Relative Efficiency of Experimental Designs in Evaluation of Plant Genetic Resources

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An experiment was conducted in a Reinforced Alpha Design (RAD) with 50 accessions and 5 control treatments of tomato during the *Rabi* season of 2006. The data on 9 distinct descriptors namely, plant height, primary branches, flower clusters per plant, flowers per cluster, days to first fruit set, fruits per plant, fruit weight per plant, locules per fruit and pericarp thickness were recorded on 12 randomly selected plants per accession. The designs, namely, Completely Randomized Design, Randomized Block Design and Augmented Block Design were derived from RAD. A comparison of the relative efficiency of these four designs revealed that in plant genetic resources evaluation for identification of promising material in case of insufficient seed quantity, one should adopt a two phase strategy using reinforced alpha design. During phase I (first year), when sufficient seed quantity is not available, one replication of reinforced alpha design may be used. During phase II (second year), the second replication of the design may be deployed.

Key Words: Augmented block design, Evaluation, Reinforced alpha design, Relative efficiency, Tomato

Introduction

In plant genetic resources evaluation, the germplasm is assembled either through explorations or introductions from exotic sources, and exchange programmes etc. Germplasm curator generally comes across situations when he has to test or evaluate the performance of the new germplasm/superior selections etc. with the existing released varieties (control treatments). Normally, the seed quantity of newly collected material is very limited in comparison to check varieties. Augmented Block Design (ABD) suggested by Federer (1956) has been extensively used by the germplasm evaluators for testing the new lines with the check varieties. Hence, an appropriate and efficient experimental design is required to handle such a situation. Relative efficiencies of some of the incomplete block designs have been compared with data on uniformity trials (Gomez, 1969; Johnson and Murphy, 1943; Sarma *et al*., 1985; Zuber, 1942). In the present investigation, the efficiency of experimental designs, *viz.*, Completely Randomized Design (CRD), Randomized Block Design (RBD), Reinforced Alpha Design (RAD), and ABD used in evaluation of germplasm material have been compared. For this purpose, the experiment was laid in RAD and other designs were derived from it.

Materials and Methods

A total of 50 accessions and 5 controls of tomato from diverse eco-geographical regions were grown during the *Rabi* season of 2006 at NBPGR Experimental Farm, Issapur, New Delhi. The accessions were grown in 6 row plots of 2.25 m length with a row spacing of 60 cm and plant spacing of 45 cm in an Reinforced Alpha Design. The material was grown in 2 replications with 5 blocks each of size 15 in each replication. Each block contained 10 accessions and 5 controls, *viz.*, Pusa Upkar, Pusa Sheetal, Pusa Ruby, Pusa Gaurav and Pusa Early Dwarf. One could have used an alpha design for 55 treatments. Since, the interest in the present study is in making test vs. control treatment comparisons, therefore, RAD comprised 2 replications with each 50 accessions with 10 blocks of size 10 each and each of the 5 control treatments were added in each of the blocks of the RAD (Table 1). Thus, each accession was replicated twice and each control was replicated 10 times. Data were recorded for a set of 9 distinct descriptors, *viz.*, plant height, primary branches, flower clusters per plant, flowers per cluster, days to first fruit set, fruits per plant, fruit weight per plant, locules per fruit and pericarp thickness on 12 randomly selected plants per accession. The following three designs were derived from RAD.

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Completely Randomised Design (CRD): Each of the 50 accessions being replicated 2 times and the controls 5 times each.

Randomised Block Design (RBD): Each of the 50 accessions replicated 2 times and the controls replicated equal number of times in 2 replications, *i.e.*, 10 times each.

Augmented Block Design (ABD): One hundred treatments (2 times same set of 50 accessions) being laid out in 10 blocks and 5 controls being randomized in each block.

The randomization was done as per procedure of RAD and not as per the derived design. Therefore, the designs can not be analyzed as derived designs. However, for the sake of illustration purpose of use of RAD in reducing the experimental error and increasing the precision of treatment comparisons, the data was analyzed from experiments as per respective experimental designs. For a detailed discussion on alpha design, a reference may be made to Prasad *et al*. (2007), Prasad *et al.* (2008) and http://www.iasri.res.in/design/Alpha/Home.htm.

The data were subjected to statistical analysis for the corresponding design. The amount of information obtained from a design D is $(n_e+1)/(n_e+3)s_e^2$ with n_e and s_e^2 representing the error degrees of freedom and the corresponding error mean square (Fisher, 1947). Then the relative efficiency of design D_1 with another design D_2 is the ratio of amounts of information in D_1 to D_2. When the treatment differences were significant for a descriptor, number of accessions superior to the best check and the number of pairs of treatment combinations having significant differences were determined. In ABD, for determining significant pairs of accessions, we have considered set 1 comprised first five blocks of replication 1 and set 2 comprised five blocks of replication 2. The significant pairs of accessions were determined in each set. The common pair of accessions between set 1 and 2 is reported.

For computing the number of significant comparisons among all possible pairs of treatment combinations, 2 critical differences between pair of treatments (i) accessions being replicated 2 times, and (ii) accession and control were computed in CRD and RBD. In ABD, 3 critical differences were computed, *viz.* (i) between 2 accessions occurring in the same block, (ii) between 2 accessions occurring in different blocks, and (iii) between an accession and control (Federer, 1956). The breeder is normally interested in identifying accessions superior to best control variety.

In ABD, while performing the analysis of variance, sum of squares for treatments was adjusted for block effects and blocks sum of squares was unadjusted. In addition, when treatment differences and block differences were significant, treatment means (other than control varieties) were adjusted for the block effects before making comparison between treatments.

Results and Discussion

All the accessions differed significantly (Table 2) for all the descriptors in all the designs except for number of locules per plant and pericarp thickness in CRD, RBD, and RAD. Replications were effective in controlling the error variation for primary branches, flower clusters per plant, days to first fruit set, fruits per plant, fruit weight per plant, locules per fruit and pericarp thickness in RBD and RAD. This resulted in increasing the efficiency of the design and more numbers of significant pair of treatment combinations in RBD over CRD. But there was more or less no change in number of treatments superior to the control variety. In RAD, blocks within replication were significant in controlling the error variation for all descriptors except plant height. Further, in ABD, 10 effective blocks were formed. This resulted in significant block effects and also more efficiency for ABD over RBD.

The relative efficiency (RE) of RAD ranged from 106.82%-354.63% over CRD and 104.5%-353.99% over RBD (Table 3). The RE of RAD was considerably higher than ABD for all the descriptors except for plant height and number of flowers per cluster. However, it was nearly same for primary branches and flower clusters per plant. The RE of ABD was considerably higher than CRD and RBD for all the descriptors except for fruit weight per plant and pericarp thickness where it was nearly similar. The RE of RBD over CRD was just above 100% for all the descriptors.

The number of significant pairs of treatments were the highest for primary branches (480), days to first fruit set (403), and fruit weight per plant (1064) in RAD and for plant height (672), flower clusters per plant (715), flowers per cluster (618), fruits per plant (492), and locules/fruit (5) in ABD (Table 4). The numbers of significant pairs in CRD and RBD were approximately equal for different descriptors. In RAD, the numbers of superior accessions over control were 23, 8, 18, 4,

Table 1. Layout of Reinforced Alpha Design (RAD) parameters: (v=50, b=10, k=10, r=2)

	Replication 1									
Block 1	50 (EC29627)	5 (EC742)	10 (EC29969)	15 (EC6486)	20 (EC3736)	25 (EC3815)	30 (EC32614)	35 (EC7345)	40 (EC32276)	45 (EC34480)
Block 2	1 (EC2585)	6 (EC35373)	11 (EC35257)	16 (EC13574)	21 (EC13274)	26 (EC2491)	31 (EC35242)	36 (EC5888)	41 (EC2699)	46 (EC4955)
Block 3	2 (EC35265)	7 (EC23528)	12 (EC16343)	17 (EC3366)	22 (EC35203)	27 (EC7785)	32 (EC32019)	37 (EC1087)	42 (EC35240)	47 (EC31767)
Block 4	3 (EC1191)	8 (EC35244)	13 (EC2653)	18 (EC33203)	23 (EC35236)	28 (EC8822)	33 (EC35272)	38 (EC14181)	43 (EC1753)	48 (EC8630)
Block 5	4 (EC2990)	9 (EC35382)	14 (EC5424)	19 (EC32577)	24 (EC32271)	29 (EC7371)	34 (EC27885)	39 (EC16368)	44 (EC35216)	49 (EC8590)
	Replication 2									
Block 6	50 (EC29627)	6 (EC35373)	12 (EC16343)	18 (EC33203)	24 (EC32271)	25 (EC3815)	33 (EC35272)	37 (EC1087)	44 (EC35216)	46 (EC4955)
Block 7	1 (EC2585)	7 (EC23528)	13 (EC2653)	19 (EC32577)	20 (EC3736)	26 (EC2491)	34 (EC27885)	38 (EC14181)	40 (EC32276)	47 (EC31767)
Block 8	2 (EC35265)	8 (EC35244)	14 (EC5424)	15 (EC6486)	21 (EC13274)	27 (EC7785)	30 (EC32614)	39 (EC16368)	41 (EC2699)	48 (EC8630)
Block 9	3 (EC1191)	9 (EC35382)	10 (EC29969)	16 (EC13574)	22 (EC35203)	28 (EC8822)	31 (EC35242)	35 (EC7345)	42 (EC35240)	49 (EC8590)
Block10	4 (EC2990)	5 (EC742)	11 (EC35257)	17 (EC3366)	23 (EC35236)	29 (EC7371)	32 (EC32019)	36 (EC5888)	43 (EC1753)	45 (EC34480)

Note: Text within parenthesis indicates accession number

Table 2. Analysis of variance for different descriptors in tomato

Source					Plant height (cm)				No. of primary branches			
	CRD	RBD	RAD	ABD	CRD	RBD	RAD	ABD	CRD	RBD	RAD	ABD
	Degrees of freedom				Mean square				Mean square			
Treatments	54	54	54	104	1293.75**	1293.75**	1293.75**	2002.03**	9.68**	9.68**	9.68**	775.11**
Blocks/replication	-	1	1	9	-	541.99	541.99	2512.42**	-	27.52**	27.52**	11.50**
Blocks within replications	-	-	8	-	-	-	257.17	-	-	-	10.84**	
Error	95	94	86	36	177.31	173.43	165.64	81.41	4.63	4.39	3.79	4.03
Source	No. of flower clusters per plant				No. of flowers per cluster				Days to first fruit set			
	CRD	RBD	RAD	ABD	CRD	RBD	RAD	ABD	CRD	RBD	RAD	ABD
	Mean square				Mean square				Mean square			
Treatments	179.24**	179.24**	179.24**	807.22**	2.83**	2.83**	2.83**	2.22**	38.32**	38.32**	38.32**	87.49**
Blocks/replication	-	1351.5**	1351.5**	77.82	-	0.01	0.01	964.49**	-	149.83**	149.83**	1060.25**
Blocks within replications	-	-	315.84**		-	-	1.47**	-	-	-	43.63**	
Error	92.02	78.63	56.56	50.83	0.56	0.56	0.48	0.31	16.32	14.90	12.23	13.31
Source	No. of fruits per plant				Fruit weight per plant (g)				No. of locules per fruit			
	CRD	RBD	RAD	ABD	CRD	RBD	RAD	ABD	CRD	RBD	RAD	ABD
	Mean square				Mean square				Mean square			
Treatments	2.30**	2.30**	2.30**	823.59**	153.02**	153.02**	153.02**	708.25**	0.22	0.22	0.22	776.37**
Blocks/replication	-	4.57**	4.57**	0.72**	-	23.05*	23.05*	58.47**	-	3.13**	3.13**	0.62**
Blocks within replications	-	-	1.4**	-	-	-	172.62**	-	-	-	0.62**	
Error	0.35	0.30	0.20	0.22	19.83	19.79	5.58	21.27	0.23	0.20	0.16	0.16
Source	Pericarp thickness (mm)											
	CRD	RBD	RAD	ABD								
	Mean square											
Treatments	0.004	0.004	0.004	1017.60**								
Blocks/replication	-	0.012*	0.012*	0.002								
Blocks within replications	-	-	0.01**	-								
Error	0.003	0.003	0.002	0.003								

*: P<=0.05; **: P<=0.01; CRD: Completely Randomized Design; RBD: Randomized Block Design; RAD: Reinforced Alpha Design; ABD: Augmented Block Design

Table 3. Relative efficiency of ddesigns

Design A versus Design B	Plant height (cm)	No. of primary branches	No. of flower clusters / plant	No. of flowers/ cluster	Days to first fruit set	No. of fruits/ plant	Fruit weight/ plant (g)	No. of locules/ fruit	Pericarp thickness (mm)
RAD vs. CRD	106.82	121.91	162.35	116.42	133.16	175.00	354.63	143.45	149.68
RAD vs. RBD	104.50	115.61	138.76	116.45	121.60	150.00	353.99	124.76	149.72
RAD vs. ABD	50.64	92.60	92.60	66.54	112.13	113.34	392.76	103.04	154.56
ABD vs. RBD	206.36	149.85	149.85	174.99	108.44	132.09	90.13	121.09	96.87
ABD vs. CRD	210.94	175.32	175.32	174.96	118.75	154.08	90.29	139.22	96.85
RBD vs. CRD	102.22	117.00	117.00	99.98	109.51	116.64	100.18	114.98	99.98

CRD: Completely Randomized Design; RBD: Randomized Block Design; RAD: Reinforced Alpha Design; ABD: Augmented Block Design

Table 4. Numbers of pairs of treatment combinations having significant differences and the number of significant pair of accessions superior to the best control variety

Design	Plant height (cm)	No. of primary branches	No. of flower clusters/ plant	No. of flowers/ cluster	Days to first fruit set	No. of fruits/ plant	Fruit weight/ plant (g)	No. of locules/ fruit	Pericarp thickness (mm)
CRD-A	625	280	230	233	343	431	747	–	–
CRD-B	9	1	6	1	0	3	1	–	–
RBD-A	631	292	272	233	372	398	746	–	–
RBD-B	9	1	9	1	0	3	1	–	–
AD-A	672	480	361	289	403	460	1064	–	–
AD-B	23	8	18	4	0	7	2	–	–
ABD-A	892	412	715	618	399	492	916	5	0
ABD-B	11	0	6	0	0	2	1	0	0

A: Refers to the number of significant pairwise comparisons among all possible treatment combinations; B: Refers to number of accessions superior to the best control variety

7 and 2 for plant height, primary branches, flower clusters per plant, flowers per cluster, fruits per plant, and fruit weight per plant, respectively. These were substantially higher in RAD for all the descriptors in comparison to other designs where as these were almost same for ABD (11, 0, 6, 0, 2 and 1), RBD (9, 1, 9, 1, 3 and 1), CRD (9, 1, 6, 1, 3 and 1) for plant height, primary branches, flower clusters per plant, flowers per cluster, fruits per plant, and fruit weight per plant, respectively.

The above study suggests that in plant genetic resources evaluation for identification of promising material, when sufficient seed quantity is not available, one should adopt a two phase strategy through the use of Reinforced Alpha Design. During phase I (first year), in case of insufficient seed quantity, one replication of Reinforced Alpha Design may be used. During phase II (second year), the second replication of the design may be deployed. However, a preliminary knowledge about the potential superior germplasm, promising, over the control treatment can be had from the data of first year. The analysis of this data can be performed in an ABD. In situations, when sufficient seed quantity is available, the experimenter should use an RAD and RBD should be discouraged.

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