Field Trial Designs in Plant Breeding

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Abstract: Field trial design is a crucial consideration in efficient, cost-effective plant breeding programs. Design properties and specific designs relevant to plant breeding are considered.

Good field designs are important at all major stages of a plant breeding program from the earlier stages of screening hundreds, possibly thousands of entries through identification of a few elite selections for release. At each stage, good field designs are critical to ensure an efficient cost-effective operation and to ensure the best possible genetic material is identified. Designs can range from unreplicated trials at a single location to multi-environment trials that could involve possibly hundreds of locations over a series of years. In most cases, interest focuses on genotypes as the only treatment factor where designs are generally comparative in nature and arranged to ideally minimize the variance of differences between genotypes or to maximize gains from selection.

Field designs are based on concepts of replication, control of variation among plots and randomization, where replication allows valid estimation of error variance, control of plot variation reduces error variance and randomization allows unbiased estimates of means and variances. (Giesbrecht and Gumpertz, 2004; Hinkelman and Kempthorne, 2006; Mead, 1990). Assuming the scope of inference and plot size/shape issues have been considered, the main focus of what remains generally will be how to arrange entries in the field to minimize the impact of error variance.

The major reason for grouping plots into uniform blocks is to reduce plot-to-plot variation and to improve the precision of the experiment. Failure to adequately block a field experiment can result in unacceptably large error variance and/or biased estimates of genotype effects (See Mead, 1997 for an example). Effective control of error variance usually requires relatively small blocks. Trials with a large number of entries set out in a complete block experiment where there is considerable variability among plots within a block will likely result in very poor, possibly unusable, information on genotypes. To control field variation, especially with a large number of entries, it is essential to make use of incomplete block designs.

Plant breeding trials are typically developed to give an unbiased evaluation of all test entries, and ideally to ensure equal variances of all paired differences. When incomplete block designs are used, achieving the equal variance criteria results in balanced incomplete block designs (BIBD). BIBDs require that all pairs of entries appear together in a block equally often (Giesbrecht and Gumpertz, 2004; Hinkelman and Kempthorne, 2006; Mead, 1990). Generally, balance is often possible in smaller trials, however in trial with a large number of entries, balance is normally not achievable, meaning that some differences between pairs of entries will be estimated more precisely than others. Yet in most plant breeding trials, this disparity in precision across treatment pairs is small and not a major problem, as long as the incomplete blocking is effective (Mead, 1990). Another important concept in field trial design is resolvability. A field trial is resolvable if it is laid-out in complete replicate blocks, each replicate being split into a number of incomplete blocks. Resolvable designs are useful since the entries in the same incomplete block in one replicate are spread across incomplete blocks in another replicate. In addition, resolvable designs are often easier to manage since all entries are together in complete replicates.

One category of resolvable incomplete block designs are the Lattice designs originally proposed by Yates (1936). These designs require that the number of entries is a square of the blocksize and achieve balance if enough replicates are possible. If not, simple, triple, quadruple lattices are partially balanced designs with two, three or four replicates respectively and have been used extensively in plant breeding trials. Due to the restriction on the number of genotypes that may be evaluated, there have been a number of proposed lattice type of designs, the most popular being the alpha designs developed by Patterson and Williams (1976) (Giesbrecht and Gumpertz, 2004; Hinkelman, and Kempthorne, 2006). Alpha designs are resolvable incomplete block designs where the number of entries is a multiple of block size. Although these designs cannot achieve balance, they are used extensively in plant breeding primarily because they are quite flexible regarding the number of entries to be evaluated and the appropriate size of incomplete block and they allow for good error control. In addition, these designs can be simply adapted to situation where the number of entries is not an exact multiple of block size by omitting treatments from an alpha design with a larger number of treatments. Design generation is available by using the method given in Patterson and Williams (1976) or by using available software (Alpha+, Williams and Talbot, 1993; CycDesign 3.0, Whitaker et al., 2001. Agrobase Software, Mulitze, 2004).

Although it is well documented that incomplete block designs can greatly improve the efficiency of plant breeding field trials, recent work has suggested that allowing for block differences in two directions can further improve precision (Wright, 2002; Kempton et. al, 1994). Row-column designs ideally are constructed so entries are orthogonal to both rows and columns, such as with a Latin square design. If orthogonality is not possible in both directions, then hopefully it is achieved in one direction and balance (as in BIB) is obtained in the other direction. If orthogonality is not possible, then one attempts to achieve balance in both directions if possible. A Lattice square is a special case of a RC design where balance is not achieved in either direction, but that each pair of treatments appears together in at least one row or column (Hinkelman and Kempthorne, 2006). One approach useful in plant breeding is to start with an alpha design arranged with rows as incomplete blocks and then rearrange the order of the entries in each row to balance as best as possible the entries across the columns. In this case, the alpha design is said to be latinized (Williams, 1986). Efficient row-column designs useful in plant breeding may be obtained using CycDesign (Whitaker, et. al., 2001).

Up to this point, I have assumed that each entry must be assigned to at least two plots. In the early stages of a plant breeding program, expected genetic gains may be increased by screening a large number of genotypes in contrast to having more precise comparisons of a fewer number of genotypes (Bos, 1983; Gauch and Zobel, 1996). This consideration will likely make it necessary to evaluate many entries where there may not be sufficient seed to replicate each. Federer proposed augmented designs where a set of check entries are replicated an equal number of times in a specified field design and an additional set of new or test entries are included in the experiment only once (see eg Federer, 2002; 2005). Any type of block design can be used for the check treatments with the test entries being added or ‘augmented’ to the blocks and the standard error for a difference between test entries or checks may simply computed. This approach provides a very efficient means of screening test entries and has a considerable amount of flexibility. For row-column designs, the experiment is 'latinized' so that entries do not occur more than once in a row or column. Federer (2002) proposed using this approach with augmented lattice squares while Williams and John (2003) used a latinized α-α designs (α designs for both the rows and columns) to extend the idea of the augmented lattice squares using CycDesigN (Whitaker et. al, 2001). The analyses of augmented designs is relatively simple (Scott and Milliken,1993; Federer 2001, 2002, 2005).

Similar to augmented designs are unreplicated designs where field variation may be controlled using several different approaches. Traditional unreplicated designs control local variation using a single replicated check variety distributed often systematically across the field. The approach is flexible and simple to use in that genotypes need not be randomized, visual evaluation is possible and the test genotypes are adjusted using the mean yield of the neighboring checks. The problems with the approach are that results can strongly depend on which check is chosen, how the genotypes are adjusted and the frequency and location of the check plots. The frequency of check plots that maximizes genetic gain depends on the heritability and how effective the presence of more checks control field variation (Kempton and Gleeson, 1997). A number of methods are available for adjusting genotypes yields using check plots with most using environmental indices or covariates over the field (Kempton, 1984; Besag and Kempton, 1986;Cullis et al, 1989; Hooks et. al., 2007). Appropriate field arrangements of checks have been considered by a number of workers but generally depend on the spatial pattern in the field (Kempton, 1984; Besag and Kempton, 1986; Cullis et. al, 1989; Sebolai, 2005; Martin et. al, 2006). To evaluate as many test genotypes as possible, some researchers have omitted all checks and used mass or grid selection field designs where each genotype is compared with neighboring genotypes (Gardner, 1961; Schutz and Cocherham, 1966; Bos, 1983; Annicchiarico, 2004). Although such designs are easy to apply and understand, better designs likely can be developed using spatial information (Cullis, et. al, 2006).

Multi-environment testing is a crucial step in the development of superior genotypes adapted to a wide range of environmental conditions. The design of efficient multi-environment testing programs to maximize information subject to available resources and practical limitations has been considered by a number of workers. Minimizing the variance of differences between genotypes, and using multi-environment variance components for a number of different crops, Talbot found that using 2 years at 12 sites with 2 replicates was reasonable for most crops, with more years increasing precision more than more locations (Talbot, 1984). When maximizing genetic improvement, similar results were found for corn single crosses (Sprague and Federer, 1951). Generally, the results are based on the assumption of good field designs at each site and will hold with any type of design and with different designs at each site (see eg Federer et. al, 2001).

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