

Original Article

Comparison of Methods of Estimating Variance Components in Dual Channel Microarray Applied in Split-Plot Designs

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Received: 02 June 2023

Revised: 05 July 2023

Accepted: 17 July 2023

Published: 31 July 2023

Abstract - The quality of estimation of variance components depends on the design used as well as on the unknown values of the variance components. In this article, three designs (split-plot design with the whole-plot arranged in complete randomized design and sub-plot arranged in randomized complete block design (SPD (CRD, RCBD)), split-plot design with the whole-plot arranged in complete randomized design and the sub-plot arranged in Latin square design (SPD (CRD, LSD)), and split-plot design with the whole-plot arranged in Latin square design and sub-plot arranged in randomized complete block design (SPD (LSD, RCBD))) for assessing gene expression in dual channel microarray were evaluated and compared. Three methods of sample pairing (vertical loop method (design A), cross loop method (design B) and horizontal loop method (design C)) in dual channel microarray applied on split-plot designs for obtaining variance components was used for the comparison, in order to ascertain which method gives the minimal variance components for the effects of interest. The results showed that design A had the least variance for comparing all the treatment effects except for the whole-plot treatment on SPD (CRD, RCBD), design C had the least variance for comparing all the treatment effects except for the whole-plot treatment on SPD (CRD, LSD), and design C had the least variance for comparing all the treatment effects except for the column treatment on SPD (LSD, RCBD).

Keywords - Microarray experiment, Dual channel microarray, Split plot design, Variance components.

1. Introduction

Microarray is an important tool used in measuring gene expression where different genes are arranged in arrays for identifying genes whose level of expression changes in response to treatments [11, 17, 2]. Microarray experiments are necessary for biologist in understanding genetic limitation, estimating high performance gene expression and gene sequences descriptions for natural organism populations [1, 14, 5]. The dual-channel microarray or two-colour microarray is a microarray that usually contains two different colours called cyanine 3 (green) and cyanine 5 (red) to measure the expression of genes. The dual-channel microarray offers some unique advantage like the opportunity to compare and hybridize on the same microarray slide, two target samples under identical washing and hybridization conditions and also, makes it possible to use analysis of variance to evaluate the effects of each factor [4].

The dual-channel microarray experiment for testing the effects of the difference between the levels of treatments can be performed in any experimental designs such as complete randomized design (CRD), randomized complete block design (RCBD), Latin square design (LSD) and split-plot design (SPD). SPD is an incomplete block design in which some effects are confounded with block but inter-block information is used to obtaining information for the confounded effects. Applying dual-channel microarray on SPD makes confounding more complex because some important effects may be confounded with arrays. The information between the arrays can then be use in estimating the effects that are confounded with array when there is sufficient replication [9]. Many studies on microarrays have been carried out but not limited to the following [9, 7, 18, 6, 15]. Microarray experiments are costly in terms of raw materials, availability of samples, time-consuming and the limited number of replications giving rise to unreliable variance estimates, therefore special care is required when working on microarray experiments.



[16] provided an overview of the various issues to be considered in the design of microarray experiments like; experimental design, data preparation, image analysis, normalization, quality control and statistical inference in minimizing the effect of unwanted variation and increasing the precision of the parameter estimates of interest. The precise estimates of the parameters of interest can be obtained using the method of variance components estimation. Variance components estimates the contribution of each random effect to the variance of the dependent variable. The most important issue in estimating variance components lies on the appropriate formulation of the model or the equivalent formulation of the variance table analysis that is forming all the appropriate treatment combinations, [13] and also, the ability to identify the best design that can accurately evaluate gene expression differences between treatments. [12] and [10] discussed the importance of using appropriate design to get most precise parameter estimates in the underlying statistical model, so a well-designed experiment is an important and fundamental step in planning a microarray experiment to assess differential expression of genes under study.

In order to ascertain the best design method that is suitable for comparing the parameter effects of interest, this paper evaluated the variance components estimates using three methods called, vertical loop method (design A), cross loop method (design B) and horizontal loop method (design C) associated with dual channel microarray applied on different arrangement of SPD: SPD with the whole-plot arranged in CRD and sub-plot arranged in RCBD, SPD with the whole-plot arranged in CRD and the sub-plot arranged in LSD, and SPD with the whole-plot arranged in LSD and sub-plot arranged in RCBD.

2. Split-Plot Designs

[3] described different ways of arranging the whole-plot and sub-plot treatments in SPD. Let the whole-plot be treatment A and sub-plot be treatments B. For the purpose of this work, three ways of arrangement were considered: SPD with the whole-plot arranged in CRD and sub-plot arranged in RCBD denoted by SPD (CRD, RCBD). In this design, the levels of treatment A are randomly assigned to the whole-plot and the levels of the sub-plot treatment B are randomly assigned to the sub-plots within each whole-plot; SPD with the whole-plot arranged in CRD and the sub-plot arranged in LSD denoted by SPD (CRD, LSD). Here each subject say A are randomly assigned to the whole-plots, each level of the whole-plot forms the row for assigning treatment B with the order of application forming the column; SPD with the whole-plot arranged in LSD and sub-plot arranged in RCBD denoted by SPD (LSD, RCBD). Here, treatment A are arranged in an $a \times a$ Latin square in accordance with the randomization procedures of the LSD. In each whole-plot the levels of the B-factor are applied to the split-plots according to a RCBD. The models for SPD (CRD, RCBD), SPD (CRD, LSD) and SPD (LSD, RCBD) are given by (1), (2) and (3) respectively.

$$Y_{ijk} = \mu + \alpha_i + w_{ij} + \beta_k + (\alpha\beta)_{ik} + \varepsilon_{ijk} \quad (1)$$

$$i = 1, 2, \dots, a; j = 1, 2, \dots, r; k = 1, 2, \dots, b$$

$$\sum_i \alpha_i = 0; \sum_k \beta_k = 0; w_{ij} \sim N(0, \sigma_{wp}^2); \varepsilon_{ijk} \sim N(0, \sigma_e^2)$$

where, μ is the mean response; α_i is the effect of treatment A assigned to the whole-plot; w_{ij} is the whole-plot error; β_k is the effect of treatment B assigned to the sub-plot; $(\alpha\beta)_{ik}$ is the interaction of the whole-plot and sub-plot treatments; ε_{ijk} is the sub-plot error.

$$Y_{ijk(l)} = \mu + \alpha_i + w_{ij} + \gamma_k + \beta_l + (\alpha\beta)_{il} + (\alpha\gamma)_{ik} + \varepsilon_{ijk(l)} \quad (2)$$

$$i = 1, 2, \dots, a; j = 1, 2, \dots, r; l, k = 1, 2, \dots, b$$

$$\sum_i \alpha_i = 0; \sum_l \beta_l = 0; \sum_k \gamma_k = 0; w_{ij} \sim N(0, \sigma_{wp}^2); \varepsilon_{ijk(l)} \sim N(0, \sigma_e^2)$$

where, μ is the mean response; α_i is the effect of treatment A assigned to the whole-plot; w_{ij} is the whole-plot error; γ_k is the k^{th} order effect; β_l is the effect of treatment B assigned to the sub-plot; $(\alpha\beta)_{il}$ is the interaction between the whole-plot and sub-plot treatments; $(\alpha\gamma)_{ik}$ is the interaction of treatment A and order effect; $\varepsilon_{ijk(l)}$ is the sub-plot error.

$$Y_{ijkl} = \mu + \rho_i + \gamma_j + \alpha_k + w_{ijk} + \beta_l + (\alpha\beta)_{kl} + \varepsilon_{ijkl} \quad (3)$$

$$i, j, k = 1, 2, \dots, a; l = 1, 2, \dots, b$$

$$\sum_i \rho_i = 0; \sum_k \alpha_k = 0; \sum_l \beta_l = 0; \sum_j \gamma_j = 0; w_{ijk} \sim N(0, \sigma_{wp}^2); \varepsilon_{ijkl} \sim N(0, \sigma_e^2)$$

where, μ is the mean response; ρ_i is the i^{th} effect of the row; γ_j is the j^{th} effect of the column; α_k is the effect of treatment A assigned to the whole-plot; w_{ijk} is the whole plot error; β_l is the effect of treatment B assigned to the sub-plot; $(\alpha\beta)_{kl}$ is the interaction between the whole-plot and sub-plot treatments; ε_{ijkl} is the sub-plot error.

3. Sample Pairing on Dual Channel Microarray Experiment Applied on Split-plot Designs

Sample pairing is the arrangement of samples or the way samples are assigned in a microarray experiment. The pairing of samples to the microarray is done in such a way that unbiased minimal variance estimates of the effects of interest are ensured, [8]. The sample pairing was done on different arrangement of the split-plot designs; SPD (CRD, RCBD), SPD (CRD, LSD) and SPD (LSD, RCBD) where two treatment (*A* & *B*) was considered with 2 levels each. The levels of treatment *A* are assigned to the whole-plot factor while the levels of treatment *B* are assigned to the sub-plot within the whole-plot. For each of the Split-plot designs, three methods of sample pairing; vertical loop (design A), cross loop (design B), and horizontal loop (design C) were applied respectively. The samples are paired onto the levels of treatment *A* and *B* assigned to the whole-plot and sub-plot respectively with the arrow indicating the samples paired on individual array. The arrowhead represents the sample dyed with cy5 (red colour) and the arrow tail represents the sample dyed with cy3 (green colour). The two dyes are added to the fixed effect of the whole-plot and sub-plot factors of the experiment giving three fixed effects and two arrays used per replicate with a total of twelve arrays for the entire experiment. The overall design of the experiment gives design with whole-plot factor, sub-plot factor and dyes. The assignment of treatment levels within each replication can only accommodate four treatment combinations per replicate, therefore the design is an incomplete design with two types of replication denoted by replication type I and II respectively depending on the common effect of the treatment combinations and three replications within each type of replication. The structure of the design depends on the design used for the whole-plot factor, sub-plot factor, and the assigning of the arrays as can be seen from Figures 1 to 9. The models for dual channel microarray applied on SPD (CRD, RCBD), SPD (CRD, LSD), and SPD (LSD, RCBD) are presented in (4), (5) and (6) respectively.

$$Y_{ijklm} = \mu + \alpha_i + w_{ij} + \beta_k + \theta_m + a_{l(j)} + \varepsilon_{ijklm} \quad (4)$$

$i = 1, 2, \dots, a(\text{levels of whole - plot treatment } A); k = 1, 2, \dots, b(\text{levels of the sub - plot treatment } B);$

$j = 1, 2, \dots, r(\text{replication}); l = 1, 2, \dots, p(\text{array}); m = 1, 2, \dots, t(\text{colour})$

$$Y_{ijo(k)ml} = \mu + \alpha_i + w_{ij} + \gamma_o + \beta_k + \theta_m + a_{l(j)} + \varepsilon_{ijo(k)ml} \quad (5)$$

$i = 1, 2, \dots, a(\text{levels of treatment } A); j = 1, 2, \dots, r(\text{replication}); o = 1, 2, \dots, d(\text{levels of order});$

$k = 1, 2, \dots, b(\text{levels of treatment } B); m = 1, 2, \dots, t(\text{colours}); l = 1, 2, \dots, p(\text{array})$

$$Y_{ircjklm} = \mu + \alpha_i + \rho_r + \gamma_c + w_{ircj} + \beta_k + \theta_m + a_{l(j)} + \varepsilon_{ircjklm} \quad (6)$$

$i = 1, 2, \dots, a(\text{levels of treatment } A); j = 1, 2, \dots, r(\text{replication}); r = 1, 2, \dots, m(\text{levels of row});$

$c = 1, 2, \dots, n(\text{levels of column}); k = 1, 2, \dots, b(\text{levels of treatment } B); m = 1, 2, \dots, t(\text{colour}); l = 1, 2, \dots, p(\text{array})$

3.1. Vertical loop method (Design A) for SPD (CRD, RCBD)

The vertical loop method of sample pairing shown in Figure 1, is the pairing of samples in a vertical form within the levels of whole-plot treatment *A* and across the levels of the sub-plot treatment *B*. The assignment of treatment combinations can only accommodate four treatment combinations within a replicate, the remaining treatment combinations are moved to the next replicate. Replicate with the same treatment combinations common to each other are grouped into replication type I and the remaining treatment combinations are grouped into replication type II. The assignment of the samples using the vertical loop method showed the confounding between treatment *A* and the array, hence the model is simplified by merging the whole-plot and the array terms in the model given in (1) to a single-term.

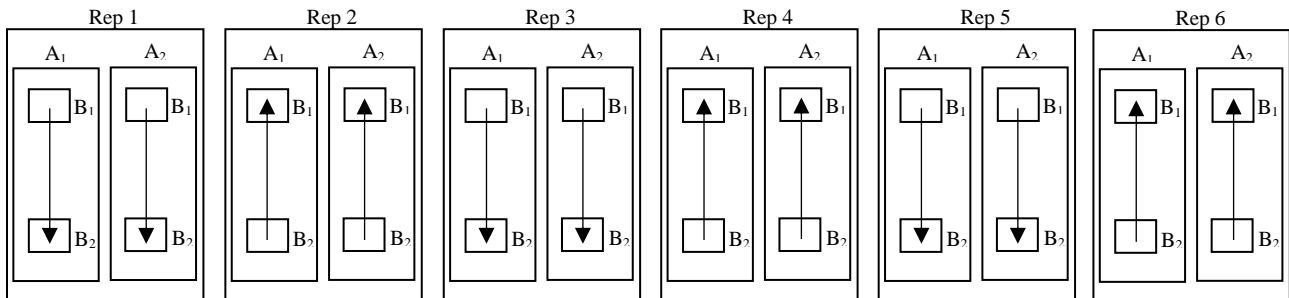


Fig. 1 Dual channel microarray applied on SPD (CRD, RCBD) using vertical loop method

From Figure 1; rep1, 3, and 5 belongs to rep type I with treatments combination (1, 1, G); where the first 1 represents level 1 of the whole-plot treatment, the second 1 denotes level 1 of the sub-plot treatment and G represents the colour dyed on the first level of the whole-plot and first level of the sub-plot treatments. Others includes: (2, 1, G), (1, 2, R), and (2, 2, R); while rep 2, 4, and 6 are in replication type II with treatments combination: (1, 1, R), (2, 1, R), (1, 2, G), and (2, 2, G). The whole plot and array effects are confounded and the resultant model is given by (7)

$$Y_{ijklm} = \mu_{ilm} + (w + a)_{ikj} + \varepsilon_{ijklm} \quad (7)$$

$i = 1,2(\text{levels of whole - plot treatment } A); j = I,II(\text{type of replication}); m = R,G(\text{colour})$

$l = 1,2 (\text{levels of sub - plot treatment } B); \text{ and } k = 1,2,3(\text{replicate within type of replication})$

such that $\sum_{ilm} \mu_{ilm} = 0; (w + a)_{ikj} \sim N(0, \sigma_{wp+array}^2); \varepsilon_{ijklm} \sim N(0, \sigma_e^2)$

where, μ_{ilm} are the fixed effect of whole-plot treatment A, sub-plot treatment B and dye (colour); $(w + a)_{ikj}$ are the confounded random effect of the whole-plot and array; ε_{ijklm} is the residual error; $\sigma_{(wp+array)}^2$ is the variance of the confounded effect of the whole-plot and array and σ_e^2 is the residual variance. The analysis of variance (ANOVA) table for design A is shown in Table 1.

3.2. Cross loop method (Design B) for SPD (CRD, RCBD)

Cross loop method of sample pairing shown by Figure 2, is the pairing of samples in a crossed form across levels of sub-plot treatment B and whole-plot treatment A respectively. Also, like the vertical loop method, replicate with the same treatment combinations are grouped into replication type I and replication type II. In addition to the subscript for denoting treatment B, a subscript is required to indicate the different arrays. The whole-plot and the array effects have subscripts showing the replication type each belongs to.

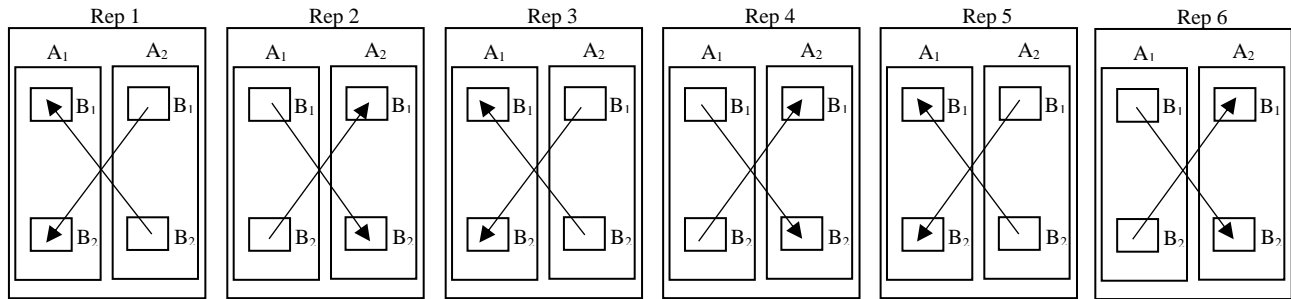


Fig. 2 Dual channel microarray applied on SPD (CRD, RCBD) using cross loop method

From Figure 2, rep1, 3, and 5 belongs to replication type I with treatments combination (1, 1, R), (1, 2, R), (2, 1, G), and (2, 2, G); while rep 2, 4, and 6 are in replication type II with treatments combination; (1, 1, G), (1, 2, G), (2, 1, R), and (2, 2, R) and represented with the model given by (8).

$$Y_{ijklmn} = \mu_{ilm} + w_{ikj} + a_{jkn} + \varepsilon_{ijklmn} \quad (8)$$

$i = 1,2(\text{levels of whole - plot treatment } A); j = I,II(\text{type of replication}); l = 1,2(\text{levels of treatment } B);$

$m = R,G(\text{colours}); n = 1,2(\text{array}) \text{ and } k = 1,2,3(\text{replication within type of replication})$

such that $\sum_{ilm} \mu_{ilm} = 0; a_{jkn} \sim N(0, \sigma_{array}^2); (w)_{ikj} \sim N(0, \sigma_{wp}^2); \varepsilon_{ijklmn} \sim N(0, \sigma_e^2)$

where, μ_{ilm} are the fixed effect of treatment A, treatment B and dye colour; w_{ikj} is the random effect of the whole-plot; a_{jkn} is the random effect of the array; ε_{ijklmn} is the random effect of the residual; σ_{array}^2 is the variance of the array and σ_{wp}^2 is the variance of the whole-plot error. The ANOVA table for design B is shown in Table 2.

3.3. Horizontal loop method (Design C) for SPD (CRD, RCBD)

Horizontal loop method of sample pairing shown by Figure 3, is the pairing done in a horizontal form within each level of sub-plot treatment *B* and across the levels of whole-plot treatment *A*. Also like the vertical and cross loop methods, replicate with the same treatment combinations are grouped into replication type I and replication type II respectively. From the assignment of the samples, the array and treatment *B* are confounded resulting in using a single subscript.

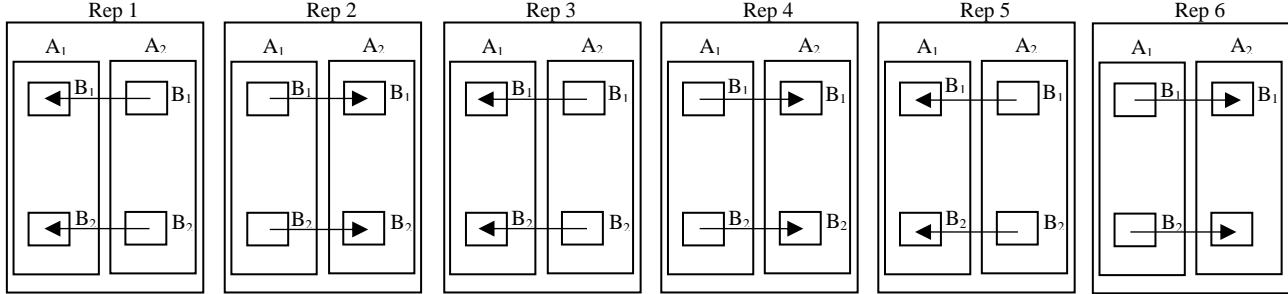


Fig. 3 Dual channel microarray applied on SPD (CRD, RCBD) using horizontal loop method

From Figure 3, we observed that rep1, 3, and 5 belongs to replication type I with treatments combination (1, 1, *R*), (1, 2, *R*), (2, 1, *G*), and (2, 2, *G*) while rep 2, rep 4, and rep 6 are in replication type II with treatments combination; (1, 1, *G*), (1, 2, *G*), (2, 1, *R*), and (2, 2, *R*) and can be represented with the model in (9).

$$Y_{ijklm} = \mu_{ilm} + w_{ikj} + a_{jkl} + \varepsilon_{ijklm} \quad (9)$$

$i = 1, 2(\text{levels of whole - plot treatment } A); j = I, II(\text{type of replication});$

$l = 1, 2(\text{levels of sub - plot treatment } B); m = R, G(\text{colour}) \text{ and } k = 1, 2, 3(\text{replicate within type of replication})$

such that $\sum_{ilm} \mu_{ilm} = 0; a_{jkl} \sim N(0, \sigma_{array}^2); (w)_{ikj} \sim N(0, \sigma_{wp}^2); \varepsilon_{ijklm} \sim N(0, \sigma_e^2)$

where, μ_{ilm} are the fixed effect of treatment *A*, treatment *B* and dye (colour); w_{ikj} is the random effect of the whole-plot; a_{jkl} is the random effect of the array; ε_{ijklm} is the random effect of the residual. The ANOVA table for design C is shown in Table 3.

Table 1. Analysis of variance table for design A on SPD (CRD, RCBD)

Source of Variation (SV)	Degree of Freedom (df)	Expected Mean Square (EMS)
Whole-plot (treatment A)	1	$\sigma_e^2 + 2\sigma_{(wp+array)}^2 + 2\varphi_A$
Sub-plot (treatment B)	1	$\sigma_e^2 + 2\varphi_B$
Treatment A*Treatment B	1	$\sigma_e^2 + \varphi_{AB}$
Colour (C)	1	$\sigma_e^2 + 4\varphi_C$
Treatment A*ColourC	1	$\sigma_e^2 + 2\varphi_{AC}$
Treatment B * ColourC	1	$\sigma_e^2 + 2\sigma_{(wp+array)}^2 + 2\varphi_{BC}$
Treatment A* Treatment B*ColourC	1	$\sigma_e^2 + 2\sigma_{(wp+array)}^2 + \varphi_{ABC}$
Reptype*Treatment A*(Repwithin)	4	$\sigma_e^2 + 2\sigma_{(wp+array)}^2$
Residual	12	σ_e^2

$$\text{where, } \varphi_A = (a - 1)^{-1} \sum_{i=1}^2 \alpha_i^2; \varphi_B = (b - 1)^{-1} \sum_{l=1}^2 \beta_l^2; \varphi_{AB} = ((a - 1)(b - 1))^{-1} \sum_{il=1}^2 (\alpha\beta)_{il}^2;$$

$$\varphi_C = (m - 1)^{-1} \sum_{m=1}^2 \theta_m^2; \varphi_{AC} = ((a - 1)(m - 1))^{-1} \sum_{im=1}^2 (\alpha\theta)_{im}^2; \varphi_{BC} = ((b - 1)(m - 1))^{-1} \sum_{lm=1}^2 (\beta\theta)_{lm}^2;$$

$$\varphi_{ABC} = ((a - 1)(b - 1)(m - 1))^{-1} \sum_{ilm=1}^2 (\alpha\beta\theta)_{ilm}^2$$

Table 2. Analysis of variance table for design B on SPD (CRD, RCBD)

SV	df	EMS
Whole-plot (treatment A)	1	$\sigma_e^2 + 2\sigma_{wp}^2 + 2\phi_A$
Sub-plot (treatment B)	1	$\sigma_e^2 + 2\phi_B$
Treatment A* Treatment B	1	$\sigma_e^2 + 2\sigma_{array}^2 + \phi_{AB}$
Colour(C)	1	$\sigma_e^2 + 2\sigma_{wp}^2 + 4\phi_C$
Treatment A * ColourC	1	$\sigma_e^2 + 2\sigma_{wp}^2 + 2\sigma_{array}^2 + 2\phi_{AC}$
Treatment B * ColourC	1	$\sigma_e^2 + 2\sigma_{array}^2 + 2\phi_{BC}$
Treatment A* Treatment B * ColourC	1	$\sigma_e^2 + \phi_{ABC}$
Reptype*Treatment A*(Repwithin)	4	$\sigma_e^2 + 2\sigma_{wp}^2$
Array*Reptype(Repwithin)	4	$\sigma_e^2 + 2\sigma_{array}^2$
Residue	8	σ_e^2

Table 3. Analysis of variance table for design C on SPD (CRD, RCBD)

SV	df	EMS
Whole-plot (treatment A)	1	$\sigma_e^2 + 2\sigma_{wp}^2 + 2\phi_A$
Sub-plot (treatment B)	1	$\sigma_e^2 + 2\sigma_{array}^2 + 2\phi_B$
Treatment A* Treatment B	1	$\sigma_e^2 + \phi_{AB}$
Colour(C)	1	$\sigma_e^2 + 2\sigma_{wp}^2 + 4\phi_C$
Treatment A * ColourC	1	$\sigma_e^2 + 2\sigma_{wp}^2 + 2\sigma_{array}^2 + 2\phi_{AC}$
Treatment B * ColourC	1	$\sigma_e^2 + 2\phi_{BC}$
Treatment A* Treatment B *ColourC	1	$\sigma_e^2 + 2\sigma_{array}^2 + \phi_{ABC}$
Reptype*TrtA*Colour (Repwithin)	4	$\sigma_e^2 + 2\sigma_{wp}^2$
Array*TrtB*Reptype(Repwithin)	4	$\sigma_e^2 + 2\sigma_{array}^2$
Residual	8	σ_e^2

3.4. Vertical Loop Method (Design A) for SPD (CRD, LSD)

The vertical loop method of sample pairing is the pairing of samples in a vertical form within the levels of the sub-plot treatment B and across the levels of the whole-plot treatment A. Only four treatment combinations are accommodated in each replicate. From assignment of the samples, a single subscript is needed unlike design B that needs another subscript to denote the array.

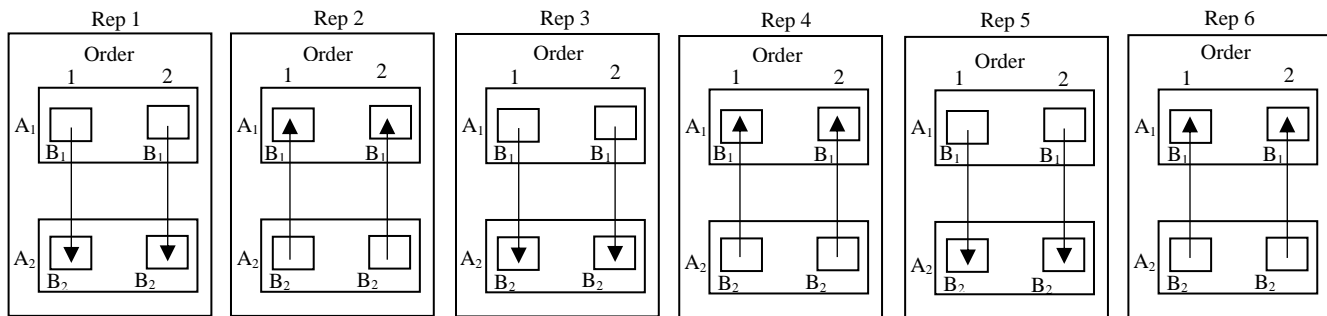


Fig. 4 Dual channel microarray applied on SPD (CRD, LSD) using vertical loop method

From Figure 4; rep1, 3, and 5 belongs to replication type I with treatments combination [1, 1(1), G]; where the first 1 represents level 1 of the whole-plot treatment, the second 1 denotes level 1 of the sub-plot treatment with the first level of order nested on the sub-plot, and G represents the colour dyed on the level 1 of the whole-plot and level 1of order nested on first level of the sub-plot treatments. Others includes: [1, 2(2), G], [2, 2(1), R], and [2, 1(2), R] while rep 2, 4, and 6 are in replication type II with treatments combination; [1, 1(1), R], [1, 2(2), R], [2, 2(1), G], and [2, 1(2), G] and is represented with the model given in (10).

$$Y_{ijko(l)m} = u_{io(l)m} + w_{ikj} + a_{jko(l)} + \epsilon_{ijko(l)} \quad (10)$$

$i = 1, 2$ (levels of whole – plot treatment A); $j = I, II$ (type of replication); $o = 1, 2$ (order effect); $l = 1, 2$ (levels of the sub – plot treatment B); $m = R, G$ (colour) and $k = 1, 2, 3$ (replicate within type of replication) where, $\mu_{io(l)m}$ are the fixed effect of treatment A, order (o) nested on treatment B, and dye colour; w_{ikj} are the random effect of the whole-plot error; $a_{jko(l)}$ are the random effect of the array; $\varepsilon_{ijko(l)}$ is the random effect of the residual. The ANOVA table for design A is shown in Table 4.

3.5. Cross loop method (Design B) for SPD (CRD, LSD)

The cross loop method of sample pairing is the pairing of samples in a cross form within the levels of treatment B at the sub-plot and across the levels of treatment A at the whole-plot. From the assignment of the samples and in addition to the subscript for denoting order nested on treatment B, a subscript is required to indicate the different arrays

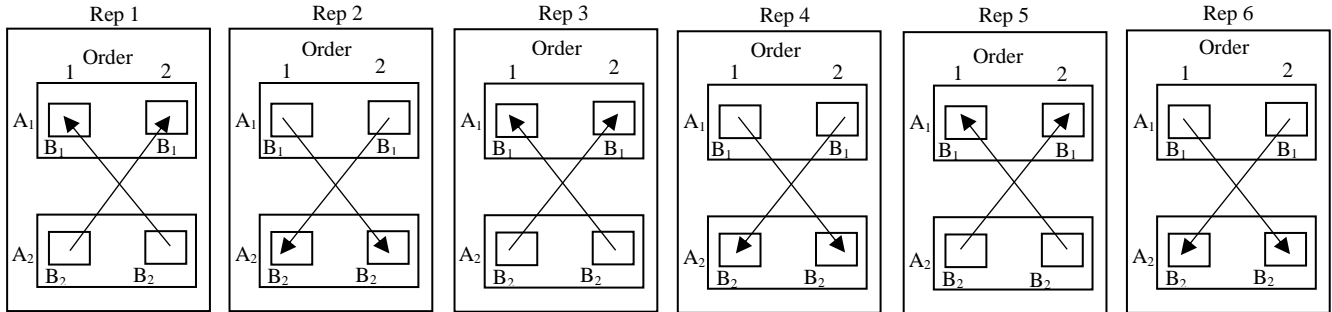


Fig. 5 Dual channel microarray applied on SPD (CRD, LSD) using cross loop method

From Figure 5; rep1, 3, and 5 belongs to replication type I with treatments combination [1, 1(1), R]; where the first 1 represents level 1 of the whole-plot treatment, the second 1 denotes level 1 of the sub-plot treatment with the first level of order nested on the sub-plot, and R represents the colour dyed on the level 1 of the whole-plot and level 1of order nested on first level of the sub-plot treatments. Others includes: [1, 2(2), R], [2, 2(1), G], and [2, 1(2), G] while rep 2, 4, and 6 are in replication type II with treatments combination; [1, 1(1), G], [1, 2(2), G], [2, 2(1), R], and [2, 1(2), R] and is represented with the model given in (11).

$$Y_{ijko(l)mn} = \mu_{io(l)m} + w_{ikj} + a_{jkn} + \varepsilon_{ijko(l)mn} \quad (11)$$

$i = 1, 2$ (levels of whole – plot treatment A); $j = I, II$ (type of replication); $o = 1, 2$ (order effect);

$l = 1, 2$ (levels of sub – plot treatment B); $m = R, G$ (colour); $n = 1, 2$ (array) and $k = 1, 2, 3$ (replicate within type of replication)

where, $\mu_{io(l)m}$ are the fixed effect of treatment A, order (o) nested on treatment B, and dye colour; w_{ikj} are the random effect of the whole-plot error; a_{jkn} are the random effect of the array; $\varepsilon_{ijko(l)mn}$ is the random effect of the residual. The ANOVA table for design B is shown in Table 5.

3.6. Horizontal loop method (Design C) for SPD (CRD, LSD)

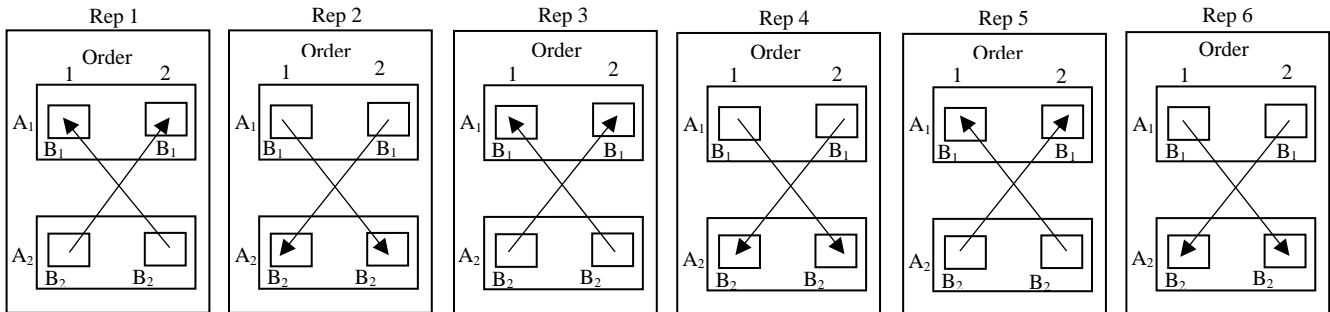


Fig. 6 Dual channel microarray applied on SPD (CRD, LSD) using horizontal loop method

The vertical loop method of sample pairing is the pairing of samples in a horizontal form within the levels of treatment A and across the levels of the sub-plot treatment B. The assignment of the samples using the vertical loop method showed the confounding between treatment A and the array, hence the model is simplified by merging the whole-plot and the array terms in the model given in (9) to a single term.

From Figure 6; repl, 3, and 5 belongs to replication type I with treatments combination [1, 1(1), R]; where the first 1 represents level 1 of the whole-plot treatment, the second 1 denotes level 1 of the sub-plot treatment with the first level of order nested on the sub-plot, and R represents the colour dyed on the level 1 of the whole-plot and level 1of order nested on first level of the sub-plot treatments. Others includes: [1, 2(2), G], [2, 2(1), G], and [2, 1(2), R] while rep 2, 4, and 6 are in replication type II with treatments combination; [1, 1(1), G], [1, 2(2), R], [2, 2(1), R], and [2, 1(2), G]. The whole-plot effect and array effect are confounded and is represented with the model given in (12).

$$Y_{ijk(o)lm} = \mu_{io(l)m} + (w + a)_{ikj} + \varepsilon_{ijk(o)lm} \quad (12)$$

$$i = 1, 2(\text{levels of whole - plot treatment A}); j = I, II(\text{type of replication}); m = R, G(\text{colour});$$

$$k = 1, 2, 3(\text{replicate within type of replication}); o = 1, 2(\text{order effect}); l = 1, 2(\text{levels of sub - plot treatment B})$$

where, $\mu_{io(l)m}$ are the fixed effect of treatment A, order (o) nested on treatment B, and dye colour; $(w + a)_{ikj}$ are the confounded random effect of the whole-plot error and the array; $\varepsilon_{ijk(o)lm}$ is the random effect of the residual. The ANOVA table for design C is shown in Table 6.

Table 4. Analysis of variance table for design A on SPD (CRD, LSD)

SV	df	EMS
Treatment A	1	$\sigma_e^2 + 2\sigma_{wp}^2 + 4\varphi_A$
Treatment B	1	$\sigma_e^2 + 2\sigma_{array}^2 + 4\varphi_B$
Treatment A *Treatment B	1	$\sigma_e^2 + 2\varphi_{AB}$
Order(O)	1	$\sigma_e^2 + 4\varphi_o$
Treatment A * Order	1	$\sigma_e^2 + 2\sigma_{wp}^2 + 2\varphi_{AO}$
Colour(C)	1	$\sigma_e^2 + 2\sigma_{wp}^2 + 6\varphi_C$
Treatment A * Colour	1	$\sigma_e^2 + 2\sigma_{wp}^2 + 2\sigma_{array}^2 + 4\varphi_{AC}$
Treatment B *Colour	1	$\sigma_e^2 + 4\varphi_{BC}$
Treatment A *Treatment B * Colour	1	$\sigma_e^2 + 2\sigma_{array}^2 + 2\varphi_{ABC}$
Order * Colour	1	$\sigma_e^2 + 2\sigma_{array}^2 + 4\varphi_{OC}$
Treatment A *Order *Colour	1	$\sigma_e^2 + 2\sigma_{wp}^2 + 2\sigma_{array}^2 + 2\varphi_{AOC}$
Reptype*TreatmentA*Colour(Repwithin)	4	$\sigma_e^2 + 2\sigma_{wp}^2$
Array*Treatment B*Reptype(Repwithin)	4	$\sigma_e^2 + 2\sigma_{array}^2$
Residual	4	σ_e^2

$$\text{where, } \varphi_A = (a - 1)^{-1} \sum_{i=1}^2 \alpha_i^2; \varphi_B = (b - 1)^{-1} \sum_{l=1}^2 \beta_l^2; \varphi_{AB} = ((a - 1)(b - 1))^{-1} \sum_{il=1}^2 (\alpha\beta)_{il}^2;$$

$$\varphi_o = (b - 1)^{-1} \sum_{o=1}^2 \gamma_o^2; \varphi_{AO} = ((a - 1)(b - 1))^{-1} \sum_{io=1}^2 (\alpha\gamma)_{io}^2; \varphi_{AC} = ((a - 1)(m - 1))^{-1} \sum_{im=1}^2 (\alpha\theta)_{im}^2;$$

$$\varphi_C = (m - 1)^{-1} \sum_{m=1}^2 \theta_m^2; \varphi_{BC} = ((b - 1)(m - 1))^{-1} \sum_{lm=1}^2 (\beta\theta)_{lm}^2;$$

$$\varphi_{ABC} = ((a - 1)(b - 1)(m - 1))^{-1} \sum_{ilm=1}^2 (\alpha\beta\theta)_{ilm}^2; \varphi_{OC} = ((b - 1)(m - 1))^{-1} \sum_{om=1}^2 (\gamma\theta)_{om}^2;$$

$$\varphi_{AOC} = ((a - 1)(b - 1)(m - 1))^{-1} \sum_{iom=1}^2 (\alpha\gamma\theta)_{iom}^2$$

Table 5. Analysis of variance table for design B on SPD (CRD, LSD)

SV	df	EMS
Treatment A	1	$\sigma_e^2 + 2\sigma_{wp}^2 + 4\phi_A$
Treatment B	1	$\sigma_e^2 + 4\phi_B$
Treatment A *Treatment B	1	$\sigma_e^2 + 2\sigma_{array}^2 + 2\phi_{AB}$
Order(O)	1	$\sigma_e^2 + 4\phi_o$
Treatment A * Order	1	$\sigma_e^2 + 2\sigma_{array}^2 + 2\phi_{AO}$
Colour(C)	1	$\sigma_e^2 + 2\sigma_{wp}^2 + 6\phi_C$
Treatment A * Colour	1	$\sigma_e^2 + 2\sigma_{wp}^2 + 2\sigma_{array}^2 + 4\phi_{AC}$
Treatment B *Colour	1	$\sigma_e^2 + 2\sigma_{array}^2 + 4\phi_{BC}$
Treatment A *Treatment B * Colour	1	$\sigma_e^2 + 2\phi_{ABC}$
Order * Colour	1	$\sigma_e^2 + 2\sigma_{array}^2 + 4\phi_{OC}$
Treatment A *Order *Colour	1	$\sigma_e^2 + 2\sigma_{wp}^2 + 2\sigma_{array}^2 + 2\phi_{AOC}$
Reptype *Treatment A*(Repwithin)	4	$\sigma_e^2 + 2\sigma_{wp}^2$
Array*Reptype(Repwithin)	4	$\sigma_e^2 + 2\sigma_{array}^2$
Residual	4	σ_e^2

Table 6. Analysis of variance table for design C on SPD (CRD, LSD)

SV	df	EMS
Treatment A	1	$\sigma_e^2 + 4\phi_A$
Treatment B	1	$\sigma_e^2 + 4\phi_B$
Treatment A *Treatment B	1	$\sigma_e^2 + 2\phi_{AB}$
Order(O)	1	$\sigma_e^2 + 4\phi_o$
Treatment A * Order	1	$\sigma_e^2 + 2\phi_{AO}$
Colour(C)	1	$\sigma_e^2 + 6\phi_C$
Treatment A * Colour	1	$\sigma_e^2 + 4\phi_{AC}$
Treatment B *Colour	1	$\sigma_e^2 + 2\sigma_{(wp+array)}^2 + 4\phi_{BC}$
Treatment A *Treatment B * Colour	1	$\sigma_e^2 + 2\sigma_{(wp+array)}^2 + 2\phi_{ABC}$
Order * Colour	1	$\sigma_e^2 + 4\phi_{OC}$
Treatment A *Order *Colour	1	$\sigma_e^2 + 2\phi_{AOC}$
Reptype *Treatment A*(Repwithin)	4	$\sigma_e^2 + 2\sigma_{(wp+array)}^2$
Residual	8	σ_e^2

3.7. Vertical loop method (Design A) for SPD (LSD, RCBD)

The vertical loop method of sample pairing is the pairing of samples in a vertical form between each level of the sub-plot treatment B within the levels of the whole-plot treatment A. Only four treatment combinations are accommodated in each replicate.

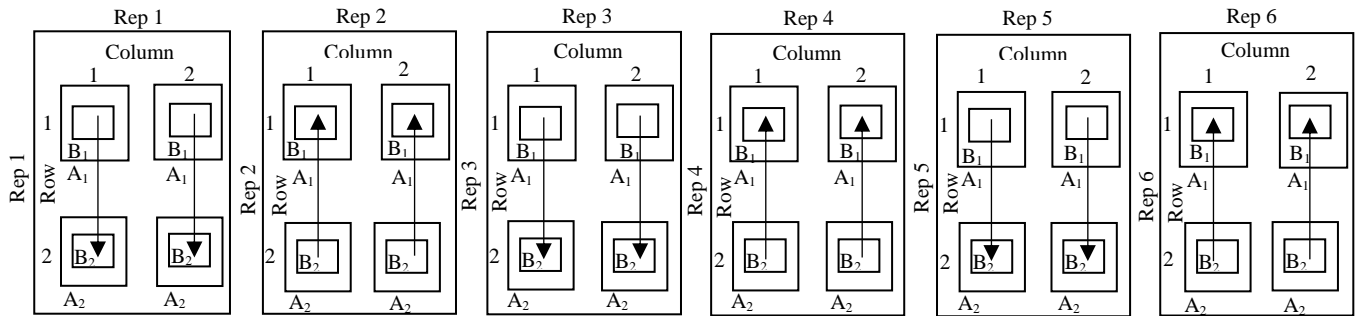


Fig. 7 Dual channel microarray applied on SPD (LSD, RCBD) using vertical loop method

From Figure 7; rep1, 3, and 5 belongs to replication type I with treatments combination (1, 1, 1, 1, G); where the first 1 represents level 1 of the whole-plot treatment, the second 1 denotes level 1 of the row, the third 1 denotes level 1 of column, the fourth 1 denotes level 1 of the sub-plot treatment, and G represents the colour dyed on the level 1 of the sub-plot within level 1 of row and column on the first level of whole-plot treatments. Others includes: (2, 1, 2, 2, G), (2, 2, 1, 1, R), and (1, 2, 2, 2, R) while rep 2, 4, and 6 are in replication type II with treatments combination: (1, 1, 1, 1, R), (2, 1, 2, 2, R), (2, 2, 1, 1, G), and (1, 2, 2, 2, G) and is represented with the model given in (13)

$$Y_{ircjklm} = \mu_{irclm} + w_{irckj} + a_{jkl} + \varepsilon_{ircjklm} \quad (13)$$

$i = 1, 2(\text{levels of whole - plot treatment } A); j = I, II(\text{type of replication}); r = 1, 2(\text{row effect});$
 $c = 1, 2(\text{column effect}); l = 1, 2(\text{levels of sub - plot treatment } B); m = R, G(\text{colour});$
 $k = 1, 2, 3(\text{replicate within type of replication})$

where, μ_{irclm} are the fixed of treatment A, row effect, column effect, treatment B, and dye colour; w_{irckj} is the random effect of the whole-plot; a_{jkl} is the random effect of the array; $\varepsilon_{ircjklm}$ is the random effect of the residual. The ANOVA table for design A is shown in Table 7.

3.8. Cross loop method (Design B) for SPD (LSD, RCBD)

The vertical loop method of sample pairing is the pairing of samples in a cross form within the levels of the sub-plot treatment B and across the levels of the whole-plot treatment A. From the assignment of the samples and in addition to the subscript for denoting treatment B, a subscript is required to indicate the different arrays.

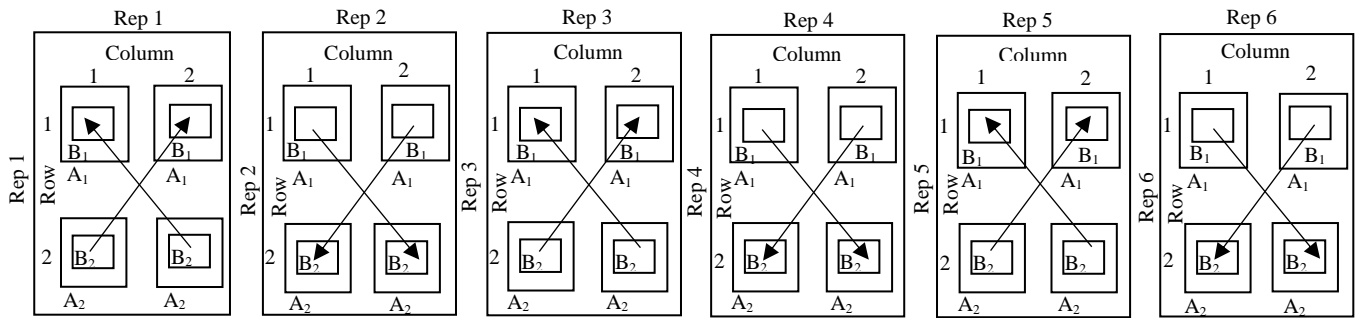


Fig. 8 Dual channel microarray applied on SPD (LSD, RCBD) using cross loop method

From Figure 8; rep1, 3, and 5 belongs to replication type I with treatments combination (1, 1, 1, 1, R); where the first 1 represents level 1 of the whole-plot treatment, the second 1 denotes level 1 of the row, the third 1 denotes level 1 of column, the fourth 1 denotes level 1 of the sub-plot treatment, and R represents the colour dyed on the level 1 of the sub-plot within level 1 of row and column on the first level of whole-plot treatments. Others includes: (2, 2, 1, 1, G), (1, 2, 2, 2, G), and (2, 1, 2, 2, R); while rep 2, 4, and 6 are in replication type II with treatments combination: (1, 1, 1, 1, G), (2, 1, 2, 2, G), (2, 2, 1, 1, R), and (1, 2, 2, 2, R) and is represented with the model given in (14)

$$Y_{ircjklmn} = \mu_{irclm} + w_{irckj} + a_{jkn} + \varepsilon_{ircjklmn} \quad (14)$$

$i = 1, 2(\text{levels of whole - plot treatment } A); l = 1, 2(\text{levels of sub - plot treatment } B);$
 $m = R, G(\text{colour}); r = 1, 2(\text{row effect}); c = 1, 2(\text{column effect}); j = I, II(\text{type of replication});$
 $k = 1, 2, 3(\text{replicate within type of replication})$

where, μ_{irclm} are the fixed of treatment A, row effect, column effect, treatment B, and dye colour; w_{irckj} is the random effect of the whole-plot; a_{jkn} is the random effect of the array; $\varepsilon_{ircjklmn}$ is the random effect of the residual. The ANOVA table for design B is shown in Table 8.

3.9. Horizontal loop method (Design C) for SPD (LSD, RBCD)

The vertical loop method of sample pairing is the pairing of samples in a horizontal form to the levels of the sub-plot treatment B within the levels of the whole-plot treatment A.

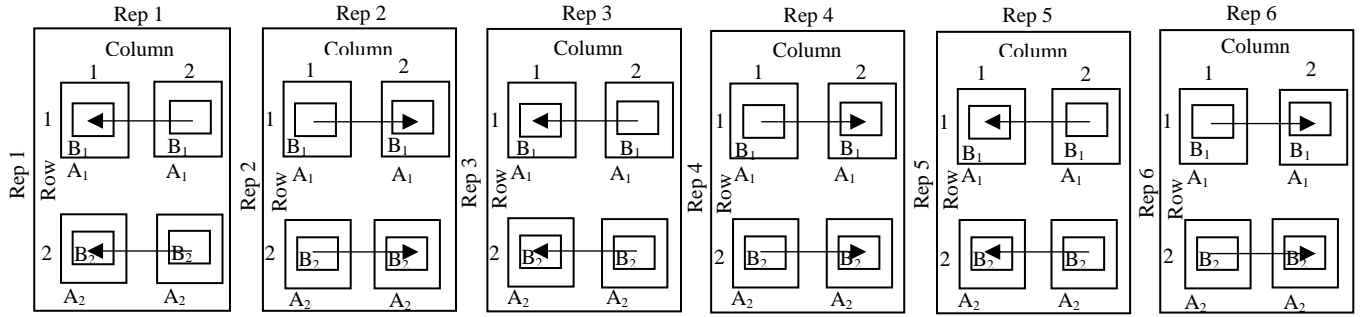


Fig. 9 Dual channel microarray applied on SPD (LSD, RCBD) using horizontal loop method

From the construction of this design; rep1, 3, and 5 belongs to replication type I with treatments combination (1, 1, 1, 1, R); where the first 1 represents level 1 of the whole-plot treatment, the second 1 denotes level 1 of the row, the third 1 denotes level 1 of column, the fourth 1 denotes level 1 of the sub-plot treatment, and R represents the colour dyed on the level 1 of the sub-plot within level 1 of row and column on the first level of whole-plot treatments. Others includes: (2, 2, 1, 1, R), (1, 2, 2, 2, G), and (2, 1, 2, 2, G); while rep 2, 4, and 6 are in replication type II with treatments combination: (1, 1, 1, 1, G), (2, 1, 2, 2, R), (2, 2, 1, 1, G), and (1, 2, 2, 2, R) and is represented with the model given in (15)

$$Y_{ircjklm} = \mu_{irclm} + w_{ircjk} + a_{jkr} + \varepsilon_{ircjklm} \quad (15)$$

$i = 1, 2$ (levels of whole – plot treatment A); $j = I, II$ (type of replication); $r = 1, 2$ (row effect);

$c = 1, 2$ (column effect); $l = 1, 2$ (levels of sub – plot treatment B); $m = R, G$ (colour);

$k = 1, 2, 3$ (replicate within type of replication)

where, μ_{irclm} are the fixed of treatment A, row effect, column effect, treatment B, and dye colour; w_{ircjk} is the random effect of the whole-plot; a_{jkr} is the random effect of the array; $\varepsilon_{ircjklm}$ is the random effect of the residual. The ANOVA table for design C is shown in Table 9.

Table 7. Analysis of variance table for design A on SPD (LSD, RCBD)

SV	df	EMS
Row	1	$\sigma_e^2 + 2\sigma_{wp}^2 + 4\varphi_R$
Column	1	$\sigma_e^2 + 2\sigma_{wp}^2 + 4\varphi_C$
Treatment A	1	$\sigma_e^2 + 2\sigma_{wp}^2 + 4\varphi_A$
Treatment B	1	$\sigma_e^2 + 2\sigma_{array}^2 + 4\varphi_B$
Treatment A *Treatment B	1	$\sigma_e^2 + 2\varphi_{AB}$
Colour(C)	1	$\sigma_e^2 + 6\varphi_{CR}$
Row * Colour	1	$\sigma_e^2 + 2\sigma_{wp}^2 + 4\varphi_{RCR}$
Column * Colour	1	$\sigma_e^2 + 2\sigma_{wp}^2 + 2\sigma_{array}^2 + 4\varphi_{CCR}$
Treatment A * Colour	1	$\sigma_e^2 + 2\sigma_{wp}^2 + 2\sigma_{array}^2 + 2\varphi_{ACR}$
Treatment B *Colour	1	$\sigma_e^2 + 4\varphi_{BCR}$
Treatment A *Treatment B * Colour	1	$\sigma_e^2 + 2\sigma_{array}^2 + 2\varphi_{ABCR}$
Row *Column *TreatmentA*(Reptype)	4	$\sigma_e^2 + 2\sigma_{wp}^2$
Treatment B *Array*Rep(Reptype)	4	$\sigma_e^2 + 2\sigma_{array}^2$
Residual	4	σ_e^2

$$\text{where, } \varphi_R = (r - 1)^{-1} \sum_{r=1}^2 \rho_r^2; \varphi_C = (c - 1)^{-1} \sum_{c=1}^2 \gamma_c^2; \varphi_A = (a - 1)^{-1} \sum_{i=1}^2 \alpha_i^2; \varphi_B = (b - 1)^{-1} \sum_{l=1}^2 \beta_l^2; \\ \varphi_{AB} = ((a - 1)(b - 1))^{-1} \sum_{il=1}^2 (\alpha\beta)_{il}^2; \varphi_{CR} = (m - 1)^{-1} \sum_{m=1}^2 \theta_m^2; \varphi_{RCR} = ((r - 1)(m - 1))^{-1} \sum_{rm=1}^2 (\rho\theta)_{rm}^2;$$

$$\varphi_{CCR} = ((c - 1)(m - 1))^{-1} \sum_{cm=1}^2 (\gamma\theta)_{cm}^2; \varphi_{ACR} = ((a - 1)(m - 1))^{-1} \sum_{im=1}^2 (\alpha\theta)_{im}^2;$$

$$\varphi_{BCR} = ((b - 1)(m - 1))^{-1} \sum_{lm=1}^2 (\beta\theta)_{lm}^2; \varphi_{ABCR} = ((a - 1)(b - 1)(m - 1))^{-1} \sum_{ilm=1}^2 (\alpha\beta\theta)_{ilm}^2$$

Table 8. Analysis of variance table for design B on SPD (LSD, RCBD)

SV	df	EMS
Row	1	$\sigma_e^2 + 2\sigma_{wp}^2 + 4\varphi_R$
Column	1	$\sigma_e^2 + 2\sigma_{wp}^2 + 4\varphi_C$
Treatment A	1	$\sigma_e^2 + 2\sigma_{wp}^2 + 4\varphi_A$
Treatment B	1	$\sigma_e^2 + 4\varphi_A$
Treatment A *Treatment B	1	$\sigma_e^2 + 2\sigma_{array}^2 + 2\varphi_{AB}$
Colour(C)	1	$\sigma_e^2 + 6\varphi_{CR}$
Row * Colour	1	$\sigma_e^2 + 2\sigma_{wp}^2 + 4\varphi_{RCR}$
Column * Colour	1	$\sigma_e^2 + 2\sigma_{wp}^2 + 4\varphi_{CCR}$
Treatment A * Colour	1	$\sigma_e^2 + 2\sigma_{wp}^2 + 2\sigma_{array}^2 + 2\varphi_{ACR}$
Treatment B *Colour	1	$\sigma_e^2 + 2\sigma_{array}^2 + 4\varphi_{BCR}$
Treatment A *Treatment B * Colour	1	$\sigma_e^2 + 2\varphi_{ABCR}$
Row *Column *TreatmentA*(Reptype)	4	$\sigma_e^2 + 2\sigma_{wp}^2$
Array*Rep(Reptype)	4	$\sigma_e^2 + 2\sigma_{array}^2$
Residual	4	σ_e^2

Table 9. Analysis of variance table for design C on SPD (LSD, RCBD)

SV	df	EMS
Row	1	$\sigma_e^2 + 2\sigma_{wp}^2 + 4\varphi_R$
Column	1	$\sigma_e^2 + 2\sigma_{wp}^2 + 4\varphi_C$
Treatment A	1	$\sigma_e^2 + 2\sigma_{wp}^2 + 4\varphi_A$
Treatment B	1	$\sigma_e^2 + 4\varphi_B$
Treatment A *Treatment B	1	$\sigma_e^2 + 2\varphi_{AB}$
Colour(C)	1	$\sigma_e^2 + 2\sigma_{wp}^2 + 6\varphi_{CR}$
Row * Colour	1	$\sigma_e^2 + 2\sigma_{wp}^2 + 2\sigma_{array}^2 + 2\varphi_{RCR}$
Column * Colour	1	$\sigma_e^2 + 2\sigma_{wp}^2 + 4\varphi_{CCR}$
Treatment A * Colour	1	$\sigma_e^2 + 2\sigma_{wp}^2 + 2\sigma_{array}^2 + 2\varphi_{ACR}$
Treatment B *Colour	1	$\sigma_e^2 + 2\sigma_{wp}^2 + 2\sigma_{array}^2 + 4\varphi_{BCR}$
Treatment A *Treatment B * Colour	1	$\sigma_e^2 + 2\sigma_{wp}^2 + 2\sigma_{array}^2 + 2\varphi_{ABCR}$
Row *Column *TreatmentA*(Reptype)	4	$\sigma_e^2 + 2\sigma_{wp}^2$
Treatment B*Array*Rep(Reptype)	4	$\sigma_e^2 + 2\sigma_{array}^2$
Residual	4	σ_e^2

4. Estimation of Variance Components

In order to obtain the expected variance components for each design, the models are obtained for each treatment combinations in the k^{th} replicate of Type I and Type II based on the model defined. Each treatment combination is given in form of a model for each replication type, from the models for each treatment combinations, the treatment effects and their expected variances components were derived and computed for each design.

4.1. Models observations for the treatment combinations for design A on SPD (CRD, RCBD)

From the model defined for design A given in (7), Table 10 was computed from the four treatment combinations in each rep type. Each treatment combination is given in form of a model for each replication type, indicating the levels of the whole-plot, sub-plot, and colour.

Table 10. The observations of the models for the k replicate of type I and type II replication of vertical loop method (design A)

Replication type		Y_{B_1}	Y_{B_2}
Rep type I	Y_{A_1}	$Y_{1Ik1G} = \mu_{11G} + (w + a)_{1kI} + \varepsilon_{1Ik1G}$	$Y_{1Ik2R} = \mu_{12R} + (w + a)_{1kI} + \varepsilon_{1Ik2R}$
	Y_{A_2}	$Y_{2Ik1G} = \mu_{21G} + (w + a)_{2kI} + \varepsilon_{2Ik1G}$	$Y_{2Ik2R} = \mu_{22R} + (w + a)_{2kI} + \varepsilon_{2Ik2R}$
Rep type II	Y_{A_1}	$Y_{1IIk1R} = \mu_{11R} + (w + a)_{1kII} + \varepsilon_{1IIk1R}$	$Y_{1IIk2G} = \mu_{12G} + (w + a)_{1kII} + \varepsilon_{1IIk2G}$
	Y_{A_2}	$Y_{2IIk1R} = \mu_{21R} + (w + a)_{2kII} + \varepsilon_{2IIk1R}$	$Y_{2IIk2G} = \mu_{22G} + (w + a)_{2kII} + \varepsilon_{2IIk2G}$

where, $Y_{A_1}, Y_{A_2}, Y_{B_1}$, and Y_{B_2} represents first and second levels of treatments A and B respectively.

The same procedures used were also applied in estimating the treatments effects for design B and design C. The summary of results obtained for rep types I and II are shown in Tables 13.

Table 11. The observations of the models for the k replicate of type I and type II replication of cross loop method (design B)

Replication type		Y_{B_1}	Y_{B_2}
Rep type I	Y_{A_1}	$Y_{1Ik1R1} = \mu_{11R} + w_{1kI} + a_{1kI} + \varepsilon_{1Ik1R1}$	$Y_{1Ik2R2} = \mu_{12R} + w_{1kI} + a_{1kI} + \varepsilon_{1Ik2R2}$
	Y_{A_2}	$Y_{2Ik1G2} = \mu_{21G} + w_{2kI} + a_{1kI} + \varepsilon_{2Ik1G2}$	$Y_{2Ik2G1} = \mu_{22R} + w_{2kI} + a_{1kI} + \varepsilon_{2Ik2G1}$
Rep type II	Y_{A_1}	$Y_{1IIk1G1} = \mu_{11G} + w_{1kII} + a_{1Ik1} + \varepsilon_{1IIk1G1}$	$Y_{1IIk2G2} = \mu_{12G} + w_{1kII} + a_{1Ik2} + \varepsilon_{1IIk2G2}$
	Y_{A_2}	$Y_{2IIk1R2} = \mu_{21R} + w_{2kII} + a_{1Ik2} + \varepsilon_{2IIk1R2}$	$Y_{2IIk2R1} = \mu_{22R} + w_{2kII} + a_{1Ik1} + \varepsilon_{2IIk2R1}$

Table 12. The observation of the models for the k replicate of type I and II replication of horizontal loop method (design C)

Replication type		Y_{B_1}	Y_{B_2}
Rep type I	Y_{A_1}	$Y_{1Ik1R} = \mu_{11R} + w_{1kI} + a_{1kI} + \varepsilon_{1Ik1R1}$	$Y_{1Ik2R} = \mu_{12R} + w_{1kI} + a_{1kI} + \varepsilon_{1Ik2R}$
	Y_{A_2}	$Y_{2Ik1G} = \mu_{21G} + w_{2kI} + a_{1kI} + \varepsilon_{2Ik1G}$	$Y_{2Ik2G} = \mu_{22G} + w_{2kI} + a_{1kI} + \varepsilon_{2Ik2G}$
Rep type II	Y_{A_1}	$Y_{1IIk1G} = \mu_{11G} + w_{1kII} + a_{1Ik1} + \varepsilon_{1IIk1G}$	$Y_{1IIk2G} = \mu_{12G} + w_{1kII} + a_{1Ik2} + \varepsilon_{1IIk2G}$
	Y_{A_2}	$Y_{2IIk1R} = \mu_{21R} + w_{2kII} + a_{1Ik1} + \varepsilon_{2IIk1R}$	$Y_{2IIk2R} = \mu_{22R} + w_{2kII} + a_{1Ik2} + \varepsilon_{2IIk2R}$

Table 13. Summary of the expected variance components for the treatment effects based on vertical, cross and horizontal loop methods for SPD (CRD, RCBD)

Treatment effects	Expected variance for the treatment effects		
	DESIGN A	DESIGN B	DESIGN C
$\bar{Y}_{A_1..} - \bar{Y}_{A_2..}$	$[2\sigma_{(wp+array)}^2 + \sigma_e^2]/2k$	$[2\sigma_{wp}^2 + \sigma_e^2]/2k$	$[2\sigma_{wp}^2 + \sigma_e^2]/2k$
$\bar{Y}_{.B_1.} - \bar{Y}_{.B_2.}$	$[\sigma_e^2]/2k$	$[\sigma_e^2]/2k$	$[2\sigma_{array}^2 + \sigma_e^2]/2k$
$\bar{Y}_{..C_R} - \bar{Y}_{..C_G}$	$[\sigma_e^2]/2k$	$[2\sigma_{wp}^2 + \sigma_e^2]/2k$	$[2\sigma_{wp}^2 + \sigma_e^2]/2k$
$\bar{Y}_{A_1B_1.} - \bar{Y}_{A_2B_1.}$	$[\sigma_{(wp+array)}^2 + \sigma_e^2]/k$	$[\sigma_{(wp)}^2 + \sigma_{array}^2 + \sigma_e^2]/k$	$[\sigma_{(wp)}^2 + 2\sigma_{array}^2 + \sigma_e^2]/k$
$\bar{Y}_{A_1B_2.} - \bar{Y}_{A_2B_2.}$	$[\sigma_{(wp+array)}^2 + \sigma_e^2]/k$	$[\sigma_{(wp)}^2 + \sigma_{array}^2 + \sigma_e^2]/k$	$[\sigma_{(wp)}^2 + 2\sigma_{array}^2 + \sigma_e^2]/k$
$\bar{Y}_{A_1B_1.} - \bar{Y}_{A_1B_2.}$	$[\sigma_e^2]/k$	$[\sigma_{array}^2 + \sigma_e^2]/k$	$[\sigma_{array}^2 + \sigma_e^2]/k$
$\bar{Y}_{A_2B_1.} - \bar{Y}_{A_2B_2.}$	$[\sigma_e^2]/k$	$[\sigma_{array}^2 + \sigma_e^2]/k$	$[\sigma_{array}^2 + \sigma_e^2]/k$

4.2. Models observations for the treatment combinations for design A on SPD (CRD, LSD)

From the model defined for design A given in (10), Table 14 was computed from the four treatment combinations in each rep type.

Table 14. The observations of the models for the k replicate of type I and type II replication of vertical loop method (design A)

Replication type		Y_{B_1}	Y_{B_2}
Rep type I	Y_{A_1}	$Y_{1Ik1(1)G} = \mu_{11(1)G} + w_{1kI} + a_{1Ik1(1)} + \varepsilon_{1Ik1(1)G}$	$Y_{1Ik2(2)G} = \mu_{12(2)G} + w_{1kI} + a_{1Ik2(2)} + \varepsilon_{1Ik2(2)G}$
	Y_{A_2}	$Y_{2Ik2(1)R} = \mu_{22(1)R} + w_{2kI} + a_{1Ik2(1)} + \varepsilon_{2Ik2(1)R}$	$Y_{2Ik1(2)R} = \mu_{21(2)R} + w_{2kI} + a_{1Ik1(2)} + \varepsilon_{2Ik1(2)R}$
Rep type II	Y_{A_1}	$Y_{1IIk1(1)R} = \mu_{11(1)R} + w_{1kII} + a_{1IIk1(1)} + \varepsilon_{1IIk1(1)R}$	$Y_{1IIk2(2)R} = \mu_{12(2)R} + w_{1kII} + a_{1IIk2(2)} + \varepsilon_{1IIk2(2)R}$
	Y_{A_2}	$Y_{2IIk2(1)G} = \mu_{22(1)G} + w_{2kII} + a_{1IIk2(1)} + \varepsilon_{2IIk2(1)G}$	$Y_{2IIk1(2)G} = \mu_{21(2)G} + w_{2kII} + a_{1IIk1(2)} + \varepsilon_{2IIk1(2)G}$

The same procedures used were also applied in estimating the treatments effects for design B and design C. The summary of results obtained for rep types I and II are shown in Table 17.

Table 15. The observations of the models for the *k* replicate of type I and type II replication of cross loop method (design B)

Replication type	Y_{B_1}	Y_{B_2}
Rep type I Y_{A_1}	$Y_{1Ik1(1)R1} = \mu_{11(1)R} + w_{1ki} + a_{Ik1} + \varepsilon_{1Ik1(1)R1}$	$Y_{1Ik2(2)R2} = \mu_{12(2)R} + w_{1ki} + a_{Ik2} + \varepsilon_{1Ik2(2)R2}$
Y_{A_2}	$Y_{2Ik2(1)G1} = \mu_{22(1)G} + w_{2ki} + a_{Ik1} + \varepsilon_{2Ik2(1)G1}$	$Y_{2Ik1(2)G2} = \mu_{21(2)G} + w_{2ki} + a_{Ik2} + \varepsilon_{2Ik1(2)G2}$
Rep type II Y_{A_1}	$Y_{1IIk1(1)G1} = \mu_{11(1)G} + w_{1kII} + a_{IIk1} + \varepsilon_{1IIk1(1)G1}$	$Y_{1IIk2(2)G2} = \mu_{12(2)G} + w_{1kII} + a_{IIk2} + \varepsilon_{2IIk2(2)G2}$
Y_{A_2}	$Y_{2IIk2(1)R1} = \mu_{22(1)R} + w_{2kII} + a_{IIk1} + \varepsilon_{2IIk2(1)R1}$	$Y_{2IIk1(2)R2} = \mu_{21(2)R} + w_{2kII} + a_{IIk2} + \varepsilon_{2IIk1(2)R2}$

Table 16. The observation of the models for the *k* replicate of type I and II replication of horizontal loop method (design C)

Replication type	Y_{B_1}	Y_{B_2}
Rep type I Y_{A_1}	$Y_{1Ik1(1)R} = \mu_{11(1)R} + (w + a)_{1ki} + \varepsilon_{1Ik1(1)R}$	$Y_{1Ik2(2)G} = \mu_{12(2)G} + (w + a)_{1ki} + \varepsilon_{1Ik2(2)G}$
Y_{A_2}	$Y_{2Ik2(1)G} = \mu_{22(1)G} + (w + a)_{2ki} + \varepsilon_{2Ik2(1)G}$	$Y_{2Ik1(2)R} = \mu_{21(2)R} + (w + a)_{2ki} + \varepsilon_{2Ik1(2)R}$
Rep type II Y_{A_1}	$Y_{1IIk1(1)G} = \mu_{11(1)G} + (w + a)_{1kII} + \varepsilon_{1IIk1(1)G}$	$Y_{1IIk2(2)R} = \mu_{12(2)R} + (w + a)_{1kII} + \varepsilon_{1IIk2(2)R}$
Y_{A_2}	$Y_{2IIk2(1)R} = \mu_{22(1)R} + (w + a)_{2kII} + \varepsilon_{2IIk2(1)R}$	$Y_{2IIk1(2)G} = \mu_{21(2)G} + (w + a)_{2kII} + \varepsilon_{2IIk1(2)G}$

Table 17. Summary of the variance estimated for the treatment effects based on vertical, cross and horizontal loop methods for SPD (CRD, LSD)

Treatment effects	Estimated variance for the treatment effects		
	DESIGN A	DESIGN B	DESIGN C
$\bar{Y}_{A_1 \dots} - \bar{Y}_{A_2 \dots}$	$[2\sigma_{wp}^2 + \sigma_{array}^2 + \sigma_e^2]/3k$	$[2\sigma_{wp}^2 + \sigma_e^2]/3k$	$[2\sigma_{(wp+array)}^2 + \sigma_e^2]/3k$
$\bar{Y}_{\cdot B_1 \dots} - \bar{Y}_{\cdot B_2 \dots}$	$[\sigma_{array}^2 + \sigma_e^2]/3k$	$[2\sigma_{array}^2 + \sigma_e^2]/3k$	$[\sigma_e^2]/3k$
$\bar{Y}_{\dots O_1 \cdot} - \bar{Y}_{\dots O_2 \cdot}$	$[\sigma_{array}^2 + \sigma_e^2]/3k$	$[\sigma_e^2]/3k$	$[\sigma_e^2]/3k$
$\bar{Y}_{\dots C_R} - \bar{Y}_{\dots C_G}$	$[2\sigma_{wp}^2 + \sigma_{array}^2 + \sigma_e^2]/3k$	$[2\sigma_{wp}^2 + \sigma_e^2]/3k$	$[\sigma_e^2]/3k$
$\bar{Y}_{A_1 B_1 \dots} - \bar{Y}_{A_2 B_1 \dots}$	$[\sigma_{wp}^2 + \sigma_{array}^2 + \sigma_e^2]/2k$	$[\sigma_{wp}^2 + 2\sigma_{array}^2 + \sigma_e^2]/2k$	$[\sigma_{(wp+array)}^2 + \sigma_e^2]/2k$
$\bar{Y}_{A_1 B_2 \dots} - \bar{Y}_{A_2 B_2 \dots}$	$[\sigma_{wp}^2 + \sigma_{array}^2 + \sigma_e^2]/2k$	$[\sigma_{wp}^2 + 2\sigma_{array}^2 + \sigma_e^2]/2k$	$[\sigma_{(wp+array)}^2 + \sigma_e^2]/2k$
$\bar{Y}_{A_1 B_1 \dots} - \bar{Y}_{A_1 B_2 \dots}$	$[\sigma_{array}^2 + \sigma_e^2]/2k$	$[\sigma_{array}^2 + \sigma_e^2]/2k$	$[\sigma_e^2]/2k$
$\bar{Y}_{A_2 B_1 \dots} - \bar{Y}_{A_2 B_2 \dots}$	$[\sigma_{array}^2 + \sigma_e^2]/2k$	$[\sigma_{array}^2 + \sigma_e^2]/2k$	$[\sigma_e^2]/2k$

4.3. Models observations for the treatment combinations for SPD (LSD, RCBD) design A

From the model defined for design A given in (13), Table 18 was computed from the four treatment combinations in each rep type.

The same procedures used were also applied in estimating the treatments effects for design B and design C. The summary of results obtained for rep types I and II are shown in Table 21.

Table 18. The observations of the models for the *k* replicate of type I and type II replication of vertical loop method (design A)

Replication type	Y_{B_1}	Y_{B_2}
REP TYPE I Y_{A_1}	$Y_{111Ik1R} = \mu_{1111R} + w_{111ki} + a_{Ik1} + \varepsilon_{111Ik1R}$	$Y_{122Ik2G} = \mu_{1222G} + w_{122ki} + a_{Ik1} + \varepsilon_{122Ik2G}$
Y_{A_2}	$Y_{221Ik1R} = \mu_{2211R} + w_{221ki} + a_{Ik2} + \varepsilon_{221Ik1R}$	$Y_{212Ik2G} = \mu_{2122G} + w_{212ki} + a_{Ik2} + \varepsilon_{212Ik2G}$
REP TYPE II Y_{A_1}	$Y_{111IIk1G} = \mu_{1111G} + w_{111kII} + a_{IIk1} + \varepsilon_{111IIk1G}$	$Y_{122IIk2R} = \mu_{1222R} + w_{122kII} + a_{IIk1} + \varepsilon_{122IIk2R}$
Y_{A_2}	$Y_{221IIk1G} = \mu_{2211G} + w_{221kII} + a_{IIk2} + \varepsilon_{221IIk1G}$	$Y_{212IIk2R} = \mu_{2122R} + w_{212kII} + a_{IIk2} + \varepsilon_{212IIk2R}$

Table 19. The observations of the models for the *k* replicate of type I and type II replication of cross loop method (design B)

Replication type	Y_{B_1}	Y_{B_2}
REP TYPE I Y_{A_1}	$Y_{111Ik1R1} = \mu_{1111R} + w_{111kI} + a_{Ik1} + \epsilon_{111Ik1R1}$	$Y_{122Ik2G1} = \mu_{1222G} + w_{122kI} + a_{Ik1} + \epsilon_{122Ik2G1}$
Y_{A_2}	$Y_{221Ik1G2} = \mu_{2211G} + w_{221kI} + a_{Ik2} + \epsilon_{221Ik1G2}$	$Y_{212Ik2R2} = \mu_{2122R} + w_{212kI} + a_{Ik2} + \epsilon_{212Ik2R2}$
REP TYPE II Y_{A_1}	$Y_{111Ik1G1} = \mu_{1111G} + w_{111kII} + a_{IIk1} + \epsilon_{111Ik1G1}$	$Y_{122Ik2R1} = \mu_{1222R} + w_{122kII} + a_{IIk1} + \epsilon_{122Ik2R1}$
Y_{A_2}	$Y_{221Ik1R2} = \mu_{2211R} + w_{221kII} + a_{IIk2} + \epsilon_{221Ik1R2}$	$Y_{212Ik2G2} = \mu_{2122G} + w_{212kII} + a_{IIk2} + \epsilon_{212Ik2G2}$

Table 20. The observation of the models for the *k* replicate of type I and II replication of horizontal loop method (design C)

Replication type	Y_{B_1}	Y_{B_2}
REP TYPE I Y_{A_1}	$Y_{111Ik1R} = \mu_{1111R} + w_{111kI} + a_{Ik1} + \epsilon_{111Ik1R}$	$Y_{122Ik2G} = \mu_{1222G} + w_{122kI} + a_{Ik2} + \epsilon_{122Ik2G}$
Y_{A_2}	$Y_{221Ik1R} = \mu_{2211R} + w_{221kI} + a_{Ik2} + \epsilon_{221Ik1R}$	$Y_{212Ik2G} = \mu_{2122G} + w_{212kI} + a_{Ik1} + \epsilon_{212Ik2G}$
REP TYPE I Y_{A_1}	$Y_{111Ik1G} = \mu_{1111G} + w_{111kII} + a_{IIk1} + \epsilon_{111Ik1G}$	$Y_{122Ik2R} = \mu_{1222R} + w_{122kII} + a_{IIk2} + \epsilon_{122Ik2R}$
Y_{A_2}	$Y_{221Ik1G} = \mu_{2211G} + w_{221kII} + a_{IIk2} + \epsilon_{221Ik1G}$	$Y_{212Ik2R} = \mu_{2122R} + w_{212kII} + a_{IIk1} + \epsilon_{212Ik2R}$

Table 21. Summary of the variance estimated for the treatment effects based on vertical, cross and horizontal loop methods for SPD (LSD, RCBD)

Treatment effects	Estimated variance for the treatment effects		
	DESIGN A	DESIGN B	DESIGN C
$\bar{Y}_{A_1 \dots} - \bar{Y}_{A_2 \dots}$	$[\sigma_{wp}^2 + \sigma_e^2]/4k$	$[\sigma_{wp}^2 + 2\sigma_{array}^2 + \sigma_e^2]/4k$	$[\sigma_{wp}^2 + \sigma_e^2]/4k$
$\bar{Y}_{\cdot B_1 \dots} - \bar{Y}_{\cdot B_2 \dots}$	$[\sigma_{wp}^2 + 2\sigma_{array}^2 + \sigma_e^2]/4k$	$[\sigma_{wp}^2 + \sigma_e^2]/4k$	$[\sigma_{wp}^2 + \sigma_e^2]/4k$
$\bar{Y}_{\cdot \cdot R_1 \dots} - \bar{Y}_{\cdot \cdot R_2 \dots}$	$[\sigma_{wp}^2 + \sigma_e^2]/4k$	$[\sigma_{wp}^2 + \sigma_e^2]/4k$	$[\sigma_{wp}^2 + \sigma_e^2]/4k$
$\bar{Y}_{\dots CL_1 \dots} - \bar{Y}_{\dots CL_2 \dots}$	$[\sigma_{wp}^2 + 2\sigma_{array}^2 + \sigma_e^2]/4k$	$[\sigma_{wp}^2 + \sigma_e^2]/4k$	$[\sigma_{wp}^2 + 2\sigma_{array}^2 + \sigma_e^2]/4k$
$\bar{Y}_{\dots \dots C_R} - \bar{Y}_{\dots \dots C_G}$	$[\sigma_{wp}^2 + \sigma_e^2]/4k$	$[\sigma_{wp}^2 + \sigma_e^2]/4k$	$[\sigma_{wp}^2 + \sigma_e^2]/4k$
$\bar{Y}_{A_1 B_1 \dots} - \bar{Y}_{A_2 B_1 \dots}$	$[\sigma_{wp}^2 + 2\sigma_{array}^2 + \sigma_e^2]/3k$	$[\sigma_{wp}^2 + \sigma_{array}^2 + \sigma_e^2]/3k$	$[\sigma_{wp}^2 + \sigma_{array}^2 + \sigma_e^2]/3k$
$\bar{Y}_{A_1 B_2 \dots} - \bar{Y}_{A_2 B_2 \dots}$	$[\sigma_{wp}^2 + 2\sigma_{array}^2 + \sigma_e^2]/3k$	$[\sigma_{wp}^2 + \sigma_{array}^2 + \sigma_e^2]/3k$	$[\sigma_{wp}^2 + \sigma_{array}^2 + \sigma_e^2]/3k$
$\bar{Y}_{A_1 B_1 \dots} - \bar{Y}_{A_1 B_2 \dots}$	$[\sigma_{array}^2 + \sigma_e^2]/3k$	$[\sigma_{array}^2 + \sigma_e^2]/3k$	$[\sigma_{array}^2 + \sigma_e^2]/3k$
$\bar{Y}_{A_2 B_1 \dots} - \bar{Y}_{A_2 B_2 \dots}$	$[\sigma_{array}^2 + \sigma_e^2]/3k$	$[\sigma_{array}^2 + \sigma_e^2]/3k$	$[\sigma_{array}^2 + \sigma_e^2]/3k$

Comparing based on SPD (CRD, RCBD); the difference between the levels of the whole-plot treatment A:design A gave $[2\sigma_{(wp+array)}^2 + \sigma_e^2]/2k$ while design B and C gave $[2\sigma_{wp}^2 + \sigma_e^2]/2k$. The difference between the levels of the sub-plot treatment B: designs A and B gave $[\sigma_e^2]/2k$ while design C gave $[2\sigma_{array}^2 + \sigma_e^2]/2k$. And comparing the difference between the levels of color design A gave $[\sigma_e^2]/2k$ while design B and design C gave $[2\sigma_{wp}^2 + \sigma_e^2]/2k$. This implies that design A had the least variance for comparing all the parameters of interest except the whole-plot effect comparison and considered as the best pairing method in SPD (CRD, RCBD).

Comparing based on SPD (CRD, LSD); the difference between the levels of the whole-plot treatment A:design A gave $[2\sigma_{wp}^2 + \sigma_{array}^2 + \sigma_e^2]/3k$, design B gave $[2\sigma_{wp}^2 + \sigma_e^2]/3k$ while design C gave $[2\sigma_{(wp+array)}^2 + \sigma_e^2]/3k$. The difference between the levels of the sub-plot treatment B: designs A gave $[\sigma_{array}^2 + \sigma_e^2]/3k$, B gave $[2\sigma_{array}^2 + \sigma_e^2]/3k$ while design C gave $[\sigma_e^2]/3k$. Comparing the difference between the levels of order: Design B and C gave $[\sigma_e^2]/3k$ while design A gave $[\sigma_{array}^2 + \sigma_e^2]/3k$. And comparing the difference between the levels of color design A gave $[2\sigma_{wp}^2 + \sigma_{array}^2 + \sigma_e^2]/3k$, design

B gave $[2\sigma_{wp}^2 + \sigma_e^2]/3k$ while design C gave $[\sigma_e^2]/3k$. This implies that design C had the least variance for comparing all the parameters of interest except the whole-plot effect comparison in SPD (CRD, LSD).

Comparing based on SPD (LSD, RCBD); the difference between the levels of the whole-plot treatment A: design A and C gave $[\sigma_{wp}^2 + \sigma_e^2]/4k$, while design B gave $[\sigma_{wp}^2 + 2\sigma_{array}^2 + \sigma_e^2]/4k$. The difference between the levels of the sub-plot treatment B: design A gave $[\sigma_{wp}^2 + 2\sigma_{array}^2 + \sigma_e^2]/4k$ while design B and C gave $[\sigma_{wp}^2 + \sigma_e^2]/4k$. Comparing the difference between the levels of row: Design A, B and C gave $[\sigma_{wp}^2 + \sigma_e^2]/4k$. And comparing the difference between the levels of column design A and C gave $[\sigma_{wp}^2 + 2\sigma_{array}^2 + \sigma_e^2]/4k$ while design B gave $[\sigma_{wp}^2 + \sigma_e^2]/4k$. Comparing the difference between the levels of colour: design A, B and C gave $[\sigma_{wp}^2 + \sigma_e^2]/4k$. This implies that design C had the least variance for comparing all the parameters of interest except the column effect comparison and considered as the best pairing method in dual channel microarray applied on SPD (LSD, RCBD).

5. Conclusion

The purpose of this work was to evaluate and compare three methods of sample pairing via variance components estimates for the effects of interest in dual channel microarray applied on SPD (CRD, RCBD), SPD (CRD, LSD), and SPD (LSD, RCBD) in order to verify which method gave the least minimal variance for the treatment effects comparison. Three methods of sample pairing: vertical loop method, cross loop method and horizontal loop method were used. The estimated expected variance components showed that design A had the least variance for comparing all the treatment effects except for the whole-plot treatment on SPD (CRD, RCBD), design C had the least variance for comparing all the treatment effects except for the whole-plot treatment on SPD (CRD, LSD), and design C had the least variance for comparing all the treatment effects except for the column treatment on SPD (LSD, RCBD). Therefore, we conclude and recommend vertical loop method, horizontal loop method and horizontal loop method when performing microarray experiment on dual channel microarray applied on SPD (CRD, RCBD), SPD (CRD, LSD), and SPD (LSD, RCBD) respectively since this method gave the best precision for comparing different treatment effects.

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