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Some remarks on the analysis of variance for diallel cross experiments

Wykorzystanie analizy wariancji do oceny wyników doświadczeń z zastosowaniem krzyżowania diallelicznego

The paper deals with three approaches to an analysis of variance for data obtained in a diallel cross experiment with spring rape. The data concerning plants height of 8 inbred lines and 56 crosses between them were used to illustrate consequences of approaches to the analysis. Formally, the experiment was carried out in unbalanced lattice square with subsamples on the units. In the first approach an appropriate analysis for this design is given. In the second approach the mean from subsamples was treated as an observation in a block designed experiment. In the third approach the resolvability property was used and the statistical analysis for the experiment carried out in a complete randomized design was proposed. The consequences in a statistical inference concerning statistical characteristics (general combining ability, specific combining ability) for two experiments with F_1 and with F_2 were examined.

Key words: analysis of variance, block design, resolvable block design, lattice square, diallel cross experiment

Praca dotyczy porównania trzech sposobów wnioskowania statystycznego dla dwóch doświadczeń z genotypami rodzicielskimi i mieszańcami (pokolenia F_1 i F_2) krzyżówek diallelicznych rzepaku jarego. Każde z tych doświadczeń zostało założone w częściowo zrównoważonej kracie kwadratowej dla 8 linii rodzicielskich i 56 mieszańców. Dokonano pomiaru wysokości każdej rośliny na poletku. Dla takiego układu została przeprowadzona analiza wariancji (sposób pierwszy). W podejściu drugim analizę przeprowadzono na średnich z kilku roślin na poletku. Trzeci sposób polegał na przeprowadzeniu analizy tak jak dla doświadczeń zakładanych w układzie o losowanych blokach. Konsekwencje zastosowania tych sposobów analizy przedstawiono na przykładzie wnioskowania o ogólnej i specyficznej zdolności kombinacyjnej badanych linii rodzicielskich.

Słowa kluczowe: analiza wariancji, układ blokowy, rozkładalny układ blokowy, krata kwadratowa, doświadczenie dialleliczne

INTRODUCTION

In a breeding program it is often necessary to decide on what kind of an experimental unit (observation of considered traits) it should be based. Sometimes we take as the unit a plant or a plot. But more often, the experimental unit is a sample of plants chosen from a plot. These approaches have some consequences in statistical data analysis, as well as practical consequences for performance of the experiments and for their costs.

The different significance of the units is directly related to the experimental design. Many breeding experiments are carried out in resolvable incomplete block designs. In this design a set of incomplete blocks is divided into subsets, called superblocks, in such a way that in each superblock all treatments occur once. Then, the number of treatment replications is equal to the number of superblocks in the experiment. In such an experiment we have two systems of blocking, one connected with the usual incomplete blocks that form superblocks, and another connected with the superblocks only. This means that, with respect to the first system of blocking, we have the experiment set up in an incomplete block design, while with respect to the second system we have the experiment set up in a completely randomized block design. In this paper the consequences of the different significance of units and different approaches to the design will be examined in a diallel mating system with spring rape.

More exactly, the breeders design the experiment taking into account the available plant material and technical constraints on the experiment. But it is often necessary to remodel the data obtained (Mejza, Mejza, 1989; Mejza, 1994). The reason for that decision is usually some (random) inaccuracy in the performance of the experiment or unexpectedly large or small variability of the experimental units. This remodeling should be close to the original design of the experiment. In this paper the remodeling is carried out in the class of block designs.

The spring rape diallel cross experiment is used to illustrate our remodeling of the experimental data. This allows us to get more information that permits the use of the data obtained in the designs with no additional structure of treatments.

The aim of the diallel cross experiment is to compare the breeding value of parents inbred lines (clones) to identify the best parents and their hybrids. The inferences on parents usefulness in practical breeding are based on such characteristics as general combining ability (gca) and specific combining ability (sca).

Hence, the general hypotheses concerning parent and hybrids effects can be split into orthogonal sub-hypotheses connected with (gca), (sca), and reciprocal (re) effects.

MATERIALS AND METHODS

In the experiment we considered 8 inbred lines of spring rape S_6 generation. The lines were obtained at the IHAR Experimental Station in Borowo (Poland). Then, in the Department of Genetics and Plant Breeding, Agricultural University of Poznań, diallel crosses (method I according to Griffing's (1956) crossing systems) were made. The F_1 and

F_2 progeny between the lines (after F_1-selfing) of these crosses were used in two field experiments performed at the Agricultural University Experimental Station in Dłoń.

The experimental design used for these two experiments was a partially balanced lattice square for 8×8 treatments (8 parental lines and 56 crosses) in four replications (cf. Cochran and Cox, 1957; Clathworthy, 1973). It is noteworthy that in each lattice square all 64 genotypes occur. In this sense the design is resolvable.

In each experiment we had 256 one-row plots with a length of 1 m and with 0.5 m as the distance between rows. Usually, 15 plants per plot were measured.

During the vegetation period and after harvesting the following agronomic traits were evaluated: diameter of root neck, plant height, height to the first branch, number of branches, number of pods per plant, number of seeds per plant, number of seeds per pod, 1000 seeds weight.

In this paper we will consider plant height only.

Hence, on the basis of the diallel cross experiment described above, we would like to:
— examine the necessity of taking 15 plants per plot for measurement (this makes the experiment expensive and there are not always so many plants available);
— examine the effectiveness of applying an incomplete blocks design;
— compare the variability of the examined traits in generations F_1 and F_2;
— examine three approaches to the analysis of experiments in relation to inferences concerning breeding characteristics.

STATISTICAL ANALYSIS AND DISCUSSION

We would like to solve the above-mentioned statistical analysis problems with respect to the three approaches to the analysis of data in the diallel cross experiments under consideration.

Statistical evaluation of data was performed using the program DGH (Kala *et al.* 1997).

Approach I

Let us treat the experiment as one carried out in an incomplete block design (32 blocks) with subsamples on the plots. The generations F_1 and F_2 will be considered separately. For the analysis we have taken 8 observations from a plot (because at least 8 observations were available on each of the plots). In this approach each plant was treated as the experimental unit.

In this case we did not take into account the lattice square structure of the experiment. In the diallel cross experiment very often we have small and differing numbers of seeds. In such a case the design is not orthogonal with respect to the number of units (plants). This problem is usually overcome by choosing the same number of units from each plot. The advantage of this approach is the availability of a good estimation of error which is based on a large number of degrees of freedom.

The appropriate analyses of variance are given in Tables 1 and 2.

Table 1

Analysis of variance for F_1 spring rape diallel crosses (8 × 8). Plant height (cm)

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Ratio F
Blocks	31	5602,6		
Crosses	63	19904,8	316,0	7,9**
including:				
gca	7	11903,2	1700,4	42,6**
sca	28	5786,4	206,7	5,2**
re	28	2215,2	79,1	2,0**
Residual	1953	77933,1	39,9	
including:				
lack of fit	161	5715,5	35,5	0,9
Error (within plot)	1792	72217,6	40,3	
Total	2047	103440,5		

** Significant at the 0.01 level

Table 2

Analysis of variance for F_2 spring rape diallel crosses (8 × 8). Plant height (cm)

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Ratio F
Blocks	31	7553,6		
Crosses	63	27028,0	429,0	10,1**
including:				
gca	7	16484,4	2354,9	55,5**
sca	28	6111,0	218,2	5,1**
re	28	4432,6	158,3	3,7**
Residual	1953	82713,9	42,4	
including:				
lack of fit	161	7270,7	45,2	1,1
Error (within plot)	1792	75443,2	42,1	
Total	2047	117395,5		

Approach II

Let us treat the experiments as carried out in an incomplete block design with 32 blocks of size 8. In this case the experimental unit is the plot, while the mean for plant measurements from a plot is treated as an observation.

The analyses for this approach are given in Tables 3 and 4.

Table 3

Analysis of variance for F_1 spring rape diallel crosses (8 × 8). Plant height (cm)

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Ratio F
Blocks	31	2602,6		
Crosses	63	20650,0	327,8	9,5**
including:				
gca	7	12870,5	1838,6	53,4**
sca	28	6376,4	227,7	6,6**
re	28	1403,1	50,1	1,5
Experimental error	161	5545,3	34,4	
Total	255	28797,9		

Table 4

Analysis of variance for F_2 spring rape diallel crosses (8 × 8). Plant height (cm)

Sourceof variation	Degrees of freedom	Sum of squares	Mean squares	Ratio F
Blocks	31	3542,6		
Crosses	63	19939,3	316,5	8,2**
including:				
gca	7	13808,2	1972,6	51,1**
sca	28	3011,0	107,5	2,9**
re	28	3120,1	111,4	2,9**
Experimental error	161	6220,7	38,6	
Total	255	29702,6		

Approach III

The experiment was carried out in a partially balanced lattice design. This means that this design is resolvable and superblocks are complete blocks. Then, such a superblock experiment is carried out in a completely randomized block design.

In this case we ignore the blocks in the lattice square. We do not verify a hypothesis concerning the block effects. But looking at Tables 3 and 4, we see that block variability is relatively small. This allows us to treat the experiment according to Approach III.

The results of analysis of variance for this approach are shown in Tables 5 and 6.

Table 5

Analysis of variance for F_1 spring rape diallel crosses (8 × 8). Plant height (cm)

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Ratio F
Superblocks	3	56,9		
Crosses	63	22450,0	356,3	10,7**
including:				
gca	7	13270,0	1895,7	56,9**
sca	28	7366,0	263,1	7,9**
re	28	1814,0	64,8	1,9
Experimental error	189	6291,0	33,3	
Total	255	28797,9		

Table 6

Analysis of variance for F_2 spring rape diallel crosses (8 × 8). Plant height [cm]

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Ratio F
Blocks	3	120,6		
Crosses	63	22362,0	355,0	9,3**
including:				
gca	7	14192,5	2027,5	53,1**
sca	28	3928,5	140,3	3,7**
re	28	4241,0	151,5	3,96**
Experimental error	189	7220,0	38,2	
Total	255	29702,6		

Let us consider the earlier questions in turn. There is no need to make a measurement of every plant if we are considering such a trait as plant height. The misfit of the model is small, and in this case it improves a little the conclusions from the analysis of both F_1 and F_2 hybrids.

The blocks in this experiment did not eliminate a considerable part of the total variability. This was probably due to their small surface area. The fertility of soil was uniformly distributed in the experiment. The length of blocks may have an influence on variability between blocks. It turned out that the variability within and between replications was relatively small in the considered experiment with generations F_1 and F_2.

In our experiments the block structures do not have any influence on inferences concerning diallel cross. This means that for this trait (plant height) soil variability in the experiments was very small. For other plant traits the situation may be different.

In this paper two experiments are considered, one of them for generation F_1 and the second for generation F_2. The variability of qualitative and quantitative traits in generation F_2 is usually larger than in F_1. This results from the segregation and recombination of the genes in which the parental lines differed. These experiments confirmed this opinion. In all methods of analysis of variance, the experimental error was always larger for hybrids in generation F_2

Additionally, we draw a similar inference concerning ANOVA and breeding characteristics (gca, sca) in generations F_1 and F_2.

In all the considered variants of calculation we observe significant differences among the hybrids analyzed. Also, the effects of general and specific combining abilities for the considered lines and traits are significant at the level $\alpha = 0.01$. There are some differences in the results concerning reciprocal effects (Tables 5 and 6.).

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