



Comparative efficiency of randomized complete block design versus alpha lattice design in wheat breeding experiments

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Abstract

Evaluating large number genotypes in complete block design is impractical due to the need for all treatment combinations in each block, which increases experiment size and reduces accuracy. To minimize errors and enhance the precision and effectiveness of breeding programs, a high-quality experimental design is essential. To address this limitation, the current experiment was conducted to evaluate the suitability of alpha lattice design (ALD) compared to randomized complete block design (RCBD) in plant breeding trials. In this study, three hundred six wheat genotypes were sown in ALD of 17×18, consisting of 17 blocks with 18 genotypes each, replicated in triplicates under both irrigated and rainfed conditions. The study estimated two traits: the number of grains per spike and grain yield per plant, using data from both environments. These traits were then analyzed using both RCBD and ALD to evaluate the efficiency of ALD in comparison to RCBD. The relative efficiency based on grain yield per plant under both trials indicates that ALD was more efficient than RCBD. A relative efficiency close to one for the number of grains per spike could be attributed to the large number of entries evaluated in the RCBD. The anomalies regarding low EMS in the RCBD trial instead of the ALD could potentially be explained by this factor. Therefore, in field experiments with many entries, ALD should be preferred over RCBD.

Keywords- Wheat, Alpha lattice design, RCBD, Breeding programs, Relative efficiency

Bread wheat (*Triticum aestivum* L., 2n=6x=42) is the major cereal crop across the globe. It caters as a staple food for a large population worldwide, particularly in West Asia, North Africa and Europe. According to the FAO (2022), India ranks second in terms of global wheat production, accounting for 13.3% after China at 17%. Wheat plays a pivotal role in global food security. The grains are mainly composed of carbohydrates (~70-80%) and proteins (~8-22%) (Slafer *et al.* 2021). Wheat can grow in diverse climate, water and soil regimes. The growing global population, shrinking arable land, and shifts in climate and precipitation patterns make addressing drought stress more urgent (Valizadeh *et al.* 2014; Trenberth 2011). Wheat production in semiarid and arid regions faces constraints from drought stress, making the selection of high yielding drought-tolerant cultivars crucial for rainfed conditions.

Breeders often conduct field trials to select the best entries, choosing the optimal design and analysis method based on the population's genetic architecture. In the last few decades, the development and adoption of new experimental designs have grown significantly due to expanding applications, statistical complexity and appeal they offer. It is evident that newly originated agricultural field experimental designs were inspired by applications across a wide range of experimental studies (Hinkelmann and Kempthorne 2005). One of the key principles of experimental design is the minimizing experimental error. In field experimentation, the randomized complete block design (RCBD) is commonly used because it applies randomization, replication, and local control, dividing experimental units into homogenous blocks and ensuring each block contains a complete replication (Gupta *et al.* 2016). One disadvantage of RCBD is that

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it is only suitable for 25-30 genotypes per block due to heterogeneity within larger blocks. However, most plant breeding trials involve many entries, making incomplete blocking designs, such as an alpha lattice design (ALD), a more effective choice (Williams *et al.* 2002; Patterson and Williams 1976). Lattice designs, including balanced and partially balanced incomplete block designs, were developed for large-scale agricultural trials to compare large number genotypes with greater accuracy (Yates 1936), balanced designs often require many replications. The results suggest replacing RCBD with ALD when there are more than ten treatments, as ALD provides better control of experimental variability and improves precision by reducing mean square error, coefficient of variation, and standard error of difference.

Globally, breeding and field trials now commonly employ alpha designs due to their flexibility and capacity to accommodate any number of varieties. They are recommended for trials with large numbers of genotypes on variable soil, especially when variability between lines is very less. ALD are popular in wheat breeding for their adaptability with entry numbers, block size, and error control (Kumar *et al.* 2020). They can also handle situations where the entry count isn't an exact multiple of block size by omitting treatments. Multi-environment replicated trials are essential in crop improvement programs for evaluating numerous entries and identifying the best performers. Therefore, this study aimed to compare the efficiency of alpha lattice design (ALD) with randomized complete block design (RCBD) in evaluating grain yield and related components in wheat genotypes.

Materials and methods

Experimental design

The experimental material comprised of 306 diverse wheat genotypes including doubled haploids, landraces from North-western Himalayas, exotic lines and popular Indian cultivars. The experiment was conducted at the Experimental Farm of the Department of Genetics and Plant Breeding, CSK HPKV, Palampur, during the *rabi* season of 2021-22 in irrigated vs. rainfed trials with three replications. Employing the Alpha lattice design (Patterson and Williams 1976), randomization of 306 cultivars was conducted using PB tools software. The experimental

material was assessed for various morpho-physiological traits within an ALD framework of 17×18, comprising 17 blocks with 18 genotypes each, replicated in triplicates for each trial. In RCBD, each treatment appears once in every block, making the number of treatments equal to the block size. Additionally, as each block serves as a complete replication, the number of blocks equals the number of replicated treatments.

Each entry was planted in a 1 meter-long row, with two rows per plot, with the intra and inter-row spacing of 10 cm and 20 cm respectively. Data was recorded for two agro-morphological traits: Grains per spike (GPS) and Grain yield per plant (g) (GY). The data collected were analyzed using the “agricolae” package of R statistical software (R Core Team 2020) in both RCBD and ALD. The linear mathematical model of ALD is:

$$y_{iju} = \mu + \tau_i + \beta_j + e_{iju}$$

(y_{iju} is the response of variable; μ is the general mean effect; τ_i is the effect of the i^{th} treatment; β_j is the effect of the j^{th} block; e_{iju} are uncorrelated random error components with response)

Estimation of efficiency of ALD versus RCBD

The comparative effectiveness of the ALD in contrast to the RCBD was assessed using the error mean square (EMS) and coefficient of variation (CV) from each analysis, as per the following equations:

$$\text{Relative Efficiency (RE)} = (\text{EMS for RCBD}) / (\text{EMS for ALD})$$

$$\text{Relative Efficiency (RE)} = (\text{CV for RCBD}) / (\text{CV for ALD})$$

If the value $RE > 1$, it indicates that the ALD is more efficient than the RCBD (Masood *et al.* 2008), if $RE \approx 1$, it suggests that both designs yield similar results, while $RE < 1$ indicates that the RCBD is a more efficient.

Results and Discussion

Analysis of variance for RCBD

The ANOVA (RCBD) for individual and the pooled analysis of the studied traits are presented in Tables 1 and 2, respectively. The mean square due to replications showed significant differences for GPS and GY in both environments and pooled across environments.

Homogeneity of variances for both traits was indicated by Bartlett's test, thus enabling a pooled

ANOVA to be performed. The mean square due to environment in the pooled data revealed highly significant differences for both traits. The highly significant genotypic differences among GPS and GY in both environments and the pooled environment. Consequently, the germplasm can help identify genotypes with high potential for GPS and GY.

The large number of entries evaluated in the RCBD, can lead to higher variability within blocks and anomalies in data analysis regarding low EMS.

Analysis of variance (alpha lattice design)

The ANOVA (alpha lattice design) for individual and the pooled analysis of the studied traits are presented in Tables 1 and 2, respectively. The mean square of the blocks (replication) did not show significant differences for both GPS and GY across all individual and pooled environments. The mean

square due to replications showed significant differences for GPS and GY in both the environments. Similar with the RCBD, highly significant genotypic differences were found for both GPS and GY in both environments and across pooled environment, suggesting that the germplasm pool in this study provides a rich source of genetic diversity. This can be useful for identifying genotypes with high GPS and GY potential.

The mean square of the environment in the pooled data exhibited highly significant differences for both traits. In the pooled data, mean square due to environment revealed significant differences for both the traits. A significant replication and environment interaction was observed for the trait GPS. While, Genotype \times Environment interaction was highly significant for GY but not significant for GPS. This suggests that there was wide range of genetic

Table 1. Analysis of variance (RCBD and Alpha lattice design) for studied traits in wheat in irrigated vs. rainfed trials

Sources of variation	df	Irrigated		Rainfed	
		Grains per spike	Grain yield per plant	Grains per spike	Grain yield per plant
Randomized Complete Block Design					
Replication	2	1395.36*	15.14*	475.22*	10.56*
Genotypes	305	72.93*	9.96*	77.73*	4.91*
Error	610	29.3	1.13	19.34	0.40
Alpha Lattice Design					
Replication	2	1395.36*	15.14*	475.22*	10.56*
Blocks (Replication)	48	21.28	1.28	20.04	0.30
Genotypes	305	72.78*	9.38*	74.89*	4.64*
Error	562	29.6	1.07	19.28	0.40

Table 2. Pooled Analysis of variance (RCBD and Alpha lattice design) for studied traits in wheat in irrigated vs. rainfed trials

Sources of variation	df	Grains per spike	Grain yield per plant
Pooled RCBD			
Replication (environment)	2	1733.90*	33.00*
Genotypes	305	103.90*	11.04*
Environment	1	2,313.57*	169.21*
Pooled Error	610	24.54	0.75
Pooled ALD			
Replication (environment)	2	1686.40*	25.18*
Block	16	23.80	0.86
Genotype	305	135.60*	12.88*
Environment	1	9106.70*	1415.50*
Replication \times Environment	2	184.20*	0.52
Genotype \times Environment	305	15.10	1.57*
Residuals	1124	24.60	0.73

variations among studied genotypes and environments for GY, with varying responses to different environments. These findings indicate that the studied wheat germplasm responded to $G \times E$ interaction across different environments.

Relative efficiency of ALD versus RCBD

Relative efficiency of ALD versus RCBD (Table 3) was recorded $RE \approx 1$ for GPS suggests that both designs yield similar results, while higher values for error mean square (EMS) for GY in irrigated environment (1.06) and pooled environment (1.03), indicating that the ALD is more efficient than the RCBD. Whereas for relative efficiency based on coefficient of variation (CV) was observed $RE \approx 1$ for GPS and higher for GY in irrigated environment (1.06) and pooled environment (1.01) which indicate that analysis in ALD resulted in reducing the experimental error and thus enhancing the capability of the researcher to detect significant differences among the 306 wheat genotypes. The lower CV values under rainfed trial over irrigated for both the studied traits, suggest that rainfed conditions exhibit reduced variability, which may be attributed by the broader range of grain yields observed under irrigated environments, indicating the full expression of genetic potential of the genotypes. A relative efficiency close to one for the GPS can be associated with the large number of genotypes evaluated in the RCBD. The unexpected occurrence of low EMS in the RCBD trial, compared to the ALD, may potentially be accounted for by this factor.

The results are consistent with the findings of Abd El-Mohsen and Abo-Hegazy (2013); Idrees and Khan (2009); and Masood *et al.* (2006). In a study by Priyanka *et al.* (2023), sixty-six bread wheat varieties

were evaluated in alpha-RBD with three replications, finding significant differences for all studied traits over environments. Sood *et al.* (2021) and Thakur & Sharma (2023) also evaluated black gram and cauliflower breeding material, respectively for yield and related traits in RCBD. Sanadya *et al.* (2022) evaluated 98 (12×8) genotypes in ALD over two consecutive years (2019-2021) for green forage and seed yield per plant, found ALD more effective than RCBD and pooled data varied significantly due to genotype-environment interaction. The relative efficiency of ALD studied by Kumar *et al.* (2020) indicated that these designs were more effective than the RCB design.

The study revealed that using alternative experimental designs leads to significant improvements when working with a large number of genotypes displaying notable variability. Statistical analysis of grain yield per plant data showed that the RCBD was less effective than the alpha lattice design ALD, resulting in lower experimental accuracy. Moreover, the experimental designs utilized fewer plots compared to the traditional design of randomizing entire blocks. The relative efficiency based on EMS and CV in the pooled data increased precision for grain yield per plant, leading to better experiment management. These results indicate that ALD is more suitable for wheat experimental trials than the conventional RCBD, making it a more effective approach for agricultural research.

Conclusion

An irrigated vs. rainfed trial was carried out to compare the efficiency of ALD over RCBD in enhancing wheat breeding experiments by reducing experimental error. This was accomplished by

Table 3. Relative efficiency (RE) of Alpha lattice design over RCBD for studied traits in wheat in irrigated vs rainfed trials

Traits	Irrigated			Rainfed			Pooled		
	RCBD (EMS)	ALD (EMS)	RE	RCBD (EMS)	ALD (EMS)	RE	RCBD (EMS)	ALD (EMS)	RE
Grains per spike (EMS)	29.3	29.6	0.99	19.38	19.28	1.01	24.54	24.6	1.00
Grains per spike (CV)	8.78	8.89	0.99	7.69	7.68	1.00	8.34	8.35	1.00
Grain yield per plant (EMS)	1.13	1.07	1.06	0.4	0.4	1.00	0.75	0.73	1.03
Grain yield per plant (CV)	19.72	18.54	1.06	16.45	16.61	0.99	18.46	18.21	1.01

minimizing soil heterogeneity through smaller block sizes and adjusting the mean performance of each treatment within blocks. Additionally, notable genotypic differences were observed among genotypes, indicating that the studied germplasm may offer substantial genetic diversity for future breeding programs. In multi-environment crop field experiments for assessing economic traits involving

many entries, ALD should be preferred over RCBD.

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