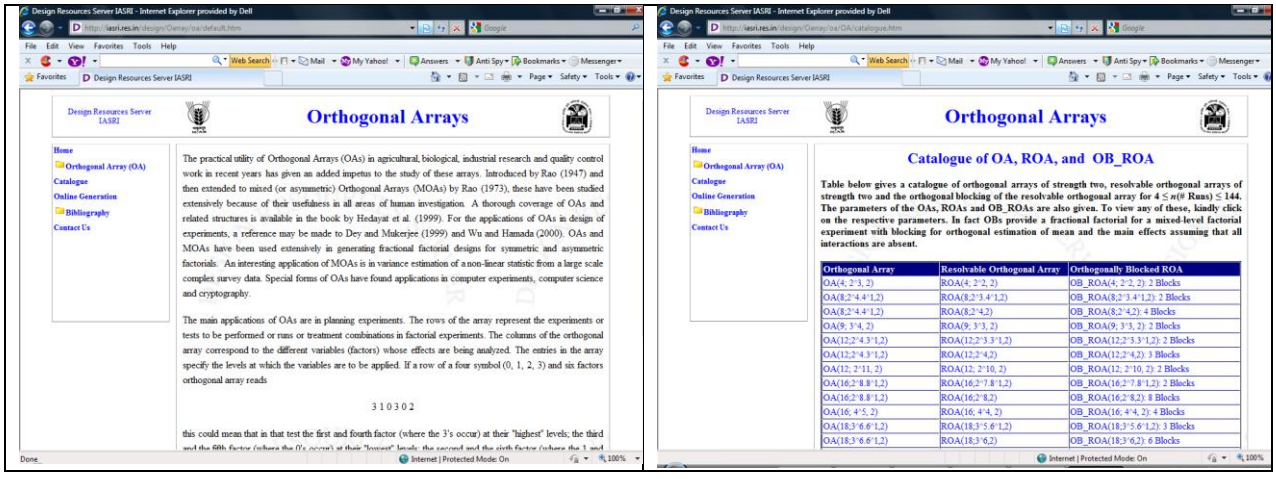
Block designs with factorial treatment structure have useful applications in designs for crop sequence experiments. Th link on block designs with factorial Treatment Structure provides a bibliography with 232 references on the subject. Catalogues of block designs with factorial treatment structure in 3-replications for number of levels for any factor at most 12 permitting estimation of main effects with full efficiency and controlling efficiency for interaction effects are also given at this link. URL for this link is www.iasri.res.in/design/factorial/factorial.htm.

Some screen shots for block designs with factorial treatment structure are



➢ **Mixed Orthogonal arrays**

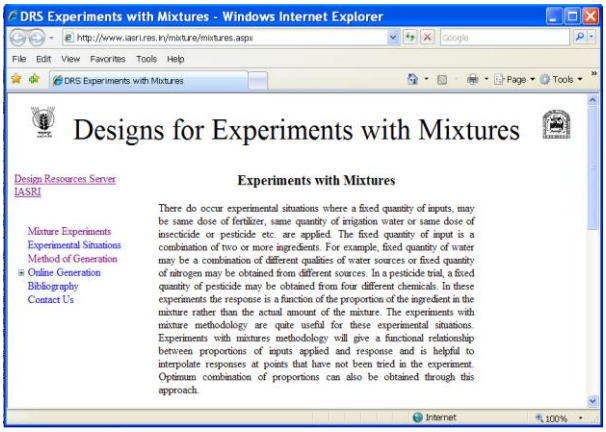
Definitions of Orthogonal arrays(OAs), mixed OA, Resolvable OA, α-resolvable OA, resolvable MOA, construction of OAs, blocking in OAs, generation of orthogonal arrays of strength two, resolvable orthogonal arrays of strength two and the orthogonal blocking of the resolvable orthogonal array for $4 \leq n(\#\text{ Runs}) \leq 144$, and bibliography on OAs.

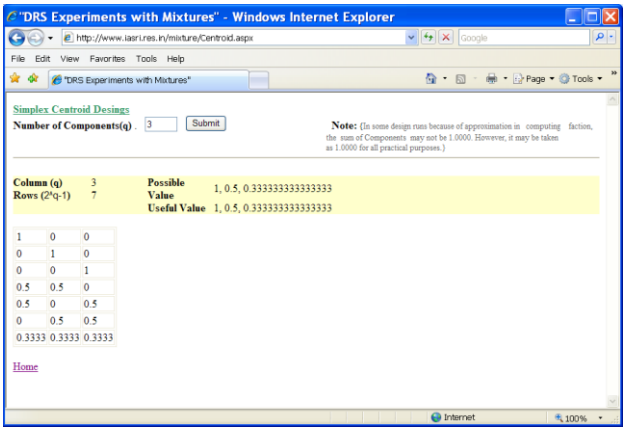


3.4 Experiments with Mixtures

Experiments with mixtures are quite useful for the experiments where a fixed quantity of inputs (may be same dose of fertilizer, same quantity of irrigation water or same dose of insecticide or pesticide etc.) are applied as a combination of two or more ingredients. In these experiments the response is a function of the proportion of the ingredients in the mixture rather than the actual amount of the mixture. A bibliography of experiments with

mixtures and online generation of simplex centroid designs are available on this page http://drs.icar.gov.in/mixture/mixtures.aspx. Some screen shots of experiments with mixtures are:



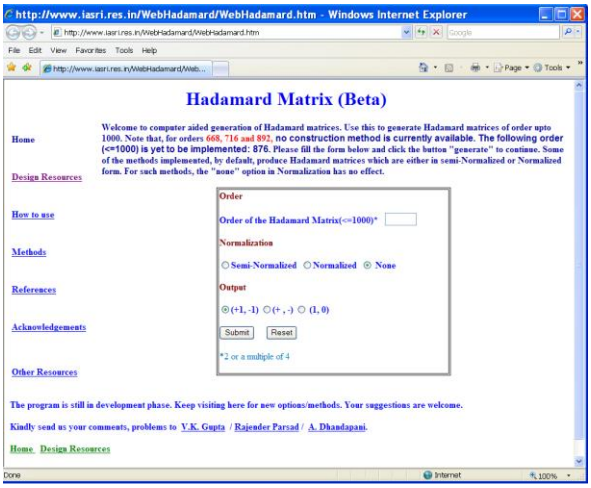


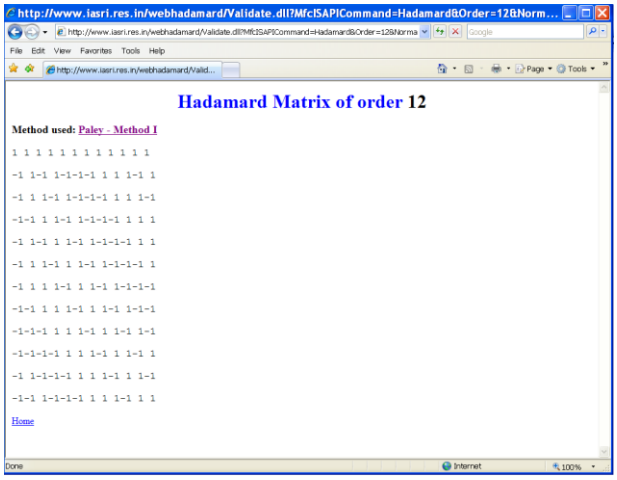
3.5 Online Design Generation- II

This link is helpful in generation of the following:

Hadamard matrix

Hadamard matrices have a tremendous potential for applications in many fields particularly in fractional factorial plans, supersaturated designs, variance estimation from large scale complex survey data, generation of incomplete block designs, coding theory, etc. One can generate Hadamard matrices for all permissible orders up to 1000 except 668, 716, 876 and 892 using the URL www.iasri.res.in/WebHadamard/WebHadamard.htm. Methods implemented produce Hadamard matrices in semi-normalized or normalized form. "None" option is also available. Hadamard matrix can be generated in (0,1); (+1,-1); or (+,-) form. The method of generation of Hadamard matrix is also given. The screen shots for generation of Hadamard matrices are

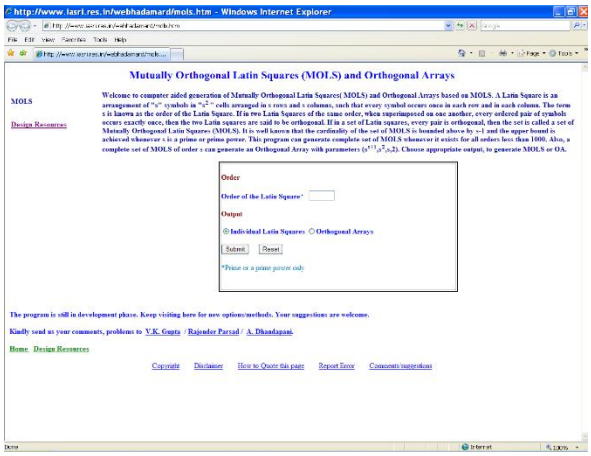


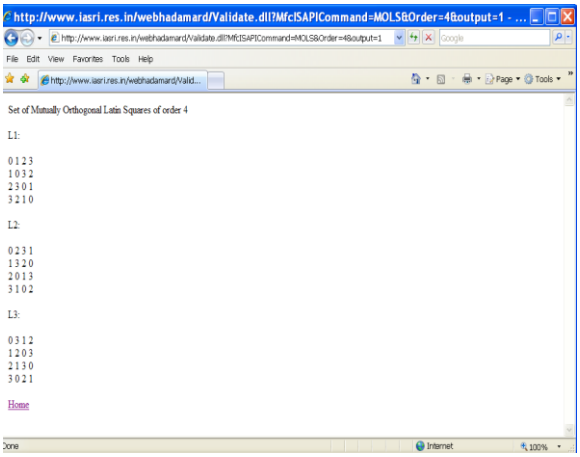


Mutually Orthogonal Latin Squares and Orthogonal arrays

Using this link one can generate complete set of mutually orthogonal Latin squares of order s, s being a prime or prime power less than 1000. One can also generate an orthogonal array

with parameters (s^{s+1}, s^2, s, 2) by choosing the output option as orthogonal arrays. The URL of this link is http://drs.icar.gov.in/WebHadamard/mols.htm. Some screen shots of mutually orthogonal Latin squares and orthogonal arrays are





3.6 Workshop Proceedings

Proceedings of 3 dissemination workshops are available for the stakeholders

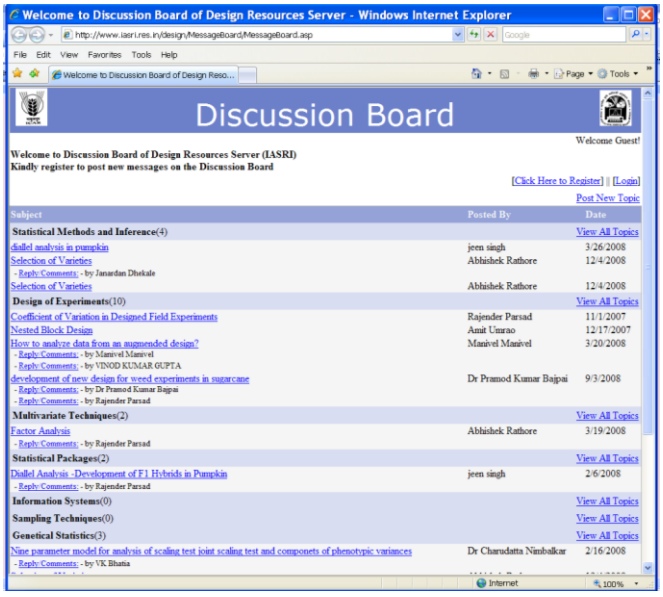
1. Design and Analysis of On-Station and On-Farm Agricultural Experiments
2. Design and Analysis of Bioassays
3. Outliers in Designed Experiments

4. Other Useful Links

The purpose of this component is to develop a network of scientists in general and a network of statisticians in particular around the globe so that interesting and useful information can be shared among the peers. It also attempts to provide a sort of advisory to the scientists. Some other useful and important links available on world wide web are also provided.

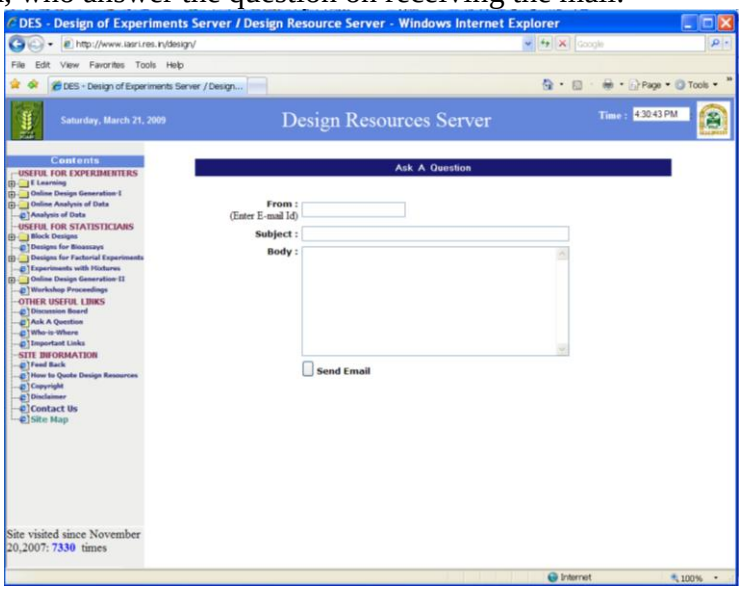
4.1 Discussion Board

The purpose of discussion board is to create a network of scientists and also to provide a platform for sharing any useful piece of research or idea with scientists over the globe. The user can use this board for learning and disseminating information after registering on the discussion board. The information can be viewed by anybody over the globe. In case there are some queries or some researchable issues, then other peers can also respond to these queries. This helps in creating a network of scientists. Number of registered participants so far is 78 (23: Agricultural Research Statisticians; 37: Experimenters; One Vice-Chancellor and 17 ISS Officers). (http://drs.icar.gov.in /MessageBoard/MessageBoard.asp).



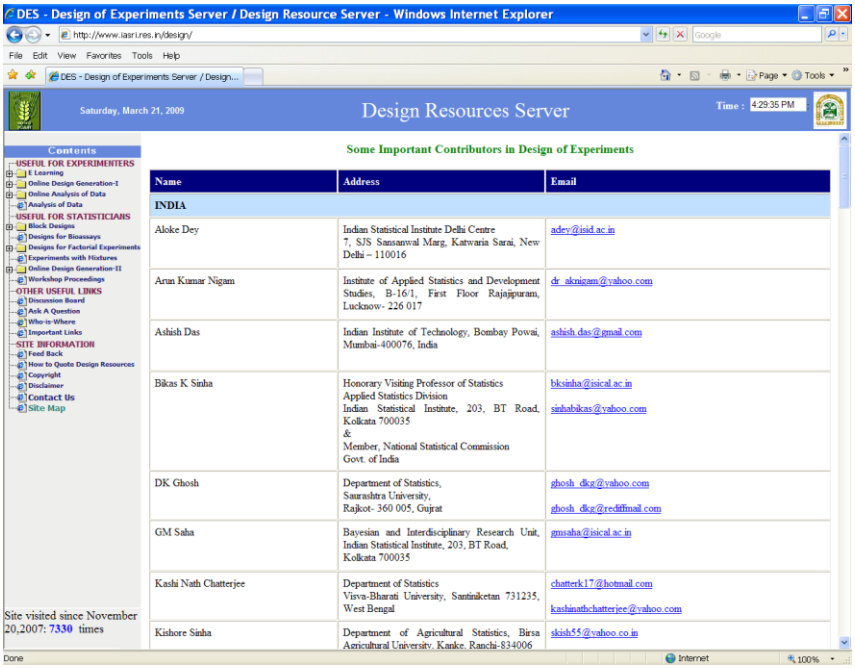
4.2 Ask a Question

The ultimate objective of this server is to provide e-learning and e-advisory services. At present this is being achieved through the link "Ask a Question". Once a user submits a question, a mail is automatically generated for Dr. Rajender Parsad, Dr. V.K. Gupta and Mrs. Alka Arora, who answer the question on receiving the mail.



4.3 Who-is-where

Addresses of important contributors in Design of Experiments including their E-mail addresses have been linked to Design Resources Server. The list includes experts from USA, Canada, Australia, UK, China, Japan, Mexico, New Zealand, Oman, Syria, Taiwan, Vietnam and India. This information is useful for all the researchers in Design of Experiments in establishing linkages with their counterparts over the globe.



4.4 Important Links

This gives links to other important sites that provide useful material on statistical learning in general and Design of Experiments in particular. Some links are as given below:

S No.	Important Links
1.	Design Resources: www.designtheory.org
2.	Statistics Glossary http://www.cas.lancs.ac.uk/glossary_v1.1/main.html
3.	Free Encyclopedia on Design of Experiments: http://en.wikipedia.org/wiki/Design_of_experiments
4.	Important Contributors to Statistics: http://en.wikipedia.org/wiki/Statistics#Important_contributors_to_statistics
5.	Electronic Statistics Text Book: http://www.statsoft.com/textbook/stathome.html
6.	On-line construction of Designs: http://biometrics.hri.ac.uk/experimentaldesigns/website/hri.htm
7.	GENDEX: http://www.designcomputing.net/gendex/
8.	Hadamard Matrices 1. *http://www.research.att.com/~njas/hadamard* 2. http://www.uow.edu.au/~jennie/WILLIAMSON/williamson.html
9.	Biplots :http://www.ggebiplot.com
10.	Free Statistical Softwares: http://freestatistics.altervista.org/en/stat.php
11.	Learning Statistics: http://freestatistics.altervista.org/en/learning.php
12.	Statistical Calculators: http://www.graphpad.com/quickcalcs/index.cfm
13.	SAS Online Doc 9.1.3: http://support.sas.com/onlinedoc/913/docMainpage.jsp
14.	University of South California: Courses in Statistics: http://www.stat.sc.edu/curricula/courses/

15.	Course on Introduction to Experimental Design: http://www.stat.sc.edu/~grego/courses/stat506
16.	Course on Experimental design: http://www.stat.sc.edu/~grego/courses/stat706

5. Site Information

This link provides information about the site on the following aspects (i) Feedback from stakeholders, (ii) How to Quote Design Resources Server, (iii) Copyright, (iv) Disclaimer, (v) Contact us, and (vi) Sitemap.

5.1 Feedback/ Comments

The feedback / comments received from the users visiting the site have been put on the server so that every user can benefit from the experience of other users. More importantly, the feedback helps in improving the contents of the site and their presentation too. We have received feedback from 19 researchers (6: Design Experts from India; 7: Experts from abroad; 4: Experimenters and 2: Agricultural Research Statisticians). The first feedback was received from Dr K Rameash, Entomologist working at ICAR Research Complex for NEH Region, Sikkim Centre, Tadong, Gangtok.

5.2 How to quote Design Resources Server

To Quote Design Resources Server, use:

Design Resources Server. *Indian Agricultural Statistics Research Institute (ICAR), New Delhi 110 012, India*. www.iasri.res.in/design (accessed last on <date>).

If referring to a particular page, then the site may be quoted as

Authors' name in 'Contact Us' list on that page. Title of page: Design Resources Server. *Indian Agricultural Statistics Research Institute (ICAR), New Delhi 110 012, India*. www.iasri.res.in/design (accessed last on <date>).

For example, page on alpha designs may be cited as
Parsad, R., Gupta, V.K. and Dhandapani, A. Alpha Designs: Design Resources Server. *Indian Agricultural Statistics Research Institute (ICAR), New Delhi 110 012, India*. www.iasri.res.in/design (accessed last on 21.03.2009).

5.3 Copyright

5.4 Disclaimer

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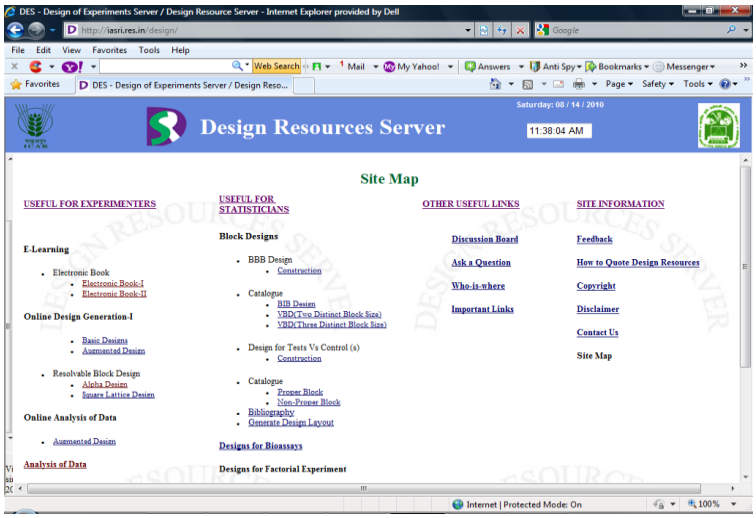
In no event will "IASRI" be liable for any loss or damage including without limitation, indirect or consequential loss or damage, or any loss or damage whatsoever arising from loss of data or profits arising out of or in connection with the use of this website.

Through this website user are able to link to other websites which are not under the control of "IASRI". The inclusion of any links does not necessarily imply a recommendation or endorsement the views expressed within them.

Every effort is made to keep the website running smoothly. However, "IASRI" takes no responsibility for and will not be liable for the website being temporarily unavailable due to technical issues beyond our control.

5.5 Site Map

This link gives a map of the various links available on the server. A user can access any of the links through this map also. A snap shot of the site map is given below:



6. Some Information on the Usage of the Server

- Design Resources Server is a copyright of IASRI (ICAR). The Server was registered under Google Analytics on May 26, 2008. For the period May 26- October 31, 2011, it has been used through 1102 cities in 113 countries spread over 6 continents. The average time on the page is 2.59 minutes.
- External links of the server are also available at:
  - http://en.wikipedia.org/wiki/Design_of_experiments
  - http://en.wikipedia.org/wiki/Hadamard_matrix
- The server has been cited at:
  - https://dspace.ist.utl.pt/bitstream/2295/145675/1/licao_21.pdf
    for lecture presentation on Unitary operators.

- Chiarandini, Marco (2008). DM811-Heuristics for Combinatorial Optimization. Laboratory Assignment, Fall 2008. Department of Mathematics and Computer Science, University of Southern Denmark, Odense.
- http://support.sas.com/techsup/technote/ts723.html
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- Server is also linked at
  - ICARDA Intranet: Biometric Services
  - CG Online learning resources- http://learning.cgiar.org/moodle/Experimental Designs and Data Analysis

7. Future Directions

The Design Resources Server created and being strengthened at IASRI aims to culminate into an expert system on design of experiments. To achieve this end, the materials available on various links need to be strengthened dynamically. Besides this, the following additions need to be made to the server in the near future:

- Online generation of
  - balanced incomplete block designs, binary balanced block designs and partially balanced incomplete block designs
  - block designs with nested factors
  - designs for crop sequence experiments
  - efficient designs for correlated error structures
  - online generation of row-column designs
  - designs for factorial experiments; fractional factorial plans
- designs for microarray experiments
- designs for computer experiments
- designs for fitting response surfaces; designs for experiments with mixtures
- split and strip plot designs
- field book of all the designs generated
- labels generation for preparing seed packets
- online analysis of data

The success of the server lies in the hands of users. It is requested that the scientists in NARS use this server rigorously and send their comments for further improvements to Dr. Rajender Parsad (rajender@iasri.res.in) / Dr. V.K. Gupta (vkgupta@iasri.res.in). The comments/ suggestions would be helpful in making this server more meaningful and useful.

Computational Tools for Drug Design And Discovery

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1. Introduction

Computational approaches in drug design, discovery and development process gaining very rapid exploration, implementation and admiration. Introducing a new drug in a market is a very complex, risky and costly process in terms of time, money and manpower. Generally it is found that drug discovery and development process takes around 10-14 years and more than 1 billion dollars capital in total. So, for reducing time, cost and risk borne factors computer aided drug design (CADD) method is widely used as a new drug design approach. It has been seen that by the use of CADD approaches we canreduce the cost of drug discovery and development up to 50%. CADD consist use of any software program-based process for establishing a standard to relate activity to structure.

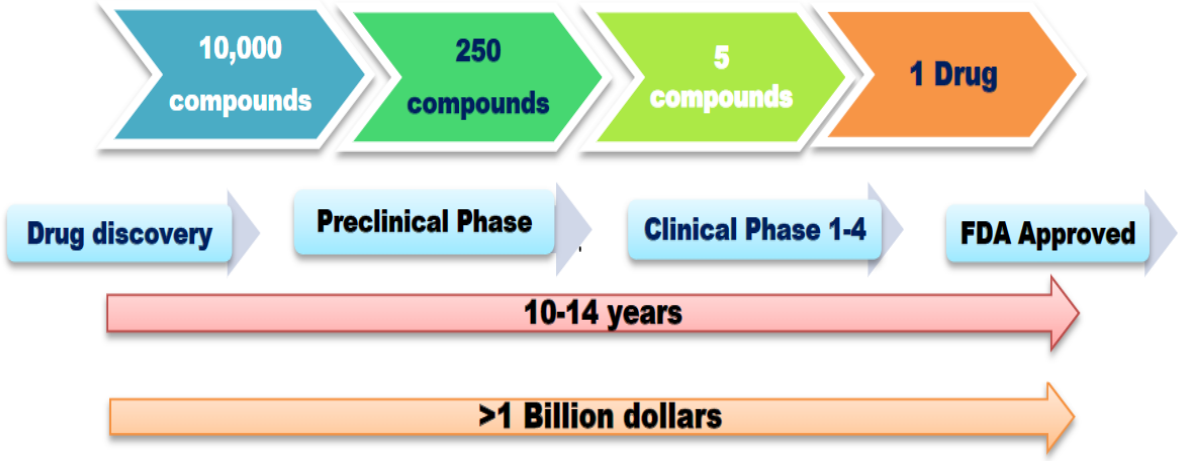

Figure 1. Trend followed in drug discovery

***In silico*:** *In silico* is an expression meaning "performed on computer or *via* computer simulation" in reference to biological experiments or the mimicking of biological processes within computer hardware and software. The phrase was coined in 1989 as allusion to the Latin phrases *in vitro, in vivo, in situ* which are commonly used in biology.

Lead compound: Chemical compound that shows promise as a treatment for a disease and may lead to development of a new drug. Thousands of compounds are tested in the laboratory to find a lead (leading) compound that may act on specific genes or proteins involved in a disease. Once a lead compound found, the chemical structure is used as starting point to make a drug that has the most benefits and the least harms.

***In silico* drug designing:** It is commonly called as computer aided drug discovery (CADD). The fundamental goal of CADD is to predict which molecules among many will bind to the

target and if so, how strongly they will, using knowledge and information on molecular mechanics and dynamics. In a drug discovery project, CADD is typically used for three main purposes:

(1) Filter large compound libraries into smaller sets of expected active compounds that can be tested experimentally

(2) Direct the optimization of lead compounds, whether to increase its affinity or improve drug metabolism and pharmacokinetics (DMPK) properties like absorption, delivery, metabolism, excretion, and the potential for toxicity (ADMET)

(3) Model novel compounds, either by "increasing" starting molecules one functional group at a time or by piecing together fragments into novel chemotypes.

2. Major types of approaches in CADD

There are mainly two types of approaches for drug design through CADD isthe following:

- ***Structure based drug design / direct approach***
- ***Ligand based drug design / indirect approach***

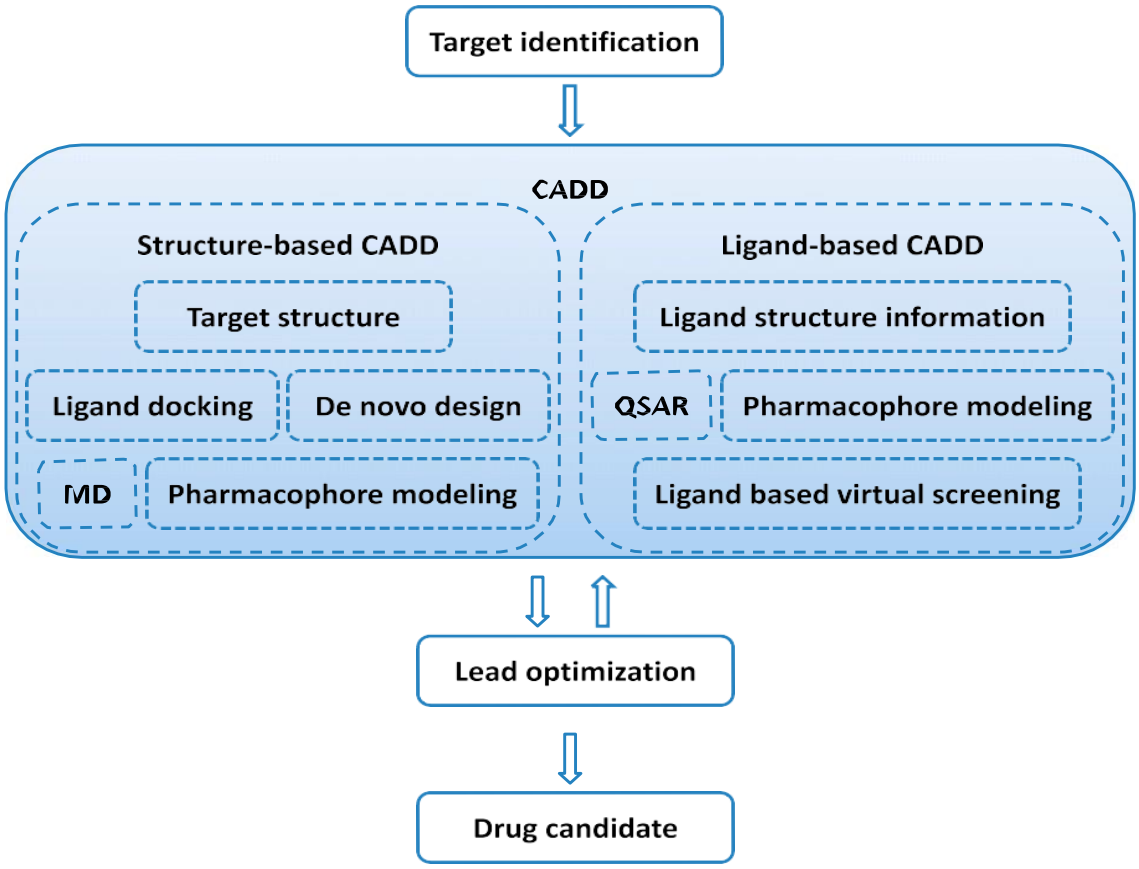

Figure 2. CADD in drug discovery/design pipeline. A therapeutic target is identified against which a drug has to be developed. Depending on the availability of structure information, a structure-based approach or a ligand-based approach is used. A successful CADD campaign will allow identification of multiple lead compounds. Lead identification is often followed by several cycles of lead optimization and subsequent lead identification using CADD. Lead compounds are tested in vivo to identify drug candidates.

Table 1. Different type of approaches in CADD

Target (receptor)	Ligand	Approach
Known	Known	Structure based drug design
Unknown	Known	Ligand based drug design
Known	Unknown	De novo Design

2.1 Structure Based Drug Design (SBDD)

Availability of 3D structure and prior knowledge on biological function(s) of target protein are pre-requisites for SBDD approach. Based on the structure of the target protein, SBDD allows design of candidate drugs that are predicted to bind to the target with high affinity and selectivity. Assumption that underlies and justifies SBDD approach is that a molecule's potential to have desired biological effects for a specific protein relies on its degree of ability to interact with binding sites on that protein.Computer-aided drug design based on a structure (SBCADD) relies on the ability to determine and analyze biological molecular 3D structures. This approach's central theory is that the ability of a molecule to interact with a specific protein and induce a desired biological effect depends on its ability to interact favorably on that protein with a particular binding site. Similar biological effects will be exerted by molecules that share these favorable interactions. Thus, by careful analysis of the binding site of a protein, novel compounds can be elucidated.Computational methods in drug discovery allow the rapid screening of a large compound library and the identification of potential binders through techniques of modeling / simulation and visualization.

A. Preparation of a Target Structure

The ideal starting point for docking is a target structure experimentally determined by X-ray crystallography or NMR techniques and deposited in the PDB.

Structural genomics has accelerated the rate of determination of target structures. 342 Sliwoski et al. was documented on the basis of comparative target protein models in the absence of experimentally defined structures (Becker et al., 2006; Warner et al., 2006; Budzik et al., 2010). Efforts were also made to incorporate information on binding properties of known ligands back into the process of comparative modeling (Evers et al., 2003; Evers and Klebe, 2004). Virtual screening success depends on the amount and quality of structural information that is known about the target as well as the small molecules that are docked. The first step is to determine the requirement for an appropriate binding pocket to be present (Hajduk et al., 2005; Fauman et al., 2011). Typically this is achieved by studying known target-ligand cocrystal structures or by using novel binding sites *in silico* methods (Laurie and Jackson, 2006).

1. Modeling in comparison. Advances in methods for biophysics. Such as the techniques of X-ray crystallography and NMR. The production of protein structures has increased. This made it possible to use structural information to guide the discovery of drugs. Computational methods are used to predict the 3D structure of target proteins in the absence

of experimental structures. Based on a template with a similar sequence, comparative modeling is used to predict target structure, leveraging that protein structure is better preserved than sequence, i.e. proteins with similar sequences have similar structures. Homology modeling is a specific type of comparative modeling that shares the same evolutionary origin in the template and target proteins. Comparative modeling involves: (1) recognition of similar proteins to act as template structures, (2) sequence alignment of target and template proteins, (3) copying co-ordinates for confidently matched regions, (4) building missing target structure atom co-ordinates, and (5) design refinement and assessment.

The steps involved in comparative modeling are shown in Figure 3. For example, PSIPRED (Buchan et al., 2010) and MODELER (Marti-Renom et al., 2000) automate the comparative modeling process.

a. Identification of template and alignment: The goal sequence is used as a request in the PDB in the first step to define model structures. A straight forward PDB-BLAST search can be used to determine templates with high sequence similarity (Altschul et al., 1990). If PDB-BLAST does not produce any hits, more advanced fold recognition methods are available (Kelley and Sternberg, 2009; Soding and Remmert, 2011). Methods such as ClustalW (Thompson et al., 1994), which is a multiple sequence alignment tool, are followed by sequence alignment. Structurally conserved regions are identified and used to build the comparative model for closely related protein structures. Building and evaluating multiple comparative models from multiple good-scoring sequence alignments improves the comparative model's quality (Chivian and Baker, 2006; Misura et al., 2006). Combining multiple templates has been shown to improve comparative models by using well-determined, mutually exclusive regions (Rai and Fiser, 2006). Selection of templates is key to successful modeling of homology. Alignment length, sequence identity, template structure resolution, and secondary structure consistency between target and templates should be carefully considered.

b. Model construction: Gaps and insertions in the original sequence alignment occur most often outside of the secondary structure elements, resulting in chain breaks (gaps or insertions) and incomplete residues (gaps) in the original target protein template. Modeling these missing regions requires linking the anchor residues on either side of the missing region, which are the N-and C-terminal residues of protein segments. There are two large classes of methods of loop modeling: (1) methods based on knowledge and (2) methods based on novo. Knowledge-based methods use protein structure loops with roughly the same anchors as those found in target models.

Loops are added to the target structure from such structures. De novo methods generate a large number of loops and use energy functions to assess the quality of predicted loops (Hillisch et al., 2004). Nonetheless, both methods solve the problem of "loop closure," i.e. detection of low-energy loop conformations from a large conformational sample space that supports the structural constraint of connecting the two anchor points. Cyclic coordinate descent (Canutescu and Dunbrack, 2003) and kinematic closure (KIC) algorithms (Mandell

et al., 2009) search for conformations that meet loop closure constraints in a target structure. Cyclic descent co-ordinate iteratively changes dihedral angles one at a time to satisfy a distance limit between anchor residues (Canutescu and Dunbrack, 2003). The KIC algorithm derives from kinematic methods that allow geometric analysis of possible configurations of a system of rigid objects connected by flexible joints. By analyzing bond lengths and bond angle constraints, the KIC algorithm generates a Fourier polynomial in N variables for N rotatable bond system (Coutsias and Seok, 2004). The loop's atomic coordinates are then determined using the polynomial equation.

c. Refining and evaluation model: By introducing ideal bond geometries and removing unfavorable contacts introduced by the initial modeling process, atomic models are refined. Refining includes minimizing models that use techniques such as molecular dynamics (Raval et al., 2012), minimizing Monte Carlo Metropolis (Misura and Baker, 2005), or genetic algorithms (Xiang, 2006). For example, in an initial low-resolution step, the ROSETTA refinement protocol fixes bond lengths and angles at ideal values and prevents steric clashes. Using a Monte Carlo minimization technique (Misura and Baker, 2005), ROSETTA then minimizes energy as a function of backbone torsional angles f, c, and v. In drug design-oriented homology models, molecular dynamics-based optimization techniques have been commonly used (Serrano et al., 2006; Li et al., 2008).

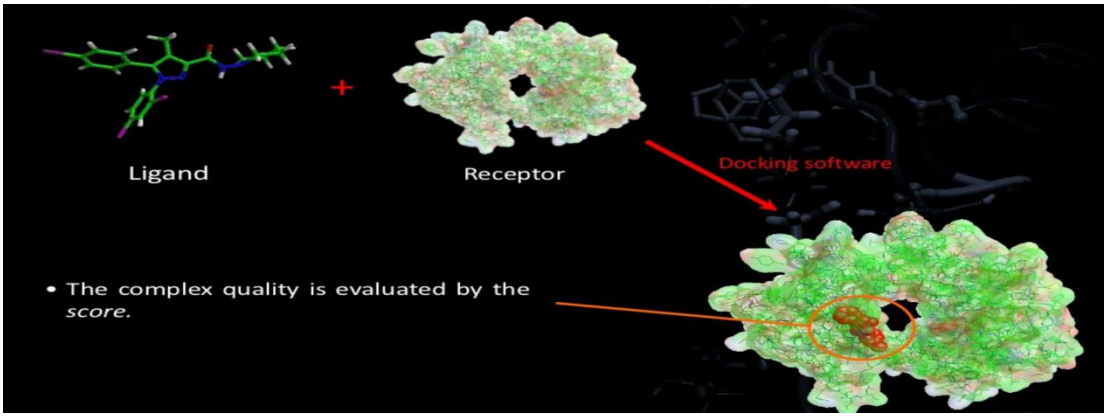

Figure 3. Structure based drug designing

Homology modelling: If there is no 3D structure information of the target, it may be possible to create homology model (this is called homology modelling) based on primary sequence similarity of the target to homologous proteins, of which 3D structure is empirically known. The 3D structure, whether it is experimental or predicted structure, of target protein provides information about chemical environment of the active site(s), enabling researchers to identify ligand(s) (drug or agrochemicals) that can bind to the active site with high affinity and selectivity. One thing that researchers should bear in mind is that, since homology modelling builds the 3D structures of proteins based on template sequences, the accuracy of the built model depends on the choice of template, alignment accuracy and refinement of the model. Generally, the models built with the templates exhibiting over 70% identities are considered to be accurate enough for drug discovery applications.

De novo design:

It is a process in which the 3D structure of receptor is used to design newer molecules. Information available about target but no existing lead compounds that can interact. This approach involves ligand optimization, it is done by analyzing the protein active site properties that could be probable area of contact by the ligand.

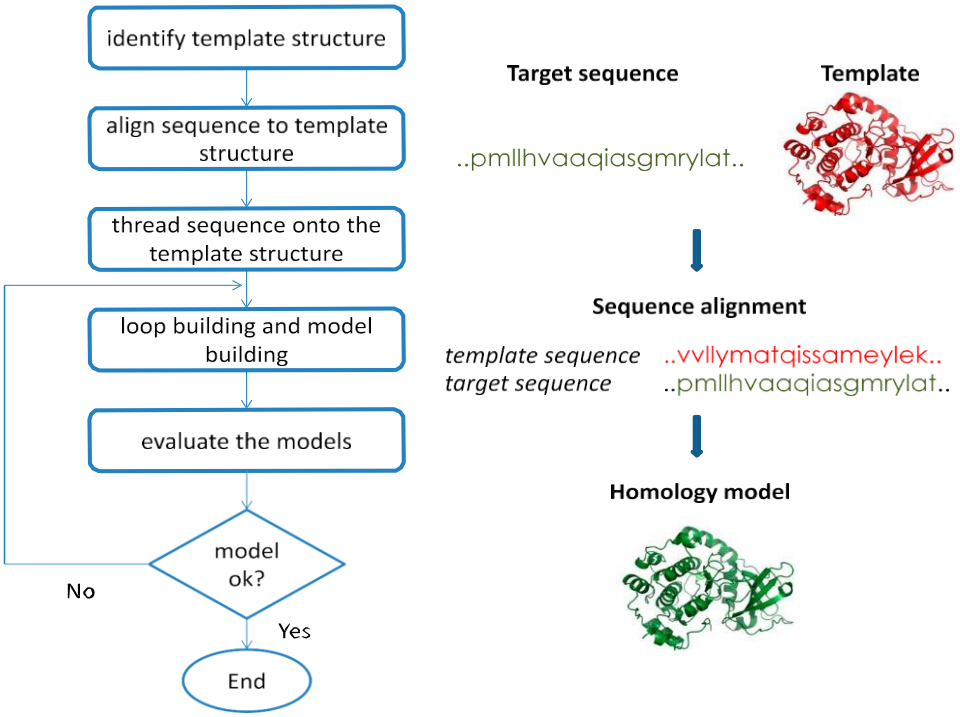

Figure 4. Scheme of homology modelling

2.2 Ligand based drug designing

In many cases, 3D structure of target protein or its homolog is not available for SBDD approach. This is true in particular for proteins that are present in cell surface or membrane due to their inherent difficulties in protein crystallization. In some cases, the use of unreliable homologous proteins (for example, low sequence identity) for homology modelling can result in high rate of false positive hits. In such situations, researcher can take LBDD. LBDD relies on knowledge of structural and chemical characteristics that molecules must have for binding to the target of interest.

Pharmacophore modelling: Pharmacophore is an abstract description of minimum, steric and electronic features that are required for interaction of target protein with ligand(s). Inference of pharmacophore using knowledge on a set of ligands (training set) that can bind to the target is called pharmacophore modelling. The process in the development of pharmacophore model involves the alignment of multiple ligands (training set), which can determine the essential chemical features that are responsible for their bioactivity. The alignment of these multiple ligands can be achieved by superimposing a set of active molecules. Such superimposed molecules are then transformed into abstract representation of different features. Pharmacophore model explains why molecules of structural diversity can bind to the common sites and have the same biological effects.

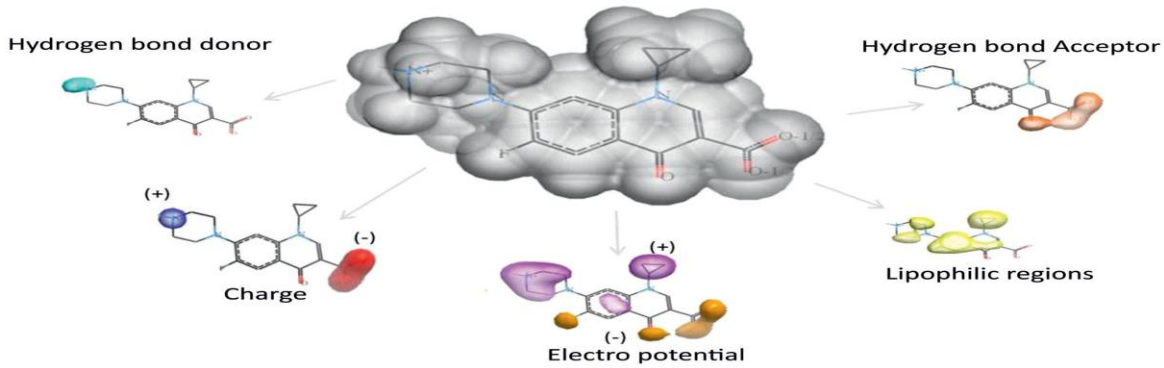

Figure 5. Features of Pharmacophore modelling

QSAR (Quantitative structure activity relationship): Quantitative structure–activity relationship (QSAR) models are regression or classification models used to predict activities of new chemical compounds based on their physio-chemical properties In general, QSAR is a regression model where it relates a set of 'predictor' variables (X) such as physio-chemical properties and molecular descriptors to the potency of the 'response' variable (Y) such as biological activity of the compound. Using this relationship, QSAR model is used to predict the activity of new compounds. The predictive ability of the QSAR model is dependent on the descriptors that were employed in the model generation.

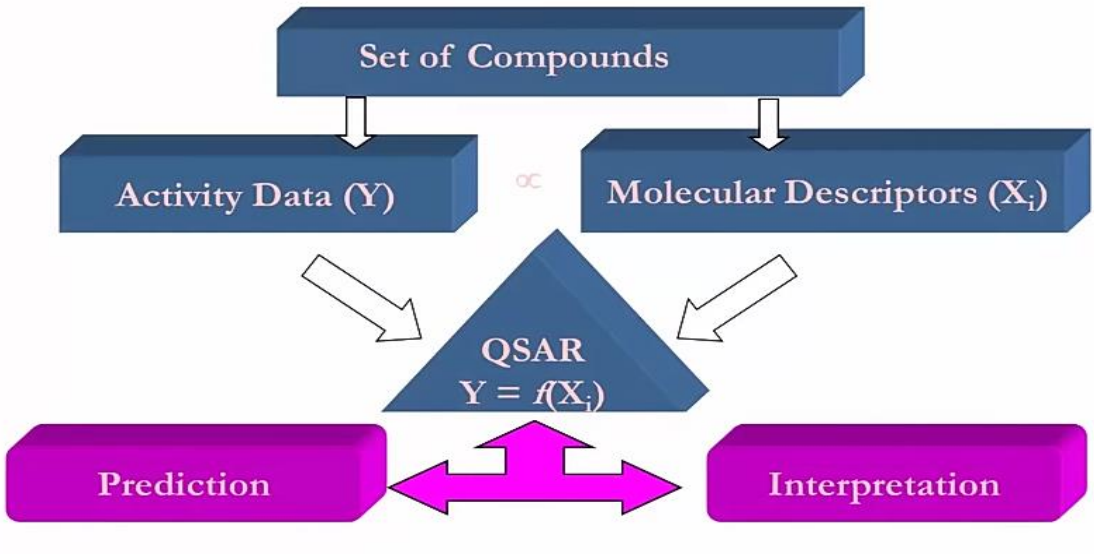

Figure 6. Prediction of QSAR

3. Molecular Docking

Once the model providing chemical environment of active sites is built, protein-ligand interactions can be explored through molecular docking, a method that predicts energetically stable orientation of ligand when it is bound to target protein. Degree of stability of interaction between molecules is the key factor to determining biological consequences of the interaction. Molecular docking reports two important

information: 1) correct conformation of a ligand-target (or ligand-receptor) complex and 2) its binding affinity which represents an approximation of the binding free energy (mathematical methods called scoring functions are used to estimate binding interaction of the protein-ligand complex). More than 30 molecular docking programs are currently available.

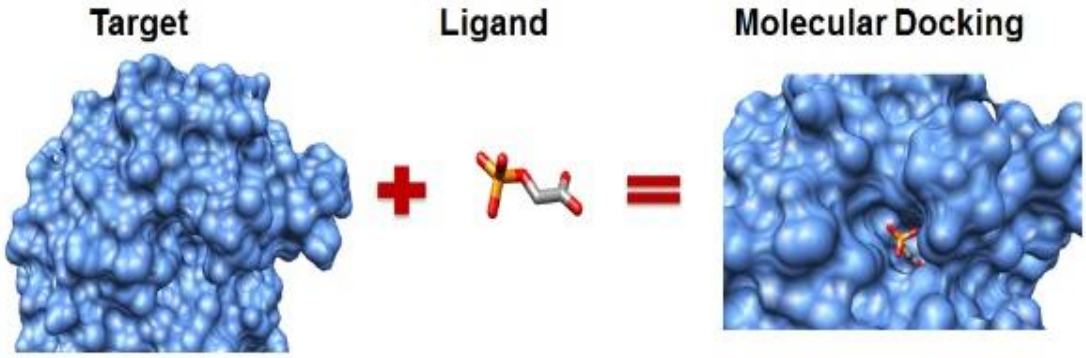

Figure 7. Molecular docking

Types of Docking: There are mainly two types of docking

Rigid docking: The ligand and protein are treated as a rigid structure during docking. Only translational and rotational degrees of freedom are considered. A large number ofconformations of each ligand are generated in advance and each is docked separately.

Flexible docking: The most common form of docking in which conformations of each molecule are generated by search algorithms during the docking process. The algorithms can avoid considering conformation that do not fit.

Figure 8. Different types of docking

4. Scoring functions in docking

In the fields of computational chemistry and molecular modelling, scoring functions are mathematical functions used to approximately predict the binding affinity between two molecules after they have been docked. Most commonly one of the molecules is a small organic compound such as a drug and the second is the drug's biological target such as a protein receptor. Scoring functions have also been developed to predict the strength of intermolecular interactions between two proteins or between protein and DNA. Scoring functions are normally parameterized (or trained) against a data set consisting of experimentally determined binding affinities between molecular species similar to the

species that one wishes to predict. For currently used methods aiming to predict affinities of ligands for proteins the following must first be known or predicted:

- **Protein tertiary structure** – arrangement of the protein atoms in three-dimensional space. Protein structures may be determined by experimental techniques such as X-ray crystallography or solution phase NMR methods or predicted by homology modelling.
- **Ligand active conformation** – three-dimensional shape of the ligand when bound to the protein
- **Binding-mode** – orientation of the two binding partners relative to each other in the complex

Classes: There are four general classes of scoring functions:

- **Force field** – affinities are estimated by summing the strength of intermolecular van der Waals and electrostatic interactions between all atoms of the two molecules in the complex using a force field. The intramolecular energies (also referred to as strain energy) of the two binding partners are also frequently included. Finally since the binding normally takes place in the presence of water, the desolation energies of the ligand and of the protein are sometimes taken into account using implicit solvation methods such as GBSA or PBSA.
- **Empirical** – based on counting the number of various types of interactions between the two binding partners. Counting may be based on the number of ligand and receptor atoms in contact with each other or by calculating the change in solvent accessible surface area (ΔSASA) in the complex compared to the uncomplexed ligand and protein. The coefficients of the scoring function are usually fit using multiple linear regression methods.
- **Knowledge-based** (also known as statistical potentials) – based on statistical observations of intermolecular close contacts in large 3D databases (such as the Cambridge Structural Database or Protein Data Bank) which are used to derive "potentials of mean force". This method is founded on the assumption that close intermolecular interactions between certain types of atoms or functional groups that occur more frequently than one would expect by a random distribution are likely to be energetically favorable and therefore contribute favorably to binding affinity.

5. Search algorithms in molecular docking

It is used to find the best conformation of the ligand and protein system. Both rigid and flexible dockings are used. There are different types of search algorithms are there and are described below:

Matching algorithms (MA):

- Molecular shape maps a ligand into an active site of a protein based on shape and chemical information
- Distance of the pharmacophore within the protein and ligand is calculated for match
- Chemical properties, like H-bond donors and acceptors considered
- Matching algorithms have the advantage of speed; used for the enrichment of active compounds from large libraries

- Matching algorithms for ligand docking are available in DOCK, FLOG, LibDock and SANDOCK program.

Incremental construction (IC):

- Put ligand into an active site in a fragmental and incremental fashion
- Ligand is divided into several fragments by breaking its rotatable bonds
- Largest fragment is considered first for docking having possible functional role
- Remaining fragments can be added incrementally
- Different orientations are generated to fit in the active site, realizes flexibility of the ligand
- IC method has been used in DOCK 4.0, FlexX, Hammerhead, SLIDE and eHiTS

Monte Carlo (MC):

- Generate poses of the ligand through bond rotation, rigid-body translation or rotation conformation, tested with an energy-based selection criterion
- Advantage of MC: change can be quite large allowing the ligand to cross the energy barriers on the potential energy surface
- Monte Carlo methods include an earlier version of AutoDock ICM , QXP

Genetic algorithm:

- Idea stems from Darwin's theory of evolution
- Df of the ligand are encoded as binary strings called genes
- Genes make up the 'chromosome' represents pose of the ligand
- Mutation causes random changes in gene and cross over exchanges gene between chromosomes
- Structure assessed by scoring function
- Genetic algorithms have used in AutoDock, GOLD, DIVALI and DARWIN

Table 2. List of molecular docking softwares

Software	Year	Organization
ADAM	1994	IMMD.lnc
Autodock	1990	The Scripps Research Institute
ICM Pro	1985	Molsoft L.L.C., La Jolla, California
Schrödinger Biologics Suite	1992	Schrödinger, LLC
Discovery Studio	-	Dassault Systèmes BIOVIA
AutoDockVina	2010	The Scripps Research Institute
BetaDock	2011	Hanyang University
1-Click Docking	2011	Mcule
AADS	2011	Indian Institute of Technology
Blaster	2019	University of California San Francisco
LightDock	2018	Barcelona Supercomputing Center
MOLS 2.0	2016	University of Madras

Molecular docking Service provider (INDIA)

- ❑ Biomed Informatics, Hyderabad: www.biomedinfo.netfirms.com
- ❑ Genomik Design Pharmaceuticals, Hyderabad:
- ❑ Aurigene Discovery Technologies, Bangalore:

6. Virtual Screening

Virtual screening (VS) is a computational technique used in drug discovery to search libraries of small moleculesin order to identify those structures which are most likely to bind to a drug target, typically a protein receptor or enzyme. It is defined as "automatically evaluating very large libraries of compounds" using computer programs. Virtual Screening can be used to select in house database compounds for screening, choose compounds that can be purchased externally, and to choose which compound should be synthesized next.

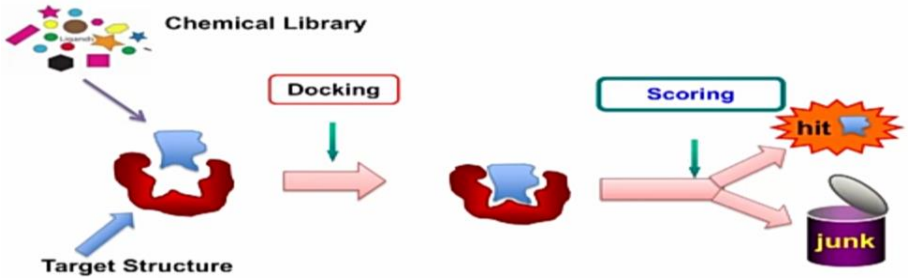

Figure 9. Virtual screening

Methods: There are two broad categories of screening techniques: ligand-based and structure-based.

Ligand based virtual screening: Given a set of structurally diverse ligands that binds to a receptor, a model of the receptor can be built by exploiting the collective information contained in such set of ligands. These are known as pharmacophore models. A candidate ligand can then be compared to the pharmacophore model to determine whether it is compatible with it and therefore likely to bind. A popular approach to ligand-based virtual screening is based on searching molecules with shape similar to that of known actives, as such molecules will fit the target's binding site and hence will be likely to bind the target.

Structure based virtual screening: Structure-based virtual screening involves docking of candidate ligands into a protein target followed by applying a scoring function to estimate the likelihood that the ligand will bind to the protein with high affinity.

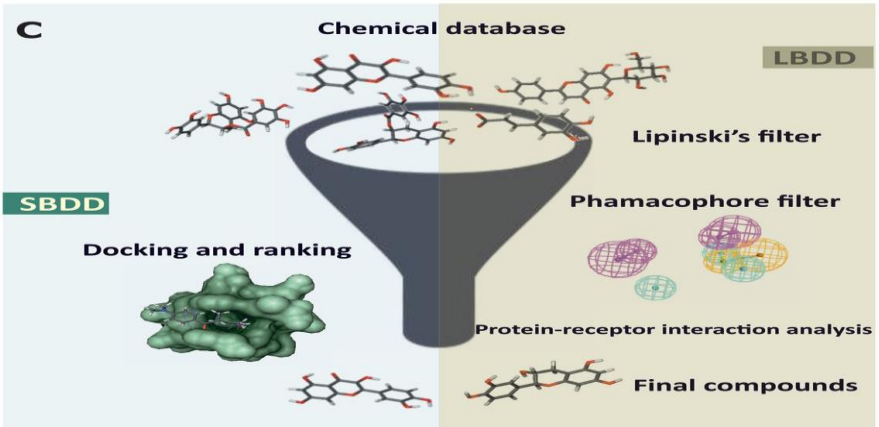

Figure 10. Types of virtual screening

***In silico* chemical library: Database of chemical compounds**.

To screen large chemical databases and prioritize compounds for synthesis, Virtual HTS uses high-performance computing. Using parallel processing clusters, current hardware and algorithms allow structural screening of up to 100,000 molecules per day (Agarwal and Fishwick, 2010). However, a virtual library must be available for screening in order to perform a virtual screen. A variety of computational and combinatorial methods can be used to construct general libraries. Some databases are listed as follows:

- Zinc: Annotated commercially available compounds
- Pubchem: Biologic activities of small molecules
- ChEMBL: ChEMBL is a manually curated database of bioactive molecules with drug-like properties, brings together chemical, bioactivity and genomic data to aid the translation of genomic information into effective new drugs.
- Chem DB: Annotated commercially available molecules
- PDB eChem: Ligands and small molecules referred in PDB
- DrugBank: Detailed drug data with comprehensive drug targetinformation
- Maybridge: Individually designed compounds, produced by innovative synthetic techniques
- WOMBAT: Bioactivity data for compounds reported in medicinalchemistry journals
- 3D MIND: Molecules with target interaction and tumor cell linescreen data
- MDDR: Drugs under development or released; descriptions oftherapeutic
- LIGAND: Chemical compounds with target and reactions data
- Accelrys Available Chemicals Directory (ACD): Consolidated catalog from major chemical suppliers

Table 4. Successful docking applications of some widely used docking software

Algorithm	Target
SEED	Plasmepsin (Friedman and Caflisch, 2009), target for malaria Flavivirus Proteases (Ekonomiuk et al., 2009a,b), target for WNV and Dengue virus Tyrosine kinase erythropoietin-producing human hepatocellular carcinoma receptor B4 (EphB4) (Lafleur et al., 2009)
FlexX	Plasmepsin II and IV inhibitors (Luksch et al., 2008), malaria Anthrax edema factor (Chen et al., 2008) Pneumococcal peptidoglycan deacetylase inhibitors (Bui et al., 2011)
Glide	Aurora kinases inhibitors (Warner et al., 2006) Falcipain inhibitors (Shah et al., 2011) Cytochrome P450 inhibitors (Caporuscioi et al., 2011)
Surflex	Topoisomerase I, anticancer (optimization)
DOCK	FK506 immunophilin (Zhao et al., 2006) BCL6, oncogene in B-cell lymphomas (Cerchietti et al., 2010)

8. Challenges

- CADD needs several basic data like three-dimensional structure of target, or its homologues and also set of ligands.
- Successful use of CADD tools naturally requires a great deal of expertise'

- Significant efforts are needed in development of new and modifications of existing tools are required for transformation of this are from art into the new advanced technology.
- Challenge to develop standardization for testing and validating the results and accurate scoring functions.
- Accurate prediction of ligand-receptor binding is still challenge.
- The process of choosing an appropriate scoring function and algorithm for a specific target and lead compound is tricky.

9. Conclusion

- Computer-aided drug design accelerate synthesis the new compounds. However experimental validation is mandatory for further confirmation.
- With today's computational resources, several million compounds can be screened in a few days.
- Pursuing a handful of promising leads for further development can save researchers considerable time and expense.
- The predictive power of CADD can help drug research programs to choose only the most promising drug candidates.
- Virtual screening, lead optimization and prediction of bioavailability and bioactivity can help and guide experimental research

Terminology in CADD:

Drug (or) ligand: The small chemical compound that can bind to protein or enzyme and can treat the disease or a small chemical compound that binds to macromolecules as signals to start (catalyze) the reaction.

Receptor (or) Target: A biological molecule (mostly macromolecules such as protein and DNA) that can receive a chemical signal (ligand) to catalyze a reaction or function.

Drug designing: A process of finding a small chemical compound that can bind to macromolecules and works as a drug.

Chemo informatics: A branch of science that deals with the study of small chemical compounds information such as properties, structures and functions.

Clefts/Cavities/Binding pockets: The space or gap regions in the protein structure. These regions are essential for the binding of small chemical compounds that acts as signal or drug molecule.

Homology modelling: Building the 3D structure of protein (target) based on the availability of experimentally (X-ray or NMR) derived 3D structures of another related (template) protein that shares the similarity.

Docking: This is a process of analyzing the binding interactions of ligand and receptor molecules.

Ligand conformation: The orientation of the ligand molecule bound in the receptor binding site.

Suggested Readings:

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NAE-Website: An Overview

http://naeagchem.icar.gov.in

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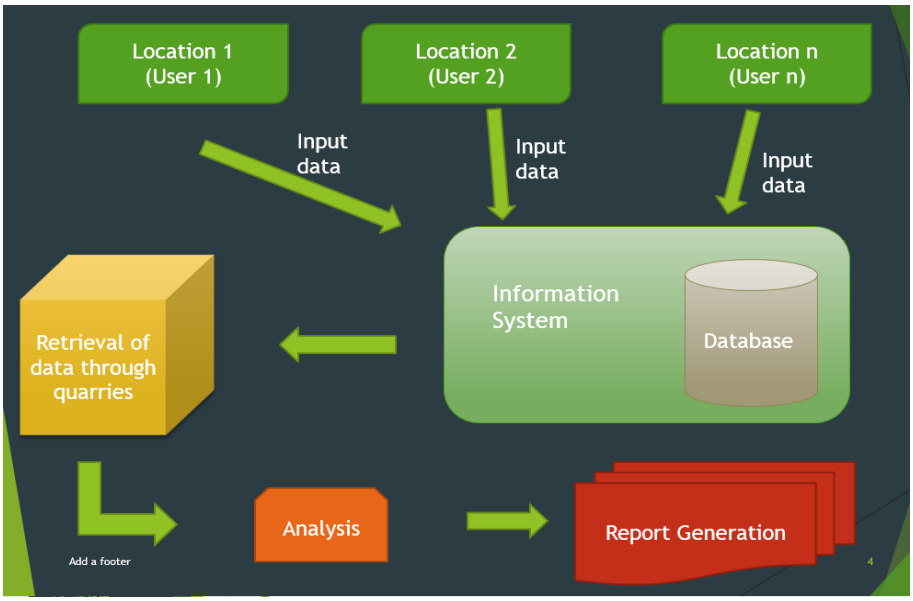
1. Introduction:

Need of information system

- Maintain Information about phytochemicals at centralized place
- Ability to carry out appropriate Statistical Analysis and Automate Uniform Reporting Process
- Provides Secure Access of Data for authorized users
- Allow different users at different location to provide inputs in database.

We have provided two Access's in this software (Information System): Restricted access and Open access. Restricted access is for the Authorized users and they can input and modify the data into the database. Open access is for Guest users who can only view the data. We have provided different restriction level for different type of users. Initially there are four types of users i.e. (admin, user-level(I), user-level(II),user-level(III)). Admin has all the rights to enter, delete, update, change settings, authenticate new users etc. User-level(I) will have the login credentials and can enter, delete and update data. User-level(II) can have the login credentials and they can only input data. User-level(III)(guest users) don't have login credentials so they cannot input anything but they make queries and view data and reports . After gathering the entire requirement, we had created a complete model of the software, which determines the complete working of it.



It has been finalized that the coding language and framework to be used in this project. We are using Python programing language with Django framework with the Pycharm IDE for the backend of the project. Currently we are using SQLlite for the Database as it is internally provided by Django framework and it will be updated to MySQL Database once the server is installed. For the

frontend we are using html, css, bootstrap and jquery to create the layout of the different pages in the system. The programming is going on in such a way, that both the backend and the frontend are implemented simultaneously. Initially the GUI has been developed to a certain extent, and then the functionalities will be added.

2. Information System: Brief report

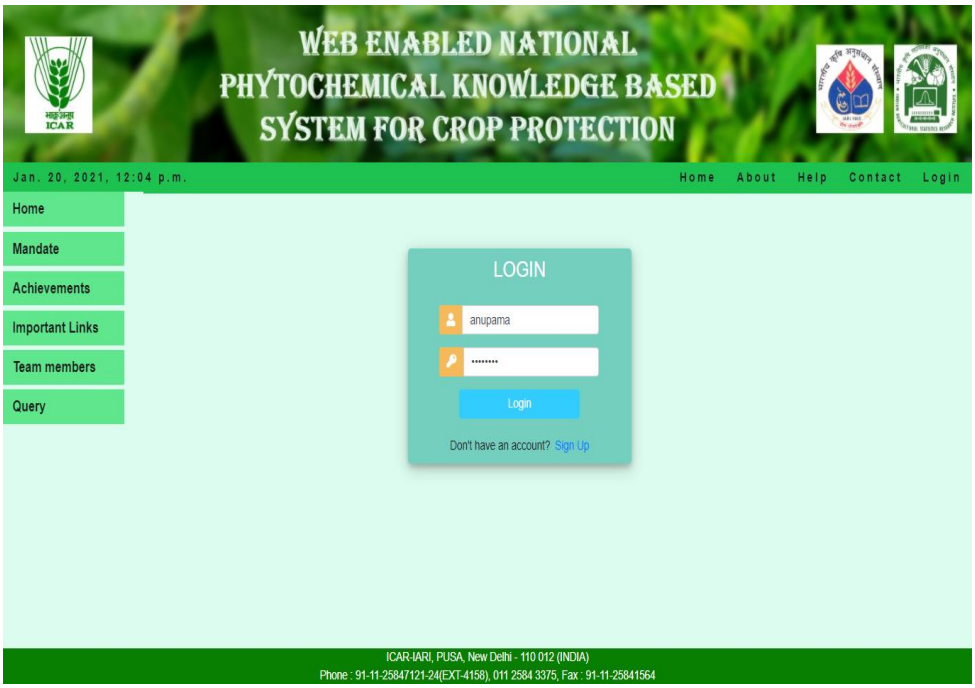
An information system has been developed and deployed in the IASRI website with URL http://naeagchem.icar.gov.in. The details and the screenshots of the information system has been described below.

(Home page)

We have divided the Home page into four division. In first division we have title of the project which is static and then in second division we have navigation bar which contains six buttons (Home, About, Help, Contact and Login) and it is

also static. Then we have side navigation bar which contains five tabs (Home, Mandate, Achievements, Important Links , Team members and Query) and it is dynamic and the number of tabs increase after login. The Fourth division is the main section of the page, which contains all the information, and it is dynamic.

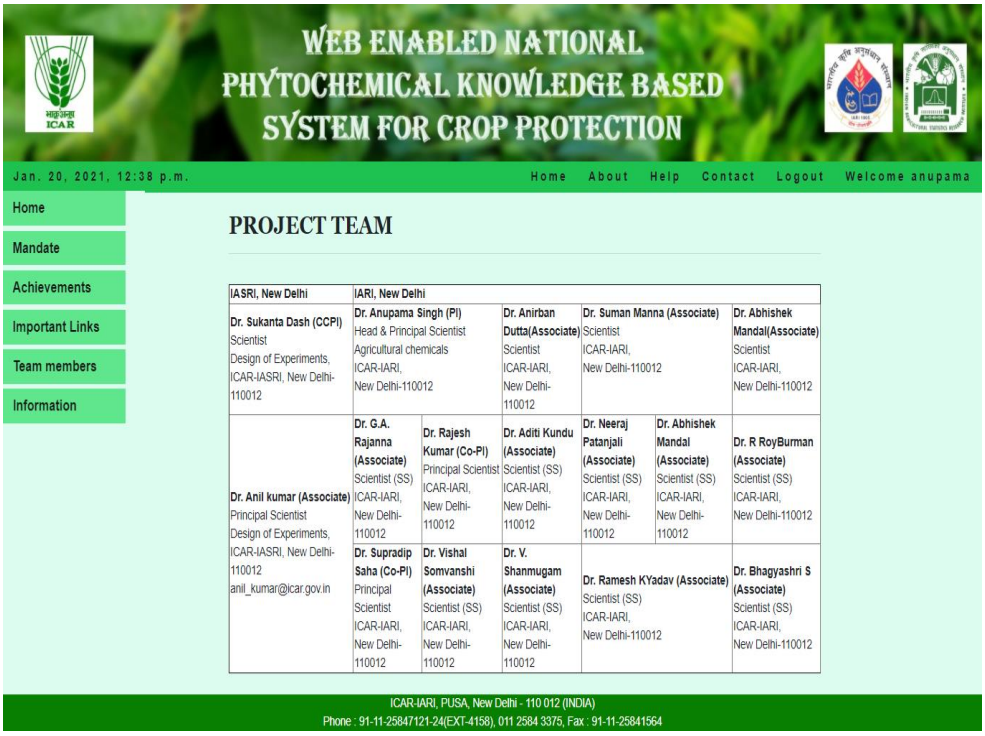
Registered and authenticated users have got login id and password with which they can login themselves to access the data input rights. After login they can access the form and input or retrieve information.



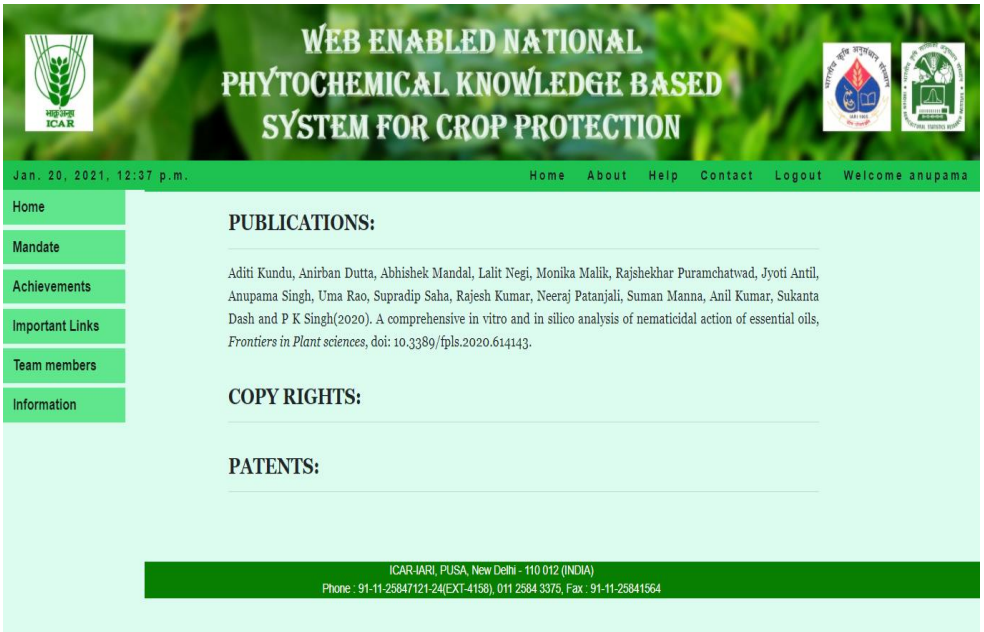
(Login page - Initially)

(After login)

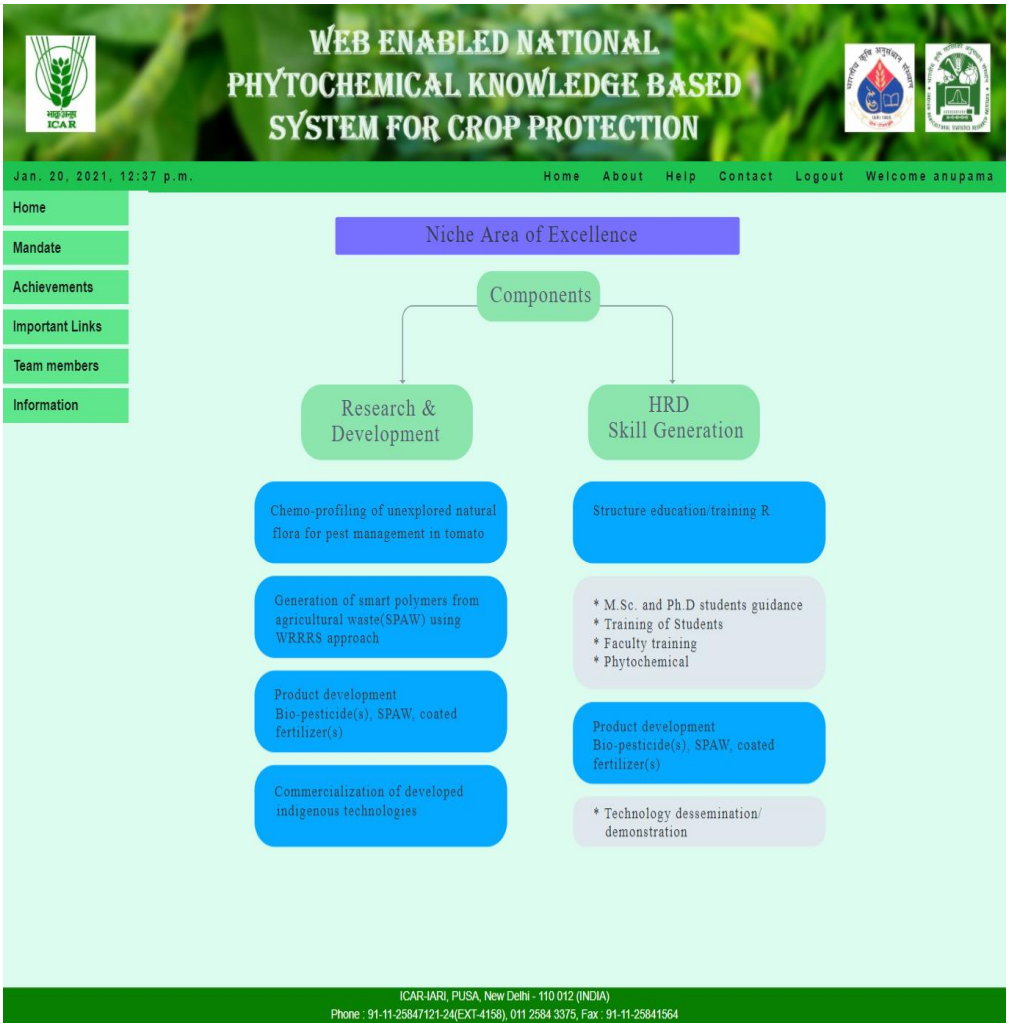
After logging in users can see they user-id at the top right of the navigation bar.



(Team Members)



(Achievements)


WEB ENABLED NATIONAL PHYTOCHEMICAL KNOWLEDGE BASED SYSTEM FOR CROP PROTECTION
Jan. 20, 2021, 12:37 p.m.
Home About Help Contact Logout Welcome anupama
Home
Mandate
Achievements
Important Links
Team members
Information
Niche Area of Excellence
Components
Research & Development
HRD Skill Generation
Chemo-profiling of unexplored natural flora for pest management in tomato
Generation of smart polymers from agricultural waste(SPAW) using WRRRS approach
Product development Bio-pesticide(s), SPAW, coated fertilizer(s)
Commercialization of developed indigenous technologies
Structure education/training R
* M.Sc. and Ph.D students guidance
* Training of Students
* Faculty training
* Phytochemical
Product development Bio-pesticide(s), SPAW, coated fertilizer(s)
* Technology dessemination/ demonstration
ICAR-IARI, PUSA, New Delhi - 110 012 (INDIA)
Phone : 91-11-25847121-24(EXT-4158), 011 2584 3375, Fax : 91-11-25841564

(Mandate)


WEB ENABLED NATIONAL PHYTOCHEMICAL KNOWLEDGE BASED SYSTEM FOR CROP PROTECTION
Jan. 20, 2021, 12:35 p.m.
Home About Help Contact Logout Welcome anupama
Home
Mandate
Achievements
Important Links
Team members
Information
ABOUT THE PROJECT
The project 'Plant source based environmentally safe crop protection and production technologies: Development and capacity building' denoted hereafter as PPPT is a Niche Area of Excellence Programme funded by Education Division, ICAR and managed by ICAR-IARI, New Delhi. The PPPT project, led by Division of Agricultural Chemicals, ICAR-IARI, is aimed at Creation of enabling environment for capacity building on safe crop protection technologies and on-farm practices that includes R&D and Education on plant Waste Recycling and Resource Recovery System (WRRRS) and creation of web enabled National Phytochemical database at the core of the scheme. The principal goal of the PPPT project is collaboratively ensuring consumer and environmental safety through capacity building on use of safe biopesticidal and resource management alternatives to synthetic options in crops. The project started on 14 th January 2019 and continues through to December 2021.
ABOUT THE SOFTWARE
NEED:
There is no such database exist in literature which contains all the information about phytochemical based crop protection and production technologies. Unavailability of these information restricts students and researchers to do further research in this area. That motivates us to develop a phytochemical knowledge based system for crop protection and production technology.
OUTCOMES:
• This software along with the database provides past and recent technology, based on crop protection and production.
• Any user like Researcher, Farmer, Student and Education staff can get benefit of viewing these technology details from this developed information system and can be used for their future research work.
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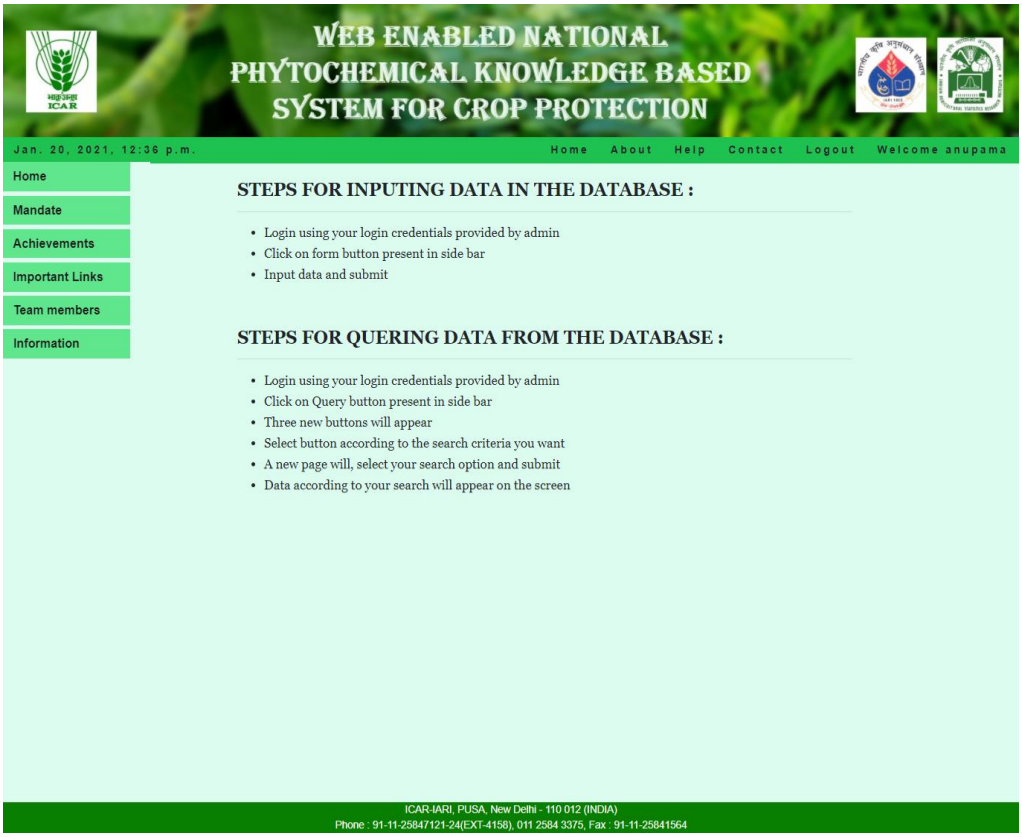
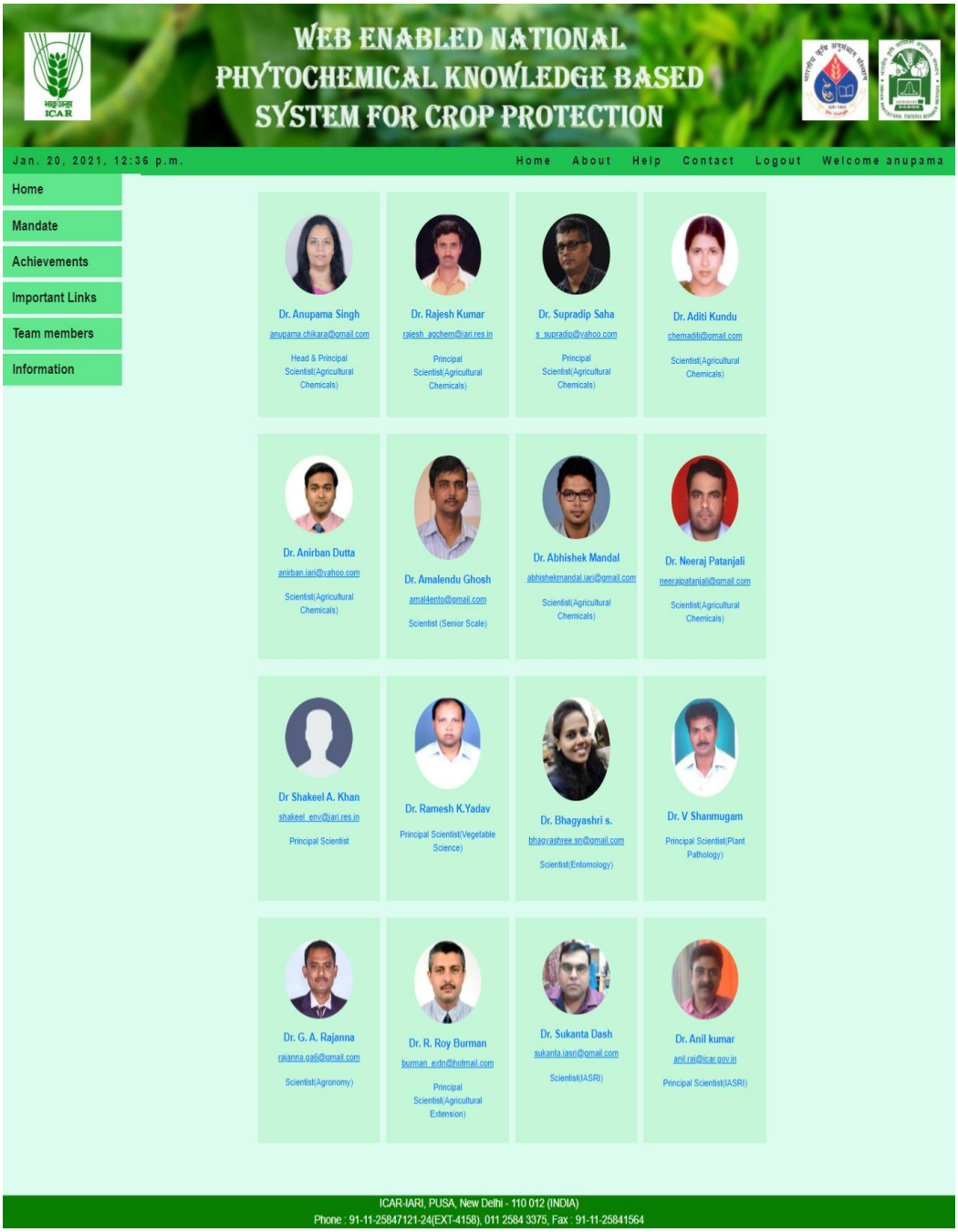
Phone : 91-11-25847121-24(EXT-4158), 011 2584 3375, Fax : 91-11-25841564

(About)



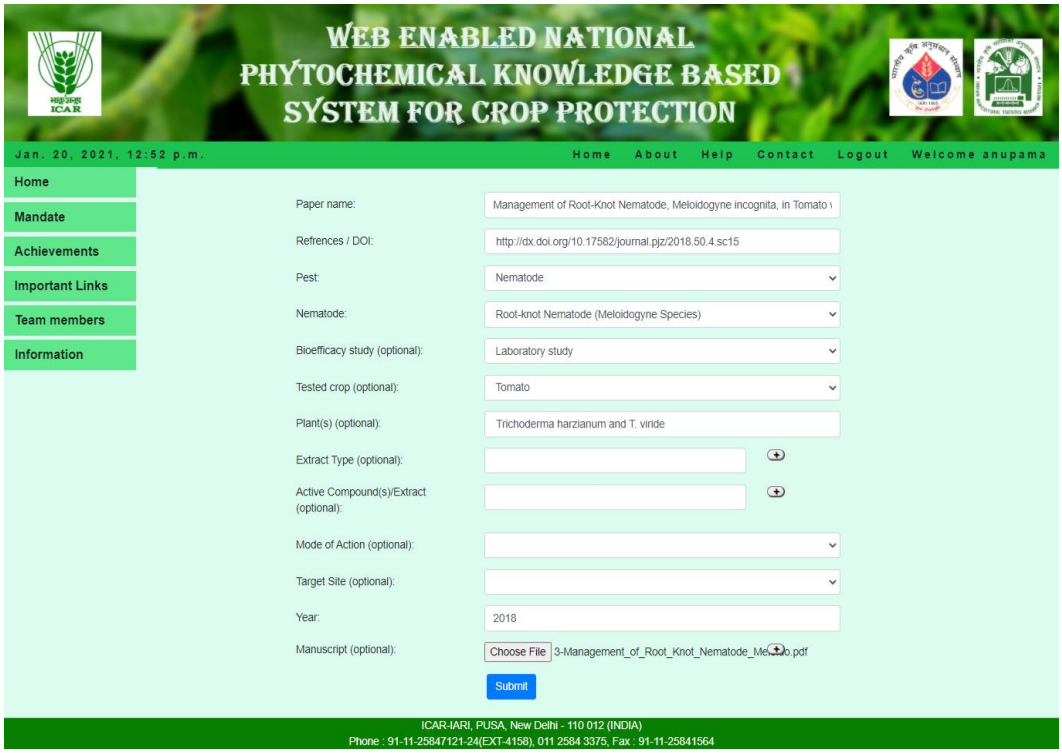
(Help)

(Important Links)



(Contact details)

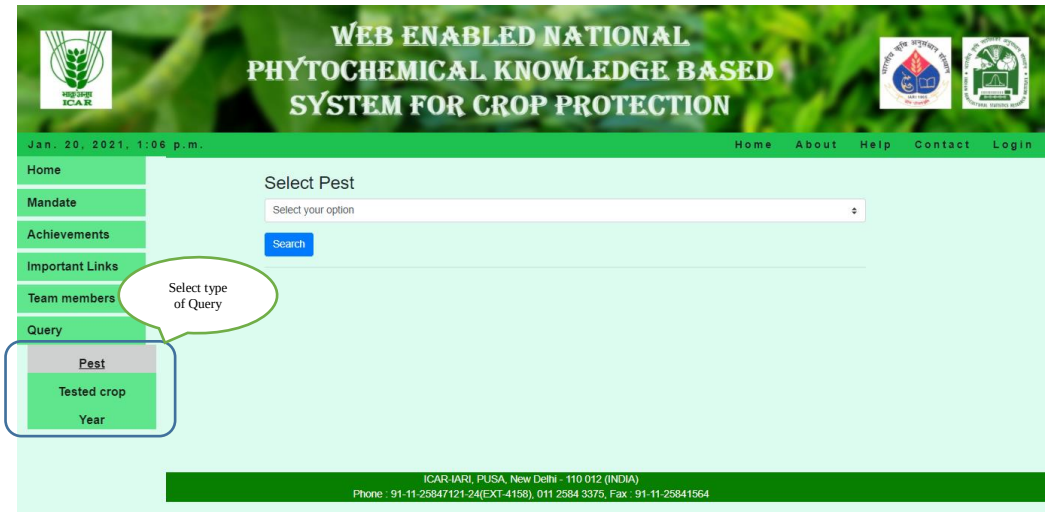
All the contacts are made hyperlinks of the respected contact detail pages.



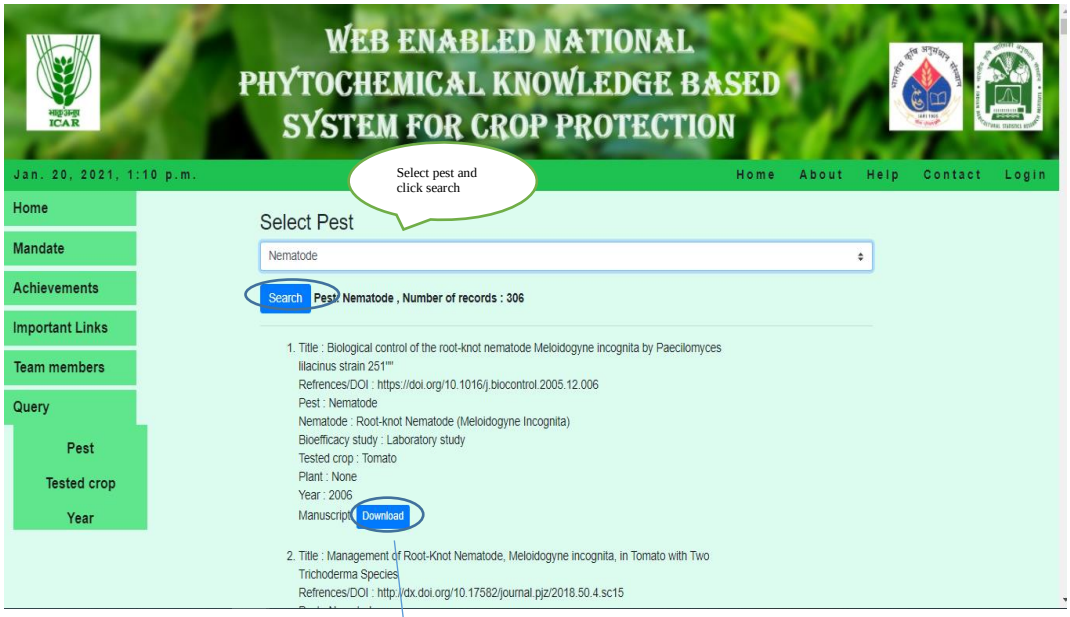
(Data entry Form layout)

The above layout show the form through which users can enter the information into the database.

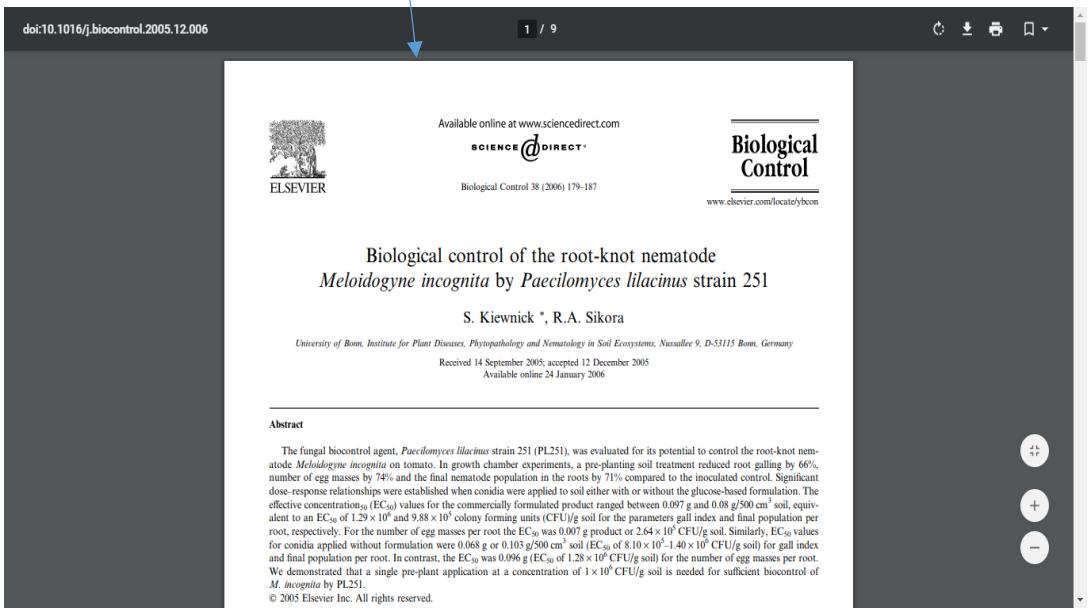
Data retrieval on the bases of type of pest, tested crop and year.
(User can query without login also)



(Types of queries)



(After querying output)



(After clicking download button-pdf)

Users can query data by accessing the query tab. Initially we have created three types of queries i.e. Information related to (i) pest (ii) crop and (iii) year. After querying users can download, the manuscript attached with the particular dataset.

3. Discussions:

This information system is made is create a database of the research's done in the field of plant source based environmentally safe crop protection and Production technologies. This database will be very helpful for everyone as it will be a

centralized information source for everyone who want's any kind of information related to the topic.

4. Future Scope:

This project has a very vast scope in future. When this project will be live many users would be able to take benefit of the database created in this project. Different users at different location would be able to access and share their experiments and would be able to contribute to the database of this information system. They will be able to generate reports and use them according to the requirement. Project can be updated in near future as and when requirement for the same arises, as it is very flexible in terms of expansion.