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Minimal cost multifactor experiments for agricultural research involving hard-to-change factors

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ABSTRACT

Multifactor experiments are very common in agricultural research. Randomizing run orders in multifactor experiments often witness in large number of factorwise level changes which will increase the cost and time of the experiments. Minimal cost multifactor experiments are such experiments where the cost of the experiment is minimum which can be achieved by choosing a factorial run order where the total number of factor level change is minimum as cost of the experiment is directly proportional to the number of level changes of factors. Here, a method of constructing minimal cost 2-level multifactor experiments with minimum number of factorwise level changes has been proposed. As for a same factorial combination, there may exist more than one minimally changed factorial run order, an exhaustive search was also performed to obtain all possible minimally changed run order for two level multifactorial experiments with three factors. Due to restricted randomization, adaption of these run orders may witness the effect of systematic time trend. Hence, the usual method of analysis may not be a feasible solution due to lack of randomization. Here, the analytical procedure of experiments using minimal cost multifactorial run order has also been highlighted based on a real experimental data. The work has been carried out at ICAR-Indian Agricultural Statistics Research Institute, New Delhi during 2019–20. The data from the real experiment used for explaining the analysis procedure has been collected from Climate Change Facility of ICAR-Indian Agricultural Research Institute farm, New Delhi, India based on experiments conducted during 2014–15.

Keywords: Exhaustive search, Hard-to-change factors, Minimum level changes, Minimal cost, Multifactor experiment, Run orders, Trend

Multifactor experiments help us in studying both main effects as well as interaction effects of factors involved in the experiments. Random execution of factorial run order is always desirable as it can avoid bias in the estimates of the effects of interest which might result due to time trend. However, random execution of run orders in multifactor experiments involving hard-to-change factors (where changing the levels of factors are difficult due to the cost structure or due to operational procedure may make the experimentation costly as the number of factor level changes is bound to increase in such situation.

In an experiment of soil microbial diversity (community level physiological profiling) through BIOLOG ecoplates for identifying the best treatment, three factors were tried, viz. (i) CO_2 [Two levels: Elevated CO_2 and Ambient CO_2], (ii) Fertilizer (Two levels: Organic and Inorganic) and (iii) Variety [two wheat varieties] [Bhowmik *et al.* 2020]. It was known in advance that changing the levels of CO_2 was very expensive. Therefore, if randomization was done on the level combinations, it would have increase the cost of the experiment to a great extent since the experiment involved hard-to-change factors. In this situation, use of a factorial design where the number of factor level changes is kept small, is advisable.

The number of factor level changes is a matter of serious concern to experimenters as one may witness situations where it is very difficult to change levels of some factors. In such situations, construction of factorial run orders where total number of factor level changes is minimum may be an alternative from cost perspective. Work have been done in these aspects [Draper and Stoneman (1968), Dickinson (1974), De León *et al.* (2005), Correa *et al.* 2009, 2012; Hilow (2013), Bhowmik *et al.* 2015, 2017, 2020; Varghese *et al.* 2017, Oprime *et al.* (2017), Pureza *et al.* 2020 etc.]. Here, some aspects of minimal cost (w.r.t. changes in factor

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levels) two level factorial design has been discussed.

MATERIALS AND METHODS

Condition for a factorial run order to be minimal cost run order: The minimum number of factorwise level changes is attained when only one factor level is changed on two successive experimental trials, or equivalently when only one sign is changed by passing from one row to the next in the design matrix. Bhowmik *et al.* (2015, 2017, 2020) have shown that if there are *k* factors with i^{th} factor is having s_i levels for $i = 1, 2, ..., k$, then the total number of level changes for the $\Pi^{K}_{i=1} S_i$ factorial design will be $(\Pi^{K}_{i=1} S_i)-1$, i.e. one less than the number of runs. Hence, for a 2^k factorial run order, i.e. for *2*-level factorial with *k* number of factors, minimum number of changes will be 2^k-1.

Method of constructing minimal cost 2^k factorial run order: Consider a 2^k factorial experiment, i.e. *2*-level factorial with *k* number of factors, Let the levels of the i^{th} [$i = 1,2,...k$] factor be denoted by ± 1 [where *-1* indicates the lower level and *+1* indicates the higher level]. The method starts with a single factor with *2* levels denoted as above, forming *2* rows and a single column. Then in order to add the 2^{nd} factor with *2* levels, repeat each row consecutively one more number of time resulting in *4* rows and single column. Now for the 2^{nd} factor, add a new column starting with entries ± 1 in ascending order of magnitude and then taking its fold over. This will give rise to a minimal cost 2^2 factorial with minimum level changes where total number of changes as *3* which is the minimum for the 2^2 factorial experiments. Similarly for minimal cost 2^3 factorial with minimum number of changes, consider the above minimal cost 2^2 factorial with minimum level changes. By following the same procedure as mentioned above, the last column, i.e. third factor is added by taking the fold over till the last entry of the column. This procedure can be extended for minimal cost 2^k factorial run order with minimum number of changes [where the total number of changes will be 2^k-1] by starting with a minimal cost 2^{k-1} factorial with minimum number of level changes. Following is an example of minimal cost 2^3 factorial with minimum number of factor level changes.

Example 1: For the minimal cost 2^3 factorial with minimum changes in factor levels, the total number of changes is *7* which is minimum possible value for a 2^3 factorial design. The total number of changes can be calculated by adding the factor-wise number of level changes of individual factor.

All possible minimal cost factorial run order through exhaustive search: Minimal cost run orders in factorial designs with minimum possible level changes are not unique and there may exist a number of such run orders. Keeping this in mind, in the present investigation exhaustive search has been implemented to obtain all possible minimal cost 2^3 factorial run order with minimum number of level changes where total changes for all the run order is *7*. The exhaustive search has been implemented using SAS 9.3 by developing a macro using Proc IML with single processor having the computational specification as follows:

Processor: Intel(R) Core(TM) i5-3470, CPU @ 3.20 GHz; RAM: 8 GB; Hard Disk Drive: 500 GB

For a 2^3 factorial design, the total possible number of run orders will be 8! = 40320. Thus, in case of 2^3 factorial design, in total 40320 number of run orders with different number of changes (including minimally changed run orders) are possible. In order to generate all possible comprehensive list of minimally changed run order for 2^3 factorial run orders, exhaustive search has been implemented which yields in total 144 number of minimal cost run orders with minimum level changes, i.e. with total number of change as 7 (i.e. 2^3-1 = 7). Out of these 144 run orders obtained through exhaustive search, there are 12 possible combinations where total number of change is 7, i.e. (1, 2, 4), (1, 4, 2), (2, 1, 4), (2, 4, 1), (4, 1, 2), (4, 2, 1), (1, 3, 3), (3, 1, 3), (3, 3, 1), (2, 2, 3), (2, 3, 2) and (3, 2, 2) respectively [numbers in brackets indicate factorwise level changes respectively]. First 6 combinations are occurring 8 numbers of times each and remaining 6 combinations are occurring 16 numbers of times each. Out of these 12 possible combinations, numbers of distinct combinations are 3 i.e. (1, 2, 4), (1, 3, 3) and (2, 2, 3) in different permutations. Every distinct combination is coming 48 number of times in different permutations. Since, cost is directly proportional to the factor level changes; therefore, for all the 144 designs generated by the exhaustive search, cost of experiments will be minimum.

Upper part of Table 1 highlights a minimal cost 2^3 factorial with minimum number of factor level changes obtained based on the above method where factor-wise number of level changes of the individual factors is 1, 2 and 4 respectively which adds up to 7. A random sample of 10 run orders out of all the 144 run orders of 2^3 minimally changed factorial design obtained through exhaustive search algorithm are also given in the lower part of Table 1.

Table 1 (i) Minimal cost 2^3 factorial run order based on the above method of construction [presence of letters indicate the higher levels of the factors with *(1)* indicate the lower levels of all the factors], (ii) Ten randomly chosen minimal cost 2^3 factorial run order out of 144 total run order obtained through exhaustive search [presence of letters indicate the higher levels of the factors with *(1)* indicate the lower levels of all the factors]

We have explained the analytical procedure for data generated through minimal cost factorial design using a real data set as follows:

Experimental site description and experimental detail: The experiment was conducted in free-air carbon dioxide enrichment (FACE) facility during *rabi* (26^{th} November, 2014-10^{th} April, 2015) at Climate Change Facility of ICAR-Indian Agricultural Research farm, New Delhi, India (28° 35′ N and 77° 12′ E, 217 m above mean sea level) with semiarid climatic condition and wheat crop was sown as test crop on 26^{th} November, 2014. The mean maximum and minimum air temperatures throughout wheat season were 25.2 and 8.8°C, respectively. Inside the FACE ring, soil texture was sandy loam (Typic Ustochrept) with

Table 1

(i) A minimal cost 2^3 factorial run order

A	B	C	Run order
-1	-1	-1	(1)
-1	-1	1	c
-1	1	1	bc
-1	1	-1	b
1	1	-1	ab
1	1	1	abc
1	-1	1	ac
1	-1	-1	a
Factorwise level changes			
1	2	4	

(ii) Ten randomly chosen minimal cost 2^3 factorial run order

Factor wise level change	*Total no. of change*	*Run order*
(1 , 2 , 4)	7	ab abc ac a (1) c bc b
(1 , 2 , 4)	7	(1) c bc b ab abc ac a
(1 , 2 , 4)	7	ac a ab abc bc b (1) c
(1 , 2 , 4)	7	bc b (1) c ac a ab abc
(1 , 3 , 3)	7	ac abc ab a (1) c bc b
(1 , 3 , 3)	7	(1) c bc b ab a ac abc
(1 , 3 , 3)	7	(1) b bc c ac a ab abc
(2 , 2 , 3)	7	abc ac a (1) c bc b ab
(2 , 2 , 3)	7	a (1) c bc b ab abc ac
(2 , 2 , 3)	7	(1) b bc abc ab a ac c

71.9% sand and 19.2% clay. The FACE ring is consisted of eight horizontal pipes which releases CO_2 and enriched air at the crop canopy level. Diameter of the ring was 8 m and it was made up of eight horizontal pipes. Carbon dioxide (CO_2) concentration inside the ring was measured by non-dispersive infrared gas analyzer (IRGA) and data was logged mechanically in the computer at each 5 min interval. Carbon dioxide (CO_2) concentration inside the FACE ring was maintained at around 550 ± 20 µmol/mol while crops grown outside the FACE ring was subjected to ambient CO_2 concentration, i.e. 400 µmol/mol. Treatments comprising chemical fertilizer with recommended dose of N-P-K (120-60-60) and FYM (total N-P-K requirement was fulfilled through FYM) was maintained throughut the crop growing season. One Aestivum wheat variety: HD 2967 and one durum wheat variety: HI 8663 were grown. In brief the treatment details are as follows:

CO_2: Elevated and ambient; Soil amendment: Inorganic fertilizer and organic fertilizer; Variety: HD 2967 and HI 8663

Carbon utilization pattern of the soil microbial community based on absorbance at 590 nano meter was recorded from each treatment combinations. The experiment was conducted in three replications. Thus, total 24 observations on carbon utilization pattern of the soil microbial community based on absorbance at 590 nano meter were available. On an average 100 cylinders are required throughout the wheat crop growing period to maintain elevated CO_2 treatment where cost of single cylinder is around ₹ 900. Thus apart from normal cultivation cost, it requires approximately ₹ 90 thousand for single FACE ring to run during the wheat crop for a single season. Also, initial setup cost for FACE is also expensive.

RESULTS AND DISCUSSION

It is to be noted that, for multi factorial experiments involving hard-to-change factors, use of minimal cost design with minimum number of changes may be useful. However, the analysis remains a matter of concern due to lack of proper randomization of run orders. One approach in this direction is to use randomization tests to identify significant factor levels, but the total number of allowable randomizations is likely to be very small. Alternatively, an Analysis of Covariance (ANCOVA) type of models by considering the influence of time trend as a covariate may be a feasible solution. Use of split plot type of model for analysis by taking the hard-to-change factor as main plot factor may be another possible alternative although in such situations only sub plot factor and main plot-sub plot interaction effects will be estimated with more precision. As in case of minimal cost factorial design with restricted randomization of run orders observation may not be independent of each other, the usual least square estimates will be biased. Hence, estimation of parameters by generalized least squares under a correlated error structure may be another possible alternative although this approach may be theoretically very difficult to understand from non statistician point of view.

From the experimental setup defined above, it can be seen that the experiment itself is a costly affair with the factor CO_2 as the difficult-to-change factor which restrict the potential of ensuring proper randomization during the planning stage of the experiment. Here, the analysis was conducted using a split plot setup by treating CO_2 as the main plot factor, whereas factorial structures of soil amendments with variety have been treated as subplot treatments. Thus, there are four subplot treatments for the present investigation. The analysis was performed using SAS 9.3. Based on the statistical analysis, it has been observed that there is a significant difference between the two types of CO_2 at 5% level of significance (P-value 0.041) with elevated CO_2 comes out to be better w.r.t. carbon utilization pattern of the soil microbial community based on absorbance at 590 nano meter. From analysis of variance (ANOVA), subplot treatments also comes out to be significant at 5% level of significance (P-value 0.023). It has been observed that among the four factorial subplot combinations of soil amendments and variety, organic fertilizer in combination with HD 2967 performs better w.r.t carbon utilization pattern of the soil microbial community based on absorbance at 590 nano meter. Based on ANOVA, the main plot and sub

Table 2 Mean table of interaction effects based on the ANOVA

Treatment	Elevated CO_2 $SP1_1$	Ambient CO_2 $SP1_1$	Row mean
Soil Amendments × Variety			
Inorganic × HD 2967	1.44[bc]	1.21[b]	1.325[b]
Inorganic × HD 8663	1.22[c]	1.05[c]	1.135[c]
Organic × HD 2967	1.51[a]	1.38[a]	1.445[a]
Organic × HD 8663	1.11[c]	1.10[c]	1.105[c]
Column mean	1.37[a]	1.185[b]	
General mean = 1.25			

plot interaction effect remains significant at 5% level of significance (P-value 0.015).

Among the interaction treatments, elevated CO_2 performs better in combination with organic fertilizer and HD *2967* w.r.t. carbon utilization pattern of the soil microbial community based on absorbance at *590* nano meter.

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