



## Efficient V-B Block Designs for CDC Method 4

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**Summary** - Some optimal incomplete block designs for complete diallel cross method 4 are known in literature. These designs require several replications for each cross and thus consume more resources such as experimental units, experimental material, time etc. So, there is a need to evolve designs which require minimum possible replications of parental lines. In this paper a method of construction of these designs is proposed by using mutually orthogonal Latin squares. These designs are connected for cross effects and perform well when compared to connected and not connected optimal designs reported by Dey and Midha (1996), Chai and Mukerjee (1999) and Gupta and Kageyama (1994), respectively.

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## I. INTRODUCTION

Orthogonal Latin squares are used for construction of Graeco Latin square, balanced incomplete block designs and square lattice designs. A set of  $p-1$  orthogonal Latin square of side  $p$  can always be constructed if  $p$  is a positive prime or power of a positive prime.

If  $p = 4t + 2$  and  $t > 1$ , then there exists pairs of mutually orthogonal Latin squares of order  $p$  (Bose, Shrikhande and Parker (1960)). From a practical view point, mutually orthogonal Latin squares are important and an exhaustive list of these squares is available in Fisher and Yates (1963). In this paper we use mutually orthogonal Latin squares in construction of mating designs for the diallel cross method 4 referred to Griffing (1956).

A diallel cross is a type of mating design used in plant breeding and animal breeding to study the genetic properties and potential of inbred lines or individuals. Let  $p$  denote the number of lines and let a cross between lines  $i$  and  $j$  be denoted by  $i \times j$ , where  $i < j = 0, 1, \dots, p-1$  and  $p(p-1)/2$  possible crosses. Among the four types of diallel discussed by Griffing (1956), method 4 is the most commonly used diallel in plant breeding. This type of diallel crossing includes the genotypes of one set of  $F_1$ 's means of the type  $(i \times j) = (j \times i)$ , but neither the parents nor the reciprocals with all possible  $v = p(p-1)/2$  crosses. This is sometimes referred to as the modified diallel. We shall refer to it as a complete diallel cross (CDC).

The problem of finding optimal mating designs for complete diallel cross experiments has received

attention in recent years; see Gupta and Kageyama (1994), Dey and Midha (1996) and Chai and Mukerjee (1999). Most of the results on optimal block designs for diallel crosses have been derived for the general combining ability (gca) under the assumptions that the model does not include parameters representing the specific combining ability (sca) Gupta and Kageyama (1994) and Dey and Midha (1996) but with few exceptions Chai and Mukerjee (1999) and Choi et al. (2002). The designs of these authors can be used to estimate specific combining ability (sca) but they demand more resources in terms of experimental units and experimental material. In such a situation there is need for designs which require minimum possible number of experimental units in conducting CDC experiments and are equally efficient in comparison to optimal block designs and randomized block designs when the model, in addition to the block effects and general combining ability, includes specific combining ability.

In the present paper we are proposing efficient variance balanced incomplete block designs for CDC experiments through mutually orthogonal Latin squares under the assumption that the model includes the parameter of specific combining ability.

## II. METHOD OF DESIGN CONSTRUCTION

It is known that when  $p$  is a prime positive integer or a power of prime positive integer, it is possible to construct  $(p-1)$  orthogonal Latin squares in such a way that they differ only in a cyclical interchange of the rows from  $2^{\text{nd}}$  to  $p^{\text{th}}$ . Such squares are taken for the construction of incomplete block designs for diallel crosses. For  $p = 6$ , such squares cannot be constructed.

Assume that there are  $p$  inbred lines and it is desired to find an incomplete block design for a mating design involving  $p(p-1)/2$  crosses. Out of  $(p-1)$  mutually orthogonal Latin square (MOLS), consider any two MOLS of semi-standard form of order  $p$  and superimposed one square over the other. We obtain one Graeco Latin square in which each cell contains ordered pairs of integers  $(i, j)$  taking values from 0 to  $p-1$ . These ordered pairs of integers occur once in a square. From Graeco Latin square remove the pairs of the type with  $i = j$  and considering other ordered pairs of integers as crosses between lines  $i$  and  $j$  and the columns as blocks. By doing so we get an incomplete block design for diallel cross experiment method 4 with parameters  $v = p(p-1)/2$ ,  $b = p$ ,  $k = p-1$ , and  $r = 2$ . The total

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number of experimental units to be allotted to  $v = p(p-1)/2$  is  $n = p(p-1)$ . Henceforth  $d(v, b, k)$  will denote the class of all block designs with  $v$  treatments,  $b$  blocks and block size  $k$ .

#### Example1:-

Let us consider the mating design for CDC experiment method 4 for  $p = 5$  parents. Consider two mutually orthogonal Latin squares  $L_1$  and  $L_2$  of semi-standard form of order 5. Superimposing one over the other square we get Graeco Latin square.

$L_1$					$L_2$				
0	1	2	3	4	0	1	2	3	4
1	2	3	4	0	2	3	4	0	1
2	3	4	0	1	4	0	1	2	3
3	4	0	1	2	1	2	3	4	0
4	0	1	2	3	3	4	0	1	2

After superimposition  $L_2$  over  $L_1$  and removing cross of the type  $i = j$  and considering columns as blocks, we obtain design  $d$  as given below:

Design  $d$

$B_1$	$B_2$	$B_3$	$B_4$	$B_5$
1×2	2×3	3×4	4×0	0×1
2×4	3×0	4×1	0×2	1×3
3×1	4×2	0×3	1×4	2×0
4×3	0×4	1×0	2×1	3×2

### III. ANALYSIS

For the analysis of data obtained from design  $d$ , we will follow Singh and Hinkelmann- (1998) two stage procedures for estimating gca and sca effects. The first stage is to consider the proposed designs to estimate cross effects, say,

$\tau = (\tau_{01}, \tau_{02}, \dots, \tau_{(p-2)(p-1)/2})$  for design  $d$  by the following model.

$$y = \mu 1 + X\tau + D\beta + e \quad (3.1)$$

Where  $y$  is an  $n \times 1$  vector of observations,  $1$  is the  $n \times 1$  vector of ones,  $X$  is the  $n \times v$  design matrix for treatments and  $D$  is an  $n \times b$  design matrix for blocks, that is, the  $(h, u)^{th}$  ( $(h, l)^{th}$ ) element of  $X$  (respectively, of  $D$ ) is 1 if the  $h^{th}$  observation pertains to the  $u^{th}$  cross (to  $l^{th}$  block), and is zero otherwise ( $h = 1, \dots, n$ ;  $u = 1, \dots, v$ ; and  $1, \dots, b$ ),  $\mu$  is a general mean,  $\tau$  is a  $v \times 1$  vector of treatment parameters,  $\beta$  is a  $b \times 1$  vector of block parameters and  $e$  is an  $n \times 1$  vector of residuals. It is assumed that vector  $\beta$  is fixed and  $e$  is normally distributed with  $E(e) = 0$ ,  $V(e) = \sigma^2 I$  and  $Cov(\beta, e') = (0)$ , where  $I$  is the identity matrix of conformable order.

$$\hat{g} = (Z' C_d C_d^{-1} C_d Z)^{-1} Z' Q_d = (Z' C_d Z)^{-1} Z' Q_d \quad (3.8)$$

Here the matrix  $(Z' C_d Z) = 2 p(p-3)/(p-1) [I_p - \frac{1}{p} 1_p 1_p']$ .

Following Tocher (1952), Raghavarao (1971) and Dey (1986), the least square method for the analysis of a proposed designs leads to the following reduced normal equations for the model (3.1).

$$C_d \tau = Q_d \quad (3.2)$$

Where  $C_d = r^{\delta} - N k^{-1} N'$  and  $Q_d = (Q_{1d}, \dots, Q_{vd}) = T - N k^{-\delta} B$

In the above expressions above  $r^{\delta}$  and  $k^{\delta}$  are diagonal matrices of order  $v \times v$  and  $b \times b$  with elements 2 and  $p$ , respectively of design  $d$ .  $N = X'D$  is the  $v \times b$  incidence matrix of the design  $d$ ;  $T = X'y$  and  $B = D'y$  are vector of cross totals and block totals of order  $v \times 1$  and  $b \times 1$  for design  $d$ , respectively.

Hence a solutions to (3.2) is given by

$$\hat{\tau} = C_d^{-} Q_d \quad (3.3)$$

Where  $C_d^{-}$  is a generalized inverses of  $C_d$  with property  $C C^{-} C = C$ . The sum of squares due to crosses are  $Q_d' C_d^{-} Q_d$  with degrees of freedom (d.f.) = rank ( $C_d$ ) for design  $d$  and expectation and variance  $Q_d$  is as

$$E(Q_d) = C_d \tau \text{ and } V(Q_d) = \sigma^2 C_d \quad (3.4)$$

Now we will utilize the above equations to estimate the genetic parameters in the proposed design. The second stage is to utilize the fact that the cross effects can be expressed in terms of gca and sca effects. So we can write

$$\tau_{ij} = g_i + g_j + s_{ij} \quad (3.5)$$

Where  $g_i$  ( $g_j$ ) is the gca for the  $i^{th}$  ( $j^{th}$ ) parent,  $s_{ij}$  ( $s_{ji}$ ) is the sca for the cross between the  $i^{th}$  and  $j^{th}$  parent ( $i < j = 0, 1, \dots, p-1$ ). In matrix notation equation (3.5) can be written as

$$\tau = Zg + s \quad (3.6)$$

Where  $Z = (z_{ui})$  ( $u = 1, 2, \dots, n$ ;  $i = 0, 1, \dots, p-1$ ) is the cross and gca relation matrix.

$z_{ui} = 2$ , if the  $u^{th}$  cross has both parents  $i$ .  
 $= 1$ , if the  $u^{th}$  cross has only one parent  $i$ .  
 $= 0$ , otherwise.

Following the approach used in Kempthorne and Curnow (1961), equation (3.2) can then be written as

$$C_d \tau = C_d Zg + C_d s \text{ or } E(Q_d) = C_d Zg + C_d s \quad (3.7)$$

Since the matrix  $C$  is singular, we use the unified theory of least square due to Rao (1973). So we get estimator of  $g$  as

$$\text{So } \hat{\mathbf{g}} = (\mathbf{Z}' \mathbf{C}_d \mathbf{Z})^{-1} \mathbf{Z}' \mathbf{C}_d \boldsymbol{\tau} \quad (3.9)$$

$$\text{Hence } \hat{\mathbf{g}} = \mathbf{H}_1 \boldsymbol{\tau}, \text{ where } \mathbf{H}_1 = (\mathbf{Z}' \mathbf{C}_d \mathbf{Z})^{-1} \mathbf{Z}' \mathbf{C}_d$$

$$\text{Now Cov } (\hat{\mathbf{g}}) = \mathbf{H}_1 \mathbf{C}_d \mathbf{H}_1' \sigma^2 = \sigma^2 (\rho-1)/2 \rho (\rho-3) \mathbf{I}_\rho \quad (3.10)$$

Since the covariance matrix of  $\hat{\mathbf{g}}$  is a constant times the identity matrix, therefore the proposed design d is variance-balanced for general combining ability effects. We thus have the following results.

#### Theorem

For a positive prime  $\rho > 3$ , if there exists a mutually orthogonal Latin square of order  $\rho$ , then there

$$\hat{\mathbf{s}} = (\mathbf{C}_d^{-1} - (\rho-1)/2 \rho (\rho-3) \mathbf{Z} \mathbf{Z}') \mathbf{Q}_d = (\mathbf{C}_d^{-1} - (\rho-1)/2 \rho (\rho-3) \mathbf{Z} \mathbf{Z}') \mathbf{C}_d \boldsymbol{\tau} = \mathbf{H}_2 \boldsymbol{\tau} \quad (3.11)$$

Where

$$\mathbf{H}_2 = (\mathbf{C}_d^{-1} - (\rho-1)/2 \rho (\rho-3) \mathbf{Z} \mathbf{Z}') \mathbf{C}_d$$

$$\text{Var } (\hat{\mathbf{s}}) = \mathbf{H}_2 \mathbf{C}_d \mathbf{H}_2' \sigma^2 \quad (3.12)$$

$$\text{Since } \mathbf{H}_1 \mathbf{1}_\rho = \mathbf{0}, \mathbf{H}_2 \mathbf{1}_\rho = \mathbf{0}, \mathbf{H}_1 \mathbf{H}_2' = \mathbf{0}, \text{rank } (\mathbf{H}_1) = \rho-1 \text{ and rank } (\mathbf{H}_2) = \rho-1.$$

It follows that  $\mathbf{g}$  and  $\mathbf{s}$  represented by treatment contrasts that carry  $\rho-1$  and  $\rho-1$  degrees of freedom respectively and that contrasts representing  $\mathbf{g}$  are orthogonal to those representing  $\mathbf{s}$ . It means the proposed design d allows for gca and sca effects to be estimated independently.

always exist variance- balanced incomplete block design for CDC experiment method 4.

Now substituting the estimate of  $\mathbf{g}$  in equation (3.6), we obtain the estimator of  $\mathbf{s}$ .

The sum of squares due to gca and sca for d are given by

$$\text{SS (gca)} = \mathbf{Q}_d' \mathbf{Z} (\mathbf{Z}' \mathbf{C}_d \mathbf{Z})^{-1} \mathbf{Z}' \mathbf{Q}_d \quad (3.13)$$

$$\text{SS (sca)} = \mathbf{Q}_d' (\mathbf{C}_d^{-1} - (\rho-1)/2 \rho (\rho-3) \mathbf{Z} \mathbf{Z}') \mathbf{Q}_d \quad (3.14)$$

The ANOVA is then given in Table 1.

Table 1 : Analysis of variance for design d

Source of variation	Degrees of Freedom	Sum of squares
Block	$\rho-1$	$\mathbf{B}' \mathbf{B} / \rho - G^2 / \rho (\rho-1)$
Crosses (adjusted for blocks)	$\text{rank } (\mathbf{C}_d)$	$\mathbf{Q}_d' \mathbf{C}_d^{-1} \mathbf{Q}_d$
gca	$\text{rank } (\mathbf{H}_1)$	$\mathbf{Q}_d' \mathbf{Z} (\mathbf{Z}' \mathbf{C}_d \mathbf{Z})^{-1} \mathbf{Z}' \mathbf{Q}_d$
sca	$\text{rank } (\mathbf{H}_2)$	$\mathbf{Q}_d' (\mathbf{C}_d^{-1} - (\rho-1)/2 \rho (\rho-3) \mathbf{Z} \mathbf{Z}') \mathbf{Q}_d$
Residual	$(n-1) - \text{rank } (\mathbf{C}_d) - \text{rank } (\mathbf{H}_1) - \text{rank } (\mathbf{H}_2)$	$\mathbf{y}' \mathbf{y} - G^2 / \rho (\rho-1) - \mathbf{B}' \mathbf{B} / \rho - \mathbf{Q}_d' \mathbf{C}_d^{-1} \mathbf{Q}_d$
Total	$n-1$	$\mathbf{y}' \mathbf{y} - G^2 / \rho (\rho-1)$

G = grand total of all n observations

#### IV. EFFICIENCY FACTOR

If instead of the proposed design d, one adopts a randomized complete block design with 2 blocks and each block contains  $\rho (\rho-1)/2$  crosses, the  $\mathbf{C}_R$ - matrix can easily shown to be

$$\mathbf{C}_R = 2 (\rho-2) (\mathbf{I}_\rho - 1/\rho \mathbf{J}_\rho) \quad (4.1)$$

Where  $\mathbf{I}_\rho$  is a identity matrix of order  $\rho$  and  $\mathbf{J}_\rho$  is a matrix of  $1$ 's. So that the variance of best linear unbiased estimate (BLUE) of any elementary contrast among the gca effects is  $\sigma_1^2 / (\rho-2)$ , where  $\sigma_1^2$  is the per observation variance in the case of randomized block experiment. It is clear from (3.10) that using design d each BLUE of any elementary contrast among gca effects is estimated with variance  $\sigma^2 (\rho-1) / \rho (\rho-3)$ . Hence efficiency factor E of design d as compared to randomized block design under the assumption of equal intra block variances is

$$E = (\sigma_1^2 / \sigma^2) \text{ is } \rho (\rho-3) / (\rho-1) (\rho-2) \quad (4.2)$$

In Tables 2, 3, and 4, we are presenting the efficiency factors of CDC by Gupta and Kageyama (1994), universally optimal and efficient block designs reported by Dey and Midha (1996) and design d in relation to randomized block design, respectively.

Table 2 : Efficiency of GK designs and designs d in comparison to RBD

S.No.	p	n	r	2k	$E_{GK}$	$E_d$
1	4	6	3	4	1.00	0.66
2	5	10	4	4	0.83	0.83
3	7	21	6	6	0.93	0.93
4	8	28	7	8	1.00	0.95
5	8	28	7	4	0.66	0.95
6	9	36	8	8	0.96	0.96
7	9	36	8	6	0.85	0.96
8	10	45	9	10	1.00	0.98
9	10	45	9	6	0.83	0.98

10	11	55	10	10	0.97	0.98
11	12	66	11	12	1.00	0.97
12	12	66	11	6	0.80	0.98
13	12	66	11	4	0.60	0.98
14	13	78	12	12	0.98	0.99
15	13	78	12	6	0.78	0.99
16	13	78	12	4	0.59	0.99
17	14	91	13	14	1.00	0.99
18	15	105	14	14	0.98	0.99
19	15	105	14	6	0.77	0.99

GK denotes Gupta and Kageyama

**Table 3 :** Efficiency of Optimal DM designs and designs d in comparison to RBD

S.No.	Ref. No.	p	$n_1$	$E_{DM}$	$n_2$	$E_d$
1	T2	5	30	0.83	20	0.83
2	T3	5	60	0.83	20	0.83
3	T4	5	90	0.83	20	0.83
4	T8	7	210	0.70	42	0.93
5	T22	7	210	0.93	42	0.93
6	T40	8	280	1.00	56	0.95
7	T41	9	252	0.96	72	0.96
8	T54	10	315	1.00	90	0.98

**Table 4 :** Efficiency of DM efficient designs and designs d in comparison to RBD

S.No.	Ref.	p	$n_1$	$E_{DM}$	$n_2$	$E_d$	S.No.	Ref	p	$n_1$	$E_{DM}$	$n_2$	$E_d$
1	T12	5	60	0.84	20	0.83	9	T58	5	60	0.84	20	0.83
2	T13	5	90	0.92	20	0.83	10	T60	5	60	0.97	20	0.83
3	T33	5	40	0.94	20	0.83	11	T94	7	210	0.84	42	0.93
4	T34	5	80	0.80	20	0.83	12	T95	7	210	0.91	42	0.93
5	T37	5	100	0.87	20	0.83	13	T77	8	196	0.98	56	0.95
6	T44	5	30	1.00	20	0.83	14	T85	9	252	1.00	72	0.96
7	T45	5	60	0.84	20	0.83	15	T91	10	405	0.92	90	0.98
8	T57	5	30	0.84	20	0.83							

DM denotes Dey and Midha , Ref means the design number reported by Dey and Midha(1996),  $n_1$  and  $n_2$  are number of experimental units required by Dey and Midha's designs and design d , respectively.  $E_{GK}$ ,  $E_{DM}$  and  $E_d$  are the efficiencies of GK, DM and design d in comparison of RCB, respectively.

## V. DISCUSSION

In Table 2, we find that for  $p = 4, 5, 8, 9, 10, 11, 12, 13$ , and 15 parental lines , the design d perform well in comparison to optimal diallel cross Gupta and Kageyama (1994). In Table 3, for  $p = 5, 7$  and 9 the performance of design d is more or less same in comparison to optimal design Dey and Midha (1996). In Table 4, for  $p = 5, 7, 8$  and 10 the design perform well in comparison to efficient designs. Since design d requires minimum possible experimental units, therefore, design d can be used in place of GK and DM designs for estimating gca and sca effects.

## VI. ILLUSTRATION

We show the essential steps of analysis of a diallel cross experiment, using an incomplete block design proposed in this paper. For this purpose, we take data from an unpublished experiment conducted by Dr. Terumi Mukai on *Drosophila melanogaster* Cockerham and Weir (1977) on page 203. For the purpose of illustration, we take data of relevant crosses from this experiment. Each cross is replicated twice. The layout and observations in parentheses are given below.

$B_1$	$B_2$	$B_3$	$B_4$	$B_5$
$1 \times 2$ (21.0)	$2 \times 3$ (16.8)	$3 \times 4$ (13.8)	$4 \times 0$ (18.8)	$0 \times 1$ (16.5)
$2 \times 4$ (15.2)	$3 \times 0$ (16.2)	$4 \times 1$ (12.2)	$0 \times 2$ (31.8)	$1 \times 3$ (13.0)
$3 \times 1$ (11.4)	$4 \times 2$ (15.4)	$0 \times 3$ (17.8)	$1 \times 4$ (13.6)	$2 \times 0$ (30.4)
$4 \times 3$ (15.2)	$0 \times 4$ (14.6)	$1 \times 0$ (15.4)	$2 \times 1$ (23.0)	$3 \times 2$ (16.3)

The following are the vector of treatment total, block total and adjusted treatment total, respectively.

$T = (31.9, 62.2, 34.0, 33.4, 44.0, 24.4, 25.8, 33.10, 30.6, 29.0)'$

$B = (62.8, 63.0, 59.2, 87.2, 76.2)'$

$Q = (-1.95, 21.35, 3.45, -4.15, 6.50, -10.35, -10.80, -1.70, -0.85, -1.50)'$

ANOVA, estimates of gca, and sca along with their standard errors are shown in Tables 5, 6 and 7.

**Table 5 :** Analysis of variance of the data

Source	D.F	Sum of squares	Mean sum of square	F
Blocks	4	137.81		
Crosses	9	418.92	46.54	53.70
g.c.a	4	341.70	85.42	98.56
s.c.a	5	77.20	15.44	17.81
Intra block error	6	5.2	0.86	
Total	19	561.93		

**Table 6 :** Estimates of the general combining ability and their estimated standard error

Parent	Estimates of (gca )	$\pm$ S E
0	-1.24	0.4147
1	-0.65	0.4147
2	-2.16	0.4147
3.	2.58	0.4147
4.	1.47	0.4147

**Table 7 :** Estimates of sca effects and their estimated standard error

SCA	Estimate of (sca)	$\pm$ S E	SCA	Estimate of (sca)	$\pm$ S E
S <sub>01</sub>	-0.63	0.4818	S <sub>13</sub>	-5.29	0.4818
S <sub>02</sub>	8.65	0.4818	S <sub>14</sub>	-5.61	0.4818
S <sub>03</sub>	3.13	0.4818	S <sub>23</sub>	-1.26	0.4818
S <sub>04</sub>	-3.04	0.4818	S <sub>24</sub>	0.52	0.4818
S <sub>12</sub>	2.58	0.4818	S <sub>34</sub>	0.95	0.4818

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