



Improving breeding efficiency through application of appropriate experimental designs and analysis models: A case of lentil (*Lens culinaris* Medikus subsp. *culinaris*) yield trials



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ABSTRACT

Lentil (*Lens culinaris* Medikus subsp. *culinaris*) is an important staple pulse and rich source of protein, especially to the economically resource-poor consumers of the developing world. Experimental and analytic technology and statistical tools are needed to enhance lentil breeding progress. One of the concerns of field experimentation is to design experiments in suitable block designs and model the data to account for any left-over trend in the field layout and for correlations in the plot errors. Elite breeding lines of lentil developed through conventional breeding methods at ICARDA were evaluated in three contrasting environments in northern Syria and Lebanon during 1999–2005. This study examines the data on seed and straw yields from 226 trials conducted in randomized complete block (RCB) and in square lattice designs. Suitable models incorporating blocking structures, linear trends and spatially correlated plot-errors were fitted to the individual datasets. The results indicated that the spatial analysis model, which accounts for the spatial pattern of the field, was better than the commonly used RCB design model. The spatial analyses gave substantial increases in precision of predicted means for the genotypes. An average efficiency of pairwise genotype means comparison over RCB was 141% for seed yield and 158% for straw yield from the trials conducted in incomplete blocks and where found superior to RCB. It also enhanced estimates of broad sense heritability on mean-basis, with an average of 72% for seed and 70% for straw yield under the superior models, compared to 62 and 55% for RCB model, respectively. The percentage genetic gain due to selection at 10% intensity was 26% for seed and 20% for straw yield based on those models, which were 2–3% higher than those from the RCB model. In general, it is recommended to continue the use of incomplete block designs for variety trials in lentils and use the most suitable spatial pattern for statistical analysis to assist field crop breeders to enhance precision in selection of desirable genotypes. These results are consistent with findings of a number of other variety trials in lentil.

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1. Introduction

Lentil (*Lens culinaris* Medikus subsp. *culinaris*) is among the important pulse crops contributing to food and nutritional security of people in Asia and Africa. Its seeds contain high levels of protein, macronutrients, micronutrients and vitamins that provide nutritional security to poor consumers who cannot afford animal products due to high prices. Additionally, lentil straw is valuable feed for animals. Growing lentils in rotations provides sustainable cereal-based cropping systems. The International Center for Agricultural Research in the Dry Areas (ICARDA) has the world mandate for lentil

improvement and is running an international breeding programme to deliver International Public Goods in the form of genetic materials. It uses diverse genetic resources in commissioning crosses and subsequent development of genetically fixed lines to deliver to national programmes through an international nursery network. In this process, a large number of genotypes are developed by cross-breeding and evaluated under various yield trials in contrasting locations. Different designs, like randomized complete block (RCB), lattice, alpha-lattice and augmented designs are used to evaluate these genetic materials. These designs have been found useful in controlling for field variation by taking into account between-block variation (Fisher, 1935; Yates, 1936; Cochran and Cox, 1957; Patterson and Williams, 1976; Kempthorne, 1983; Hinkelmann and Kempthorne, 2005). Incomplete blocks in one or in two directions can be obtained using some popular software including GenStat (Payne, 2014) and CycDesigN (VSN International, 2015).

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Although the placement of incomplete blocks in a field layout is guided by experience and understanding of the experimental field, there is still scope to account for local fertility trends and the dependence of plot errors, which are not accounted for by the classical methods of experimental design and analysis. A number of approaches are available in the literature to address various such issues arising in field conditions. Papadakis (1937) measured local fertility status of a field plot using means of neighbouring plot residuals and performed covariate adjustment to adjust the genotype effects. This concept of nearest-neighbour adjustment and use of plot residuals was exploited by a number of researchers in improving precision of selection and the inferences for genotypes (Wilkinson et al., 1983; Pearce, 1998; also see, Piepho et al., 2008 for review). Statistical inferences were further enhanced by modelling the statistical behaviour of plot residuals in one dimension in the form of an autoregressive integrated moving average (ARIMA) model (Gleeson and Cullis, 1987) or two dimensions in the form of a separable ARIMA model (Cullis and Gleeson, 1991), while other specific forms of spatial dependence can be found in Cressie (1991). A number of other factors accounting for variation, such as fertility trends described as random smoothing cubic splines (CS) in row and column directions, in addition to the dependence of plot errors in row and column directions are given in Gilmour et al. (1997). The spatial error models with block effects and trends have been applied and their performance studied on various sets of data by Grondona et al. (1996) in cereals, Sarker et al. (2001) in lentil, Singh et al. (2003) in cereals and legumes, and Malhotra et al. (2004) in chickpea.

A set of 18 models examined by Singh et al. (2003) involved first order autocorrelated error structures and were believed to provide a good coverage of spatial patterns in short layouts. While autocorrelated error structures were found useful, the model with extra measurement error or a nugget term could have been included in gauging the spatial variability, besides the use of simple models in terms of random rows and columns effects (Leiser et al., 2012). In an evaluation of a number of field trials in sorghum in Mali, West Africa, Leiser et al. (2012) explored the large number of 91 models in each trial and found that the spatial models, in form of autocorrelated error structures along rows and columns, explained the data more effectively than the classical models under RCB and lattice designs. The due spatial adjustment lead to a higher genetic gain due to selection and also resulted in change in the genotypes ranks.

Singh et al. (2003) used a model selection method using AICD, Akaike Information Criterion (Akaike, 1974) expressed as residual maximum likelihood (REML) deviance, which can be used to compare two models based on the same set of fixed effect terms. The best model was screened out of 18 models in the case of incomplete block designs and nine in the case of RCB designs. In all these models, the plot error variances were assumed constant. Singh et al. (2010) examined heterogeneity among the error variances in addition to the spatial error models discussed by Gilmour et al. (1997) and Singh et al. (2003). Competition effects (Durban et al., 2001) and spatial variability and within-row interplot competition models (Stringer et al., 2011) have been introduced for field trials.

Application of various spatial models has led to substantial increases in efficiency of pairwise comparison of genotypes in cereals and legumes. This study examines historical data on seed and straw yields from 226 lentil field trials conducted in three contrasting locations with objectives of (1) identifying spatial patterns describing the experimental fields in northern Syria and Lebanon, (2) reduction in the coefficient of variation from the standard complete block analysis method and (3) efficiency of the methods used in Singh et al. (2003). Further, (4) we examine the changes in the estimates of heritability and genetic gain when selecting the genetic material from different maturity groups.

2. Materials and methods

2.1. Environments, genetic material and experimental designs

A total of 226 experiments were conducted over seven years during 1999–2005 at three locations: (1) Breda in Syria (35°56'N, 37°10'E) with an elevation of 300 m and a long-term average annual rainfall of 266 mm; (2) Tel Hadya in Syria (36°01'N, 36°56'E) with elevation of 284 m, and rainfall of 334 mm; and (3) Terbol (33°49'N, 35°59'E), in the Beqaa valley in Lebanon, with elevation of 950 m and rainfall of 515 mm. The number of genotypes in these trials varied from 11 to 30 over the different years (Table 1). The set-ups of the experiments are presented in Table 1. The experimental designs were square lattices with 2–4 replicates when the number of genotypes was 16 and 25; and RCB designs with 2–4 replicates were used for all other numbers of genotypes. In most of these experiments, details of the field layouts were available and the spatial position of the plots could be determined. The genetically fixed materials were organized as preliminary yield trials (PYT) and advanced yield trials (AYT). The PYT materials were in the F₇ generation while that in AYT were in the F₈ generation. Data from a total of 226 trials were available on grain yields, out of which 191 trials were in lattice designs with spatial position of the plots available for 167 trials and 35 trials in RCB where spatial positions of plots were available for 33 trials. For straw yields, there were 199 trials with spatial position of the plots available for 146 out of 166 trials conducted in lattices and for 32 out of 33 trials conducted in RCB.

The agronomic practices followed were as appropriate for the local conditions. The harvested areas in the centre of the plots were 4.8 m² (2 m × 8 rows × 0.3 inter-row distance) in the advanced trials, and 3 m² (2 m × 5 rows × 0.3 inter-row distance) in the preliminary trials, and were used for yield assessment in kg/ha.

2.2. Statistical methods

We present here the method for model screening given in Singh et al. (2003) and the expressions for estimation of efficiency of experimental design, heritability and genetic advance. Consider a resolvable incomplete block design to evaluate v genotypes in b incomplete blocks in each replicate and R replicates. The block size would be $k = v/b$. Let Y_{ijl} be the response (i.e. yield) from the plot under genotype i , block j of replicate l ; $i = 1, 2, \dots, v$, $j = 1, 2, \dots, b$, and $l = 1, 2, \dots, R$. This response can also be indexed by the plot position $[r, c]$ where the above plot is in row r and column c of the $p \times q$ layout, where $r = 1, 2, \dots, p$, and $c = 1, 2, \dots, q$.

The general model assumed to explain the variation in Y is:

$$Y = \mu J + X\tau + Zu + \xi + \varepsilon$$

where Y is the vector of observed responses written as (Y_{ijl}) or $(Y_{[r,c]})$; μ is the general mean; J is vector of unity; X is the incidence matrix associated with fixed effects assumed for factors such as genotype effects and a linear fertility trend and the vector of these unknown effects is τ ; Z is the incidence matrix associated with factors with random effects, such as replicates and blocks within replicates, with effects denoted by u ; ξ is the vector of random cubic smoothing spline (CS) effects; and ε is the vector of random errors with a correlation structure.

The above model can more compactly be written as three terms:

$$Y = X^* \alpha + Z^* \beta + \varepsilon$$

where all the fixed effects are written as vector α and associated matrix X^* , and the random factor effects (other than random errors) are written as vector β and with matrix Z^* . This model form accounts for: (1) with or without incomplete blocks (replicate effect

Table 1
Distribution of number of experiments according to experimental design, locations, maturity and generation of genetic material.

	Locations		Genotypes		Replicates	Layout	(sub-total)
Number of experiments under the two experimental designs and over the locations							
Experimental Designs	Breda	Tel Hadya	Terbol	n_g		Rows \times Columns	
RCB	14 (0)	16 (2 ³)	5 (0)	11, 12, 13, 14, 15, 18, 19, 21, 23, 25 [Ⓞ] , 30	2–4	2–4 \times n_g	35 (2)
Square lattice	9 (0)	16(5)	9(0)	16	2–4	2–4 \times 16, 6 \times 8	34 (5)
Square lattice	57 (3)	64 (14)	36(2)	25	2–4	2–4 \times 25, 9 \times 10	157 (19)

⁵ Cases with no spatial information (i.e. rectangular layout was not available).

[Ⓞ] There were eight trials in RCB with 25 genotypes, which were otherwise evaluated mostly in lattices at Breda (3), Tel Hadya (3) and Terbol (2).

is always included); (2) a linear trend along rows is assumed in the form of a linear function of the column number (shows a gradient along rows), or a random CS around the line or is assumed absent; (3) plot errors are assumed to follow either first-order auto-correlation (Ar) along rows, or independently first-order Ar along rows and columns (i.e. separable Ar along rows and Ar along columns, ArAr) or to be independent. The combinations of blocking options, linear trend and plot errors gave rise to 18 models in Singh et al. (2003), which were seen likely to cover most of the spatial patterns prevailing in a crop variety evaluation field. The model details and method of selecting the best out of them have been given in Singh et al. (2003). We describe those models with their notations here in brief:

(1) Rcb: Randomized complete block design (RCB), (2) RcbAr: Rcb with first-order Ar errors along rows, (3) RcbArAr: RCB with Ar errors in plots along rows and along columns, (4) RcbL: RCB with a linear trend (L) along rows, (5) RcbAr: Rcb with a linear trend (L) along rows and first-order Ar errors along rows, (6) RcbArAr: RCB with with a linear trend (L) along rows and Ar errors in plots along rows and along columns, (7) RcbLCS: Rcb with a linear trend along rows and random cubic smoothing spline (CS) in column numbers, (8) RcbLCSAr: RCB with a linear trend along rows, a random CS in column numbers, and Ar errors along rows, (9) RcbLCSArAr: RCB with a linear trend along rows, a random CS in column numbers, and Ar errors along rows and columns. For the data from an incomplete block, another nine models were constructed by introducing incomplete blocks within replicates. An abbreviation Lat, denoting lattice design, comprises random replicate effects and block effects within replicates. For instance, LatL: Lattice with a linear trend along rows, LatArAr: Lattice with Ar errors in plots along rows and along columns, etc. The models with Ar terms were also added with an extra independent error terms, i.e., “*Units*” in the notation of Genstat software (Payne, 2014, Chapter 5, page 650) to account for a nugget effect. In majority of the trials, a replicate comprises a row, another simple model with random row effects and column effects, denoted by RowCol, was fitted. Thus on data from lattice designs, 31 models (18 models as in above, 12 models with an extra term for nugget effect, and 1 rows and columns effect model) were

with the same set of fixed effects is generally carried out using the AIC which was expressed in terms of the deviance values as $AICD = \text{deviance} + \text{twice the number of linear and non-linear variance components of the models}$. Among the models having the same set of fixed effect terms, the model with the lowest AICD value was considered to be best. Using this method, the best model for each trial was obtained. The significance of a fixed effect was assessed using the Wald test statistic produced by the REML directive. These 18 models were grouped into the models with linear trend (L) term (12 models) and those without (6 models). The best model from the group with linear trend was chosen using the AICD criterion. If the linear trend was significant (using the Wald test) then the selected model was considered best of the 18 models. If the linear trend was not significant, then the AICD was used to select the best out of the remaining 6 models without trend. If the selected model, out of 9 models for an RCB design or 18 models for an incomplete block design, had an autocorrelation structure, then it was fitted again by including a nugget effect. The difference of the deviances obtained by fitting models with and without nugget effect was tested as a chi-square distribution on one degree of freedom. In detection of a non-zero variance component of a nugget effect in the model, the limitation of likelihood ratio tests based on deviances may be addressed by introduction of a more appropriate test based on a mixture of chi-square distributions as presented in Stram and Lee (1994) and Visscher (2006). If the difference was significant then the model with nugget effect was considered for screening against RowCol model using the AIC values.

The current version of the Genstat software also provides AIC values, so the above models can equivalently be compared by directly using the AIC values, smaller the AIC value, better is the model irrespective of the fixed and random terms included in the models (Wolfinger, 1996). Thus using AIC values, selection of the best models can be carried out of 31 or 16 models for lattice or RCB data, respectively.

Efficiency of a given design and analysis duo (‘the method’) relative to RCB, and analysis based on independent errors and absence of any fertility trend (RCB) was obtained in terms of pairwise variance and was given by the expression:

$$\text{Efficiency\%} = \frac{\text{Average variance of pair-wise contrasts of genotype effects under RCB analysis}}{\text{Average variance of pair-wise contrasts of genotype effects under 'the method'}}$$

explored. The models with nugget effects will be denoted with ending in Ng. Similarly for data from RCB, a total number of 16 models were fitted.

Selection of the best model was carried out as follows. Each of the 18 models was fitted assuming fixed genotype effects and random replicate effects and block effects within replicates was carried out following Singh et al. (2003). The REML method and associated directives of GenStat were used to estimate the model parameters. The log-likelihood was computed in terms of a statistic, called the ‘deviance’ which is minus twice the REML log-likelihood ignoring a constant dependent on the fixed terms. Comparison of models

In order to estimate the broad sense heritability (h^2) on mean-basis and genetic advance/gain due to selection, we assumed the genotype effects to be random with variance σ_g^2 and plot-error variance σ_e^2 . The h^2 on mean-basis is given by $h^2 = \sigma_g^2 / (\sigma_g^2 + \sigma_e^2/r)$ and has been used by Singh et al. (2013). Singh and Ceccarelli (1995) evaluated the bias of the heritability on plot-basis using, $\sigma_g^2 / (\sigma_g^2 + \sigma_e^2)$, for data from incomplete blocks and reported bias below 10% for heritability level above 50%. This expression of heritability, h^2 , is based on the genotypic variance and the error variance resulting from the fit of the spatial or non-spatial model and therefore are adjusted for the design and error correlations. This expression does not include the variance component of control factors with random effects. Piepho and Möhring (2007) discussed

limitations of this estimate of heritability, h^2 , when the data arise from heterogeneous or correlated error structures and suggested (better) alternatives including a proposed “ad hoc” estimator of heritability, another using simulations, and that due to Cullis et al. (2006). For various challenges associated with definition and estimation of heritability from experiments on plants, one may refer to Holland et al. (2003) and Nyquist (1991). Besides the h^2 , we also computed, for comparison, two other estimates of heritability which adjust for incomplete blocks and error structures to a different degree are given by: $h^2_{Ad hoc} = \sigma_g^2 / (\sigma_g^2 + \bar{v}_{BLUE}/2)$ (Piepho and Möhring, 2007) and $h^2_C = 1 - \bar{v}_{BLUP} / (2\sigma_g^2)$ (Cullis et al., 2006), where \bar{v}_{BLUE} and \bar{v}_{BLUP} are respectively the average variance of pair-wise differences between the best linear unbiased estimates (BLUE) and best linear unbiased predictors (BLUP). The genetic gain or advance based on mean over replicates and for a selection intensity of p is $C\sigma_g h^* / \bar{Y}$ where C is a constant given by $C = \frac{1}{p\sqrt{2\pi}} e^{-z_p^2/2}$ and z_p is the upper p quantile of standard normal distribution, h^* is the square root of the heritability used (h^2 , $h^2_{Ad hoc}$ or h^2_C), \bar{Y} is mean response and r is the number of replicates (Kempthorne, 1983). The quantity C is 2.063, 1.755 and 1.4 for 5, 10 and 20% intensity of selection, respectively. Furthermore, the above genetic gain expression is based on the means of independent observations with a constant variance, i.e. the case of standard model for RCB data, this expression of genetic gain will also comprise of an additional limitation in the approximate gain for the other models. The genetic gain expressions may be denoted by GA_h , $GA_{Ad hoc}$ and GA_C for the heritability expressions, h^2 , $h^2_{Ad hoc}$ and h^2_C , respectively. Using the estimates of heritability, or the genetic gain, from various trials, a calibration of estimates resulting from one expression in terms of the other can be done using a linear regression if the correlation between them is appreciably high. Closeness of a pair of the estimates, say h^2 and h^2_C , can also be examined in terms of slopes of regression line of h^2_C on h^2 , passing through the origin, as described in standard texts (Kutner et al., 2004). Another statistics used was coefficient of variation between variables (CVV): (a) between the two estimates (h^2 and h^2_C) to compare the pair, and (b) between the three estimates

(h^2 , $h^2_{Ad hoc}$ and h^2_C) to examine closeness among the triplet. The CVV values were averaged over the trials for models other than the standard RCB model. This approach was also applied to compare the genetic gains from the three estimates of heritability.

3. Results

3.1. Coefficient of variation, efficiency, genetic advance and heritability over all experiments

The analysis of the data using the best models that were screened out of the 16 models for RCB data and 31 models for lattice design data indicated a general reduction in the coefficient of variation (CV) values for the best model, except in a few cases where CV increased (Fig. 1). As expected, the accounting for incomplete blocks, linear trends in the field, Ar between the plot errors with or without nugget effects, and rows and columns effects contributed to the reduction of experimental errors. A large number of points lying with $CV < 20\%$ on the 1:1 line (Fig. 1) show that the experimental design and model used did not capture errors beyond the RCB model. However, the efficiency and other parameters are functions of genotypic variance, and error variance as well, and these were affected differently due to presence of correlations in the plot errors.

The correlations, slopes of the regression line passing through origin, and average CVV between the three estimates of heritability h^2 , $h^2_{Ad hoc}$ and h^2_C , and the resulting gains are presented in Table 2 based the non-RCB models. For the RCB model, the three expressions are same. High correlations indicated that $h^2_{Ad hoc}$ and h^2_C can be calibrated by a linear equation in terms of h^2 , correlations exceed 0.968 for heritability and 0.998 for genetic gains. However, the slopes of the lines passing through origin were not significantly different from 1 (P -value > 0.07) for seed yield indicate that the estimates h^2 are approximating reasonably close to h^2_C and $h^2_{Ad hoc}$ as well as the associated genetic gains. Numerically, slope varied in the range 0.999–1.005 over the four cases. For straw yield, the slopes

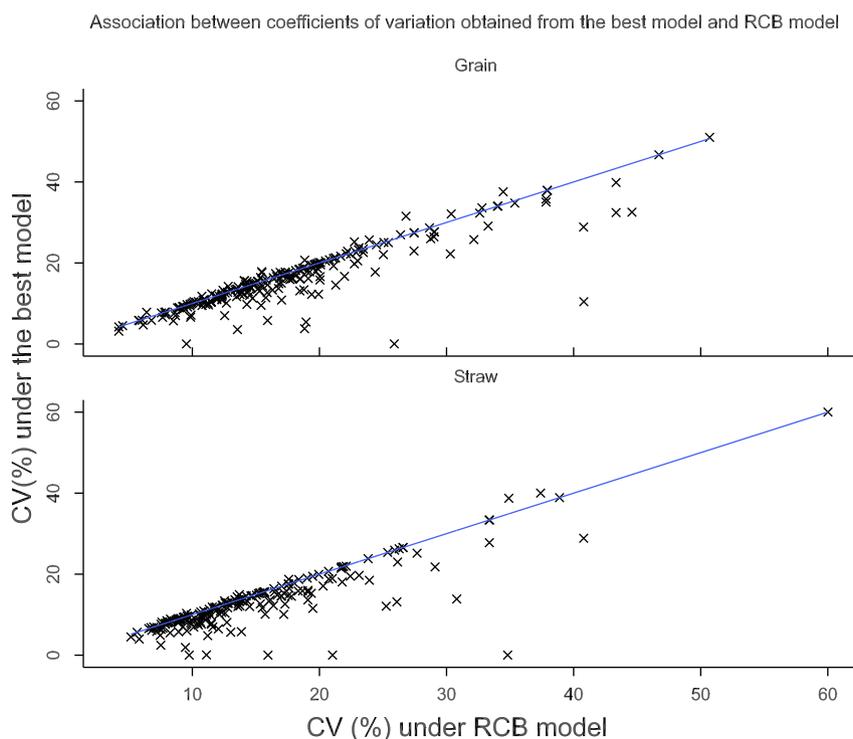


Fig. 1. Coefficient of variation (CV) under the best model vs. CV under the randomized complete block design.

Table 2
Estimates of correlation, slope and coefficient of variation between estimators of broad sense heritability on mean-basis and genetic gain at 10% selection intensity for models other than RCB model.

	Variable	Correlation (X,Y)	b^s	SE [§] (b)	tprob [°] [Slope -1 > 0]	Mean CVV [#] (X,Y) (%)	Mean CVV (All the three estimates) (%)	
Seed yield (N= 163)	Y		$X = h^2$					
	h_C^2	0.966	0.999	0.0078	0.873	6.4		
	$h_{Ad hoc}^2$	0.965	1.001	0.0079	0.875	6.6		
	All the three estimates						5.8	
	GA_C	0.998	$X = GA_h$	1.004	0.0031	0.180	3.3	
	$GA_{Ad hoc}$	0.998	1.005	0.0030	0.075	3.4		
Straw yield (N= 143)	All the three estimates						3.0	
	h_C^2	0.968	$X = h^2$	0.980	0.0083	0.020	8.5	
	$h_{Ad hoc}^2$	0.967	0.982	0.0084	0.030	8.6		
	All the three estimates						8.1	
	GA_C	0.999	$X = GA_h$	1.006	0.0030	0.042	5.3	
	$GA_{Ad hoc}$	0.999	1.007	0.0031	0.034	5.4		
	All the three estimates						5.3	

N is the number of trials.

^s b is the least square estimate of the Slope in the regression equation: $Y = \text{Slope } X$.

[§] SE: estimated standard error.

[°] tprob(|Slope-1| > 0) is the probability of Slope differing from 1 using t-distribution on N-1 degrees of freedom.

[#] CVV: Coefficient of variation between variables and GA: genetic gain (advance) are described in the text.

were significantly different from 1 at 5% but not at 1% level of significance, as the P-value was in the range 0.02–0.04 over the four cases. In this case too, we can say the expressions resulted into values close to h^2 and similar for the associated gains. The CVV values are also low, for genetic gains in the range 3.3–5.4 (for pairs with h^2) and 3.0–5.3 (for all the three estimates). However, CVV values for heritability were higher than those for the gains (6.4–8.6 for pairs with h^2 and 5.8–8.1 for all the three expressions). Therefore, the results of the three expressions were found reasonably close, particularly those supported by the slope of the linear regression.

Table 3 presents the efficiencies of the experimental design and analysis model duo, estimated gain due to selection and h^2 over all the available trials for seed (225) and straw yields (199); also shown is the number of experiments which resulted in a higher efficiency or higher gain due to selection or higher broad-sense heritability on mean basis in comparison to RCB, and the averages over such cases.

3.2. Efficiency

The best models gave on an average, over all trials, efficiency for pairwise comparison of the genotypes of 129% relative to RCB for seed and 133% for straw yields. Thus in such trials a given precision of a treatment difference that can be obtained from an RCB design can also be obtained by around a 30% reduction in the number of replications if using either an incomplete block (IB) design, e.g. a lattice design, and/or one of the spatial methods. Computations for the experiments, excluding those with RCB analyses, showed that the gain for the non-RCB design/analysis experiment cases gave an average efficiency of approximately 141% for seed (159 trials) and 148% for straw yield (139 trials). Out of such cases, the average efficiency where IB design data was analysed using spatial methods resulted in 145% for seed (116 trials) and 153% for straw yield (107 trials), indicating further reduction in resources was possible to achieve precision of genotype comparisons in contrast to experiments using an RCB design.

3.3. Heritability

The average broad-sense heritability estimates on mean-basis using h_C^2 from the best models superior to RCB (in the sense of

efficiency exceeding 100%), RCB analyses and overall the trials were 72%, 62% and 67% for seed yield, respectively, and correspondingly 70%, 55% and 62% for straw yield. Thus under the spatial models, a higher estimates of heritability was found compared to that under RCB.

3.4. Gain due to selection

The average gain due to selection of the top 10% of genotypes based on the best models superior to RCB were 26% for seed yield and 20% for straw yield, compared to and 23% and 17% if RCB was used, respectively. This showed an increase of 3% on each of the two traits when using a more efficient model than RCB. Overall the trials, the gain due to the selection was 24% for seed yield and 18% for straw yield.

3.5. Spatial patterns and efficiency, genetic advance and heritability

With a view to determining the type of spatial variability in fields where various experiments were laid out, Table 4 gives the frequencies of the models describing the field variability best, and the average efficiency, heritability h_C^2 and genetic gain due to selection. Of the 225 trials where seed yield was analysed, the RCB model was found best in 63 cases (which were designed either in RCB or IB design and the spatial information of the plots was available). In the 32 trials in RCB where spatial information was available, the best fitted models captured the spatial feature of the fields such as first-order autocorrelation between plot errors along rows (RcbAr) in seven cases (with average efficiency of 141%) and first-order autocorrelations between plot errors along rows and along columns (RcbArAr) in four cases (average efficiency of 171%), a linear trend along rows (RcbL) was detected in four cases (average efficiency of 130%) and random rows and columns effects in seven trials (average efficiency 119%) for seed yield.

In cases of lattices not only the incomplete blocks (seven trials with average efficiency of 110% for seed yield) and models with random rows and columns effects without any correlation structures (13 trials with an average efficiency of 130% for seed yield) were found useful, but also the spatial features of plots such as Ar, ArAr and local trend along rows represented by random cubic smoothing

Table 3

Number of trials and average efficiency, genetic gain for 10% selection intensity and heritability for various groups of trials.

	Best Model or RCB ⁵	Seed yield				Straw yield			
		Best trials		Experiments superior to RCB ⁶		Best trials		Trials superior to RCB	
		N	Mean	N	Mean	N	Mean	N	Mean
Efficiency (%)	IB	24	120.3	21	123.2	20	113.1	16	116.3
	SPCB	32	125.7	22	137.4	32	122.4	16	144.7
	SPIB	167	131.1	116	144.8	146	138.5	107	152.5
	(over trials superior to RCB) ⁶			159	140.9			139	147.5
	(overall trials)	225	128.9			199	133.2		
Heritability	RCB	2	0.84			1	0.63		
	IB	24	0.62	12	0.70	20	0.56	10	0.65
	SPCB	33	0.53	21	0.62	32	0.49	14	0.53
	SPIB	167	0.71	107	0.75	146	0.66	95	0.74
	(over trials superior to RCB) ⁶			140	0.72			119	0.70
(overall trials)	226	0.67			199	0.62			
(over RCBs only)	63	0.62			56	0.55			
Genetic gain (%)	RCB	2	54.6			1	28.9		
	IB	24	27.0	10	25.2	20	13.5	5	14.8
	SPCB	33	23.2	20	28.3	31	14.2	13	13.4
	SPIB	167	23.6	86	24.9	145	19.3	73	21.0
	(over trials superior to RCB) ⁶			116	25.5			91	19.6
(overall trials)	226	24.2			197	18.0			
(over RCBs only)	63	22.5			56	16.8			

⁵ RCB = randomized complete block design and analysis based on no trends in the layout and independent plot errors; IB = Incomplete block design and analysis based on no trend in the layout and independent errors; SPCB = best model out of the 16 models explored for RCB situations; SPIB = best model out of the 31 models explored for incomplete block design situations. N = number of experiments.

⁶ Based on the experiments superior to RCB in the sense of the efficiency % exceeding 100. Heritability = h_c^2 (Cullis et al., 2006). Genetic gain (%) was calculated using h_c^2 .

spline (CS). In the trials which were laid out in lattices, the incomplete block effects appeared to have been marginalized in the light of spatial features accounted for by autocorrelation between plot errors (Table 4). For example, the model RcbAr (i.e. a model without incomplete blocks) was found best in 31 of 167 fields/experiments in IB designs with spatial information. The next two models that frequently occurred were RcbL (23 fields) and RcbArAr (16 fields) with average efficiencies of 115 and 153%, respectively, for seed yield. For straw yield, the top three frequently occurring spatial patterns were RcbAr (24 of 146 fields with spatial information) with average efficiency of 142%, RcbArAr (11 fields) with efficiency of 145% and RcbCS (nine fields) with efficiency 213%. The RowCol was found best in 22 fields with 120% efficiency. In a total of three fields under either RCB or lattice design, presence of nugget effects i.e. an extra independent error term was also found significant, giving models RcbArNg, RcbArArNg and RcbLArArNg with efficiency 123, 154 and 321% respectively, for straw yield.

Over all the experiments, the average h_c^2 estimates for seed yield for the five most frequent models were 62% (63 fields best described by RCB models), 69% (38 fields under RcbAr models), and 62% (31 fields under Lat), 67% for the RcbL (27 fields) and 73% for RcbArAr models (21 fields), respectively. In the case of straw yield, the heritability under frequent models was 55% (56 fields described by RCB), 61% (28 fields under Lat model), 56% (RcbAr model in 27 fields), 59% from 26 fields with RowCol model and, 75% from 12 fields under RcbArAr model.

The genetic gain at 10% intensity of selection where RCB was found best was 14% (10 experiments in RCB) and 23% (in 51 experiments with lattices) for seed yield; such values for straw yield were 16% (16 experiments in RCB) and 17% (39 experiments in lattices). For the most frequent spatial model, the gain was 40% (RcbAr models in seven experiments conducted in RCB) and 22% (RcbAr models in 31 experiments in lattices) for seed yield. A gain of 38% was obtained from RcbLArAr model (10 experiments in lattices). There were much higher gains of 41–76% where lattice blocks were present in the spatial models LtAr, LtArAr and LtCS but were found in only one field per model. For straw yield from experiments with lattices, the most frequent spatial model RcbAr gave 15% gain

(23 experiments), followed by the RcbArAr model giving 20% gain (9 fields). The genetic gains were relatively low in the range of 5–8% for the models with nugget effects.

3.6. Heritability and genetic gains due to selection for different maturity groups

Table 5 gives the distribution of trials maturity group-wise. Over all the trials, the average seed yield h_c^2 estimates were 75, 66 and 65% for early, medium and late maturing materials, respectively. Corresponding figures for straw yield were 72, 62 and 52%, respectively. This shows a declining trend in the heritability with maturity. The gain due to the top 10% selection also followed a decreasing trend with maturity in the range of 35–21% from seed yield and 32–11% for straw yield.

4. Discussion

In field trials, experimental error variability is controlled in a number of ways, including by placement of blocks, use of possible covariates and models describing spatial variability (Fisher, 1935; Papadakis, 1937; Piepho et al., 2008; Singh et al., 2010). The effectiveness of a component of error variability is specific to the field where the experiment has been laid out. In relatively recent years, investigations have incorporated spatial variability models with or without blocks in comparison with using only blocks to account for variability. Most of the experimental designs have been developed by minimizing the average variance of pairwise comparisons of genotype effects which are assumed as fixed. However, it is more pertinent to search for experimental designs with a view to maximize the general gain due to selection from field trials. With this view, Cullis et al. (2006) have developed partially replicated (p-rep) designs considering spatial correlation structure and with genotypes effects and examined genetic gain using a simulation study. Several classes of prep designs have recently been constructed by using α -arrays (Williams et al., 2011, 2014). For generation of randomized field plan for p-rep designs on a two-way layout, an R-package (R Development Core Team, 2011) DiGger due to Coombes

Table 4
Model structures found best and average efficiency, heritability and genetic gain at 10% selection intensity.

	Model ⁵	Seed yield						Straw yield						
		Experiments where spatial information was available												
		Complete blocks		Incomplete blocks		All cases		Complete blocks		Incomplete blocks		All cases		
		N	Mean	N	Mean	N	Mean	N	Mean	N	Mean	N	Mean	
Efficiency (%)	Lat	na	na	7	109.9	31	117.9	na	na	8	129.4	28	117.7	
	LatAr	na	na	1	106.5	1	106.5	na	na	1	198.3	1	198.3	
	LatArAr	na	na	1	152.2	1	152.2	na	na	3	163.4	3	163.4	
	LatCS	na	na	1	175.1	1	175.1	na	na					
	LatCSArAr	na	na					na	na	1	225.2	1	225.2	
	LatL	na	na					na	na	1	188.7	1	188.7	
	RcbAr	7	140.7	31	144.7	38	143.9	3	123.2	24	141.5	27	139.5	
	RcbArAr	4	170.8	16	153.4	20	156.9	1	159.1	11	144.8	12	146	
	RcbArArNg									1	154.1	1	154.1	
	RcbArNg							1	122.6			1	122.6	
	Rcb	10	100	51	100	63	100	16	100	39	100	56	100	
	RcbCS			8	166.6	8	166.6			9	212.9	9	212.9	
	RcbCSAr			1	305.6	1	305.6			2	178.6	2	178.6	
	RcbCSArAr			2	328.6	2	328.6			3	253.9	3	253.9	
	RcbL	4	129.8	23	115.3	27	117.5	4	126.1	6	113	10	118.3	
	RcbLAr			2	158.2	2	158.2			7	169.2	7	169.2	
	RcbLArAr			10	170	10	170	3	191.8	7	169.9	10	176.5	
	RcbLArArNg									1	321.4	1	321.4	
	RowCol	7	119.4	13	129.9	20	126.2	4	146.2	22	119.7	26	123.7	
	(sub-total)			32		167		225			32		146	
Heritability	Lat	na	na	7	0.63	31	0.62	na	na	8	0.73	28	0.61	
	LatAr	na	na	1	0.96	1	0.96	na	na	1	0.96	1	0.96	
	LatArAr	na	na	1	0.93	1	0.93	na	na	3	0.81	3	0.81	
	LatCS	na	na	1	0.96	1	0.96	na	na					
	LatCSArAr	na	na					na	na	1	0.01	1	0.01	
	LatL	na	na					na	na	1	0.94	1	0.94	
	RcbAr	7	0.80	31	0.66	38	0.69	3	0.7443	24	0.53	27	0.56	
	RcbArAr	5	0.60	16	0.77	21	0.73	1	0	11	0.82	12	0.75	
	RcbArArNg									1	0.55	1	0.55	
	RcbArNg							1	0.4888			1	0.49	
	Rcb	10	0.44	51	0.65	63	0.62	16	0.4855	39	0.57	56	0.55	
	RcbCS			8	0.71	8	0.71			9	0.76	9	0.76	
	RcbCSAr			1	0.83	1	0.83			2	0.76	2	0.76	
	RcbCSArAr			2	0.90	2	0.90			3	0.86	3	0.86	
	RcbL	4	0.38	23	0.72	27	0.67	4	0.3045	6	0.73	10	0.56	
	RcbLAr			2	0.93	2	0.93			7	0.76	7	0.76	
	RcbLArAr			10	0.82	10	0.82	3	0.8214	7	0.87	10	0.86	
	RcbLArArNg									1	0.58	1	0.58	
	RowCol	7	0.44	13	0.74	20	0.63	4	0.3878	22	0.63	26	0.59	
	Genetic gain (%)	Lat	na	na	7	17.3	31	24.8	na	na	8	19.2	28	15.2
LatAr		na	na	1	63.7	1	63.7	na	na	1	67.9	1	67.9	
LatArAr		na	na	1	41.1	1	41.1	na	na	3	15.3	3	15.3	
LatCS		na	na	1	75.7	1	75.7	na	na					
LatCSArAr		na	na					na	na	1	0.1	1	0.1	
LatL		na	na					na	na	1	21.1	1	21.1	
RcbAr		7	39.8	31	22.2	38	25.4	3	22.27	23	15.4	26	16.2	
RcbArAr		5	25.4	16	21.9	21	22.7			11	31.8	11	31.8	
RcbArArNg										1	5.1	1	5.1	
RcbArNg								1	7.7			1	7.7	
Rcb		10	14.5	51	22.8	63	22.5	16	15.76	39	16.9	56	16.8	
RcbCS				8	22.5	8	22.5			9	20.4	9	20.4	
RcbCSAr				1	9.8	1	9.8			2	11.1	2	11.1	
RcbCSArAr				2	26.7	2	26.7			3	37.0	3	37.0	
RcbL		4	18.5	23	20.2	27	20.0	4	6.3	6	18.5	10	13.6	
RcbLAr				2	35.1	2	35.1			7	20.8	7	20.8	
RcbLArAr				10	38.5	10	38.5	3	20.69	7	28.3	10	26.0	
RcbLArArNg										1	8.3	1	8.3	
RowCol		7	20.3	13	21.2	20	20.9	4	6.65	22	16.7	26	15.1	

⁵ Rcb: Randomized complete blocks (RCB) with independent errors. RcbAr: RCB with first-order auto-correlated (Ar) errors along rows. RcbArAr: RCB with first-order auto-correlated errors in plots along rows and along columns. RcbL: RCB with linear trend along rows. RcbLAr: RCB with linear trend along rows and first-order auto-correlated errors in plots along rows. RcbLArAr: RCB with linear trend along rows and first-order auto-correlated errors in plots along rows and along columns. RcbCS: RCB with linear trend along rows and random cubic smoothing spline in column numbers. RcbCSAr: RCB with linear trend along rows, random cubic smoothing spline in column numbers, and Ar errors along rows. RcbCSArAr: RCB with linear trend along rows, random cubic smoothing spline in column numbers, and Ar errors along rows and columns. Lat: Lattice blocks with independent errors. LatAr: Lattice blocks with first-order auto-correlated errors in plots along rows. LatArAr: Lattice blocks with first-order auto-correlated errors in plots along rows and along columns. LatL: Lattice blocks with linear trend along rows. LtCS: Lattice blocks with linear trend along rows and random cubic smoothing spline in column numbers. LatCSArAr: lattice blocks with linear trend along rows, random cubic smoothing spline in column numbers, and Ar errors along rows and columns. RcbArNg: RcbAr model with extra independent error term (nugget effect). RcbArArNg: RcbArAr model with extra independent error term. RcbLArArNg: RcbLArAr model with extra independent error term. RowCol = Random rows and columns effects. N = number of experiments. na: not applicable. Blanks imply the model in the row was not found best in any trial.

Table 5
Heritability and genetic gain at 10% selection intensity using spatial methods in trials for various maturity groups.

	Maturity	Seed yield		Straw yield	
		N	Mean	N	Mean
Heritability ^s	Early	46	0.75	41	0.72
	Medium	126	0.66	112	0.62
	Late	54	0.65	46	0.52
Genetic gain (%)	Early	46	35.2	41	32.1
	Medium	126	21.6	112	15.5
	Late	54	20.8	46	10.6

^s Heritability = h_c^2 (Cullis et al., 2006). Genetic gain (%) was calculated using h_c^2 .

(2009) has been gaining ground in many plant breeding programs as well as GenStat software (Payne, 2014) and CycDesigN software (VSN International, 2015).

The spatial pattern being specific to a field, it is always a question whether there is a model that could be used by default as a contrast to the early days of models with independent errors with constant variance. A number of studies in lentils have shown suitability of different models, e.g. of 47 trials in 25 genotypes in simple/triple lattices in Sarker et al. (2001), 26 trials were better explained using spatially correlated errors on linear trends, while complete/incomplete blocks were suitable in 21 trials. The present study, which is based on a much larger number of field trials (226), also supported the role of spatial models (Tables 3 and 4)—where models based on correlated errors were frequently found appropriate. We also noted that models which used incomplete blocks were much less often appropriate, as it seemed the correlation structure compensated for the lattice blocks (Table 4), an observation also noted by Sarker et al. (2001) in a lentil study involving 53 trials. Experimental fields under these trials, exhibit a distribution of different variability patterns including the presence of trends along rows, permitting control of variability in one direction in terms of incomplete blocks as well as trends in row and column direction. The results of these trials guide that a desirable experimental design strategy would be to choose a design which insures capture of trends in two directions and serves as a better alternative to incomplete block designs with one-way blocking. The row–column designs do not pose any particular operational inconvenience as the trials generally are laid out on a rectangular field layout. For this purpose, an efficient row–column design, including a resolvable row–column design, may be implemented followed with a baseline analysis using a randomization based row–column model. There is a vast literature on efficient row–column designs constructed since the early days of development of experimental designs including Yates (1940), Pearce (1975), Singh and Dey (1979), Jonh and Eccleston (1986), Ipinyomia (1990), and standard texts (Hinkelmann and Kempthorne, 2005). Randomization plan of such designs can also be generated from the above software. Furthermore, an add-on value to the data generated by the row–column designs can be achieved by spatial modelling of error structures which could not be perceived at design stage.

In evaluation of sorghum trials in Mali, Leiser et al. (2012) covered a much wider envelop of spatial models including those with autocorrelated errors of higher order than those being presented in this study, and drew a similar conclusion that spatial models can add further value to experiment over the classical complete block design model. The lattice designs used here were in moderate number of genotypes (25 or less) while in reality plant breeders evaluate much larger number of genotypes, e.g. 70 genotypes of sorghum in trials in Mali evaluated by Leiser et al. (2012). In such situations, a row–column design with most suitably identified spatial model would be expected to result in even further higher efficiency, heritability and genetic gain over the conventional complete block designs.

Modelling field variability using the best of the 31 models led to an improvement (increase) in heritability (h_c^2) compared to that under RCB (62% on 63 trials for seed yield and 55% on 56 trials for straw yield; Table 3). A better accountability of systematic factors clearly indicated higher genetic gain compared to RCB—this implies more efficient breeding progress. The present study also examined the effect of analyses for spatial models on the association of heritability and genetic gain from trials with maturity class of the genotypes.

ICARDA has developed a suite of Online BioComputing modules which run on the Center's dedicated machines. This computing system requires a data file to be prepared specifically for an analysis and to be submitted by email. In return is received an output file containing results of the analysis. Spatial analysis, as presented in this study, can be carried out by request to the authors.

5. Conclusion

The study summarizes results of 226 lentil trials using spatial models. The statistical analysis, without any addition to the cost of experimentation, adds value to the experimental results in terms of substantial increase in precision of genotypes' predicted means and estimates of heritability and genetic gain due to selection. The search for a suitable spatial pattern contributed to enhancing the precision of the genotype means and breeding progress. Thus, field crop breeders can improve the selection efficiency and select those genotypes with genetic potential by applying appropriate statistical models without an extra cost.

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