

R. Ortiz · A. Golmirzaie

Hierarchical and factorial mating designs for quantitative genetic analysis in tetrasomic potato

Received: 19 March 2001 / Accepted: 3 July 2001

Abstract Plant breeders need to quantify additive and non-additive components of genetic variance in order to determine appropriate selection methods to improve quantitative characteristics. Hierarchical and factorial mating designs (also known as North Carolina mating designs I and II, respectively) allow one to determine these variance components. The relative advantages of these two designs in the quantitative genetics of tuber yield in tetrasomic potato were investigated. Likewise, the number of female parents to include in design I was also investigated. Data were collected from two independent experiments at two contrasting Peruvian locations: La Molina in the dry coast and San Ramon in the humid mid-altitude. In the first experiment, although design I gave a negative digenic variance (σ^2_D), this design provided almost the same estimate of narrow-sense heritability (h^2) for tuber yield as that obtained in design II (0.291 and 0.260, respectively). Therefore, design I appears to be appropriate for quantitative genetics research in tetrasomic potato, a crop in which some clones are male sterile. The easy handling of crosses (distinct random females included in the crossing scheme) is another advantage of design I relative to design II. In the second experiment, 12 males were crossed with either two or four females following a design-I mating scheme. The additive genetic variance (σ^2_A) was zero (or negative) when two females per male were included but was positive with four females. These results suggest that two females per male may not be enough for design I in tetrasomic potato. Four females per male are preferable to determine σ^2_A in design I for this tetrasomic crop.

Keywords Additive genetic variance · Heritability · North Carolina mating designs · Phenotypic variation · TPS

Introduction

Partitioning of phenotypic variation into genetic and environmental components permits breeders to make decisions for resource allocation and response to selection (Hallauer and Miranda 1981; Hill et al. 1998). Plant breeders determine their genetic response to selection (R) by the following equation $R=ih^2s_p$, where i is the selection intensity, expressed in standardized units, h^2 is the narrow-sense heritability in the reference population, and s_p is the phenotypic standard deviation of the selected characteristic. Furthermore, genetic gains in successful breeding programs result not only from a high heritability for the target trait(s) plus a high selection intensity, but also from combining a high mean with broad genetic variance for target trait(s) in the source population, a minimum number of years and sites for testing, and a dynamic selection approach.

Breeding plans for potato improvement, as for other crops, rely on information about the target population and data analysis of genetic experiments (Tarn et al. 1992). In this way the breeding plan will include logical steps such as choosing appropriate parents for crossing schemes, early or late selection in clonal generations – determined by trait h^2 , and adequate environment sampling (i.e. number of locations and years) for testing advanced breeding materials. Of course, these breeding plans depend on objectives, operation costs, cultural practices in targeted environments and even the policy affecting cultivar development and marketing. Nonetheless, the major goal of the breeding plan will always be to enhance the efficiency of the selection process.

The aim of the Centro Internacional de la Papa (CIP, Lima, Perú) has been to develop breeding populations from which national programs will select the best materi-

Communicated by P. Tigerstedt

R. Ortiz (✉)
International Institute of Tropical Agriculture
IITA c/o L.W. Lambourn & Co.
Carolyn House, 26 Dingwall Road,
Croydon CR9 3EE, United Kingdom
e-mail: R.ORTIZ@cgiar.org

A. Golmirzaie
Centro Internacional de la Papa, Apartado 1558, Lima 100, Perú

als for further cultivar development (Mendoza 1983). Therefore, its breeding plan focuses on creating breeding populations with stress tolerance (for frost and heat) and diseases or pest resistance (fungi, viruses, bacteria, nematodes and insects) rather than selecting for a elite individual clone (Mendoza 1989). One important aspect in the development of such a breeding population(s) has been to determine the extent of available genetic variation for further improvement by other local breeders in this source population. This article investigates the relative advantages of design I versus design II (Comstock and Robinson 1948, 1952) to calculate h^2 and the number of parents needed for an appropriate h^2 estimate in design I while doing population improvement for the potato.

Materials and methods

Two experiments were included in this investigation at two contrasting Peruvian locations: La Molina (12°05'S, 240 m, coastal desert) and San Ramon (11°08'S, 800 m, humid mid-altitude on the Eastern Andes slopes). In the first experiment at both La Molina and San Ramon, 100 hybrid offspring from a heterogeneous CIP breeding population were tested in design I (Comstock and Robinson 1948). In this hierarchical design each common parent was randomly mated to a different set of individuals from the non-common parent (Hill et al. 1998), i.e. a random sample of 20 male clones were individually crossed with a random sample of five female clones. In this hierarchical design the non-common parents are nested within the common parent. In this experiment, other offspring from the same breeding population were obtained following design II (Comstock and Robinson 1952) and tested in the same locations. A sample of 16 male clones were crossed with a sample of 16 female clones in four sets of four parents each in design II, which was therefore a square mating design because $n_1=n_2=4$ in this experiment. No reciprocal crosses were included in this experiment.

In the second experiment conducted at the same two locations, 48 hybrid offspring from an early CIP breeding population were tested using a design-I mating scheme. A sample of 12 males was crossed each to a random sample of four female clones. At the same locations another trial was planted using this design I but with a sample of 12 males crossed with only two random females. This experiment was undertaken to determine whether by increasing the number of males and decreasing the number of females per set would have any effect on the estimates of genetic parameters for tuber yield being calculated.

In all experiments, true potato seeds (TPS) of each offspring were planted in flats at a greenhouse nursery, and 40 seedlings were transferred after 8 weeks to single row plots in the field. The experimental layout was always a randomized block design with a maximum of three replications. Because a large number of offspring was sampled from the breeding population, those offspring sharing at least one parent were included in the same set. By following this approach a larger number of genotypes was sampled from the breeding population. Likewise, the sets increase the precision of the experiments. Furthermore the replications are recommended to be nested within sets because this layout appears to be the preferable arrangement for local control of the experimental error (Hallauer and Miranda 1981). Tuber yield per plant (kg) was the characteristic recorded in the experiment for further analysis, as indicated below.

The analyses of variance for hierarchical (design I) and factorial (design II) mating designs combined across environments and sets are given in Table 1. In design I (or the hierarchical design), the variation was split between common parents (males) and between non-common parents within common parents (or females within males). In design II (the factorial or top cross design) every male is mated to each female following a two-way analysis of variance, in which the variation can be partitioned into differences between males and females, and the interaction between them (Hill et al. 1998). Because different sets of parents were used as males and females the analysis of variance for each design included a source of variation due to the sets. However, the expectations of the mean squares of males, females, and their interaction are the same for the components of variance and covariance of the relatives. Table 2 provides full- and half-sib covariances and their associations with components of variance of design I and design II respectively in polysomic tetraploid species with non-inbred parents and assuming that higher order variances (σ^2_T and σ^2_Q) are nil

Table 1 Pooled analysis of variance for hierarchical (design I) and factorial (design II) mating designs combined across environments and sets

Item	df	Mean square	Expected mean square
Hierarchical design			
Environments (E)	e-1	M1	
Reps(R)/E	(r-1)e	M2	
Sets(S)/R×E	(s-1)rs	M3	
Males (M)/S	(m-1)s	M4	$\sigma_e^2 + r\sigma_{f/m/SE}^2 + rf\sigma_{m/SE}^2 + er\sigma_{f/m/s}^2 + erf\sigma_{m/s}^2$
Females(F)/M/S	(f-1)ms	M5	$\sigma_e^2 + r\sigma_{f/m/SE}^2 + er\sigma_{f/m/s}^2$
M/S×E	(m-1)s(e-1)	M6	$\sigma_e^2 + r\sigma_{f/m/SE}^2 + rf\sigma_{m/SE}^2$
F/M/S×E	(f-1)ms(e-1)	M7	$\sigma_e^2 + r\sigma_{f/m/SE}^2$
Pooled error	es(r-1)(mf-1)	M8	σ_e^2
Total	esrnf-1		
Factorial design			
Environments (E)	e-1	M1	
Sets (S)	s-1	M2	
E×S	(e-1)(s-1)	M3	
Reps/S×E	(r-1)es	M4	
Males (M)/S	(m-1)s	M5	$\sigma_e^2 + r\sigma_{mf/SE}^2 + rf\sigma_{m/SE}^2 + er\sigma_{mf/s}^2 + erf\sigma_{m/s}^2$
Females (F)/S	(f-1)s	M6	$\sigma_e^2 + r\sigma_{mf/SE}^2 + rm\sigma_{f/SE}^2 + er\sigma_{mf/s}^2 + erm\sigma_{f/s}^2$
F×M/S	(m-1)(f-1)s	M7	$\sigma_e^2 + r\sigma_{mf/SE}^2 + er\sigma_{mf/s}^2$
M/S×E	(m-1)s(e-1)	M8	$\sigma_e^2 + r\sigma_{mf/SE}^2 + rf\sigma_{m/SE}^2$
F/S×E	(f-1)s(e-1)	M9	$\sigma_e^2 + r\sigma_{mf/SE}^2 + rm\sigma_{f/SE}^2$
F×M/S×E	(f-1)(m-1)s(e-1)	M10	$\sigma_e^2 + r\sigma_{mf/SE}^2$
Pooled error	es(r-1)(mf-1)	M11	σ_e^2
Total	es(rmf-1)		

(Wricke and Weber 1986). These analyses were followed to determine h^2 in all experiments.

Although the original North Carolina mating designs were developed for diploid species, they can be extended to polysomic tetraploids assuming that the coefficient of double reduction (α) approaches zero (i.e. no chromatid segregation) and meiosis is regular (Dudley and Moll 1969; Dabholkar 1999). Recent analyses in potato suggest that loci with a major effect on tuber yield occur predominantly between centromeres and proximal crossovers (Tai and De Jong 1997; Buso et al. 1999), i.e. with chromosome segregation for tuber yield loci because of reduced levels of recombination (Peloquin et al. 1999). As in diploid species the estimation procedure assumes a random sample from the breeding population. The observed mean squares of the analysis of variance (ANOVA) are equated to their expectations, which are linear functions of the unknown variance components (Searle 1971), to obtain ANOVA estimates of variance components.

Results

The analyses of variance of tuber yield for designs I and II included in the first experiment are given in Table 3. Both means and the coefficient of variation for each mat-

ing design were almost the same, though the sources of variation for the interaction of the environment with the genetic components were significant only for design I.

The variance components for both designs are listed in Table 4. The male variance ($\sigma_{m/s}^2$) was twice the size in design II than in design I (0.008 and 0.004, respectively) but the female variance ($\sigma_{f/m/s}^2$) was very small (0.0003). The male×female interaction ($\sigma_{f/m/s}^2$ and $\sigma_{m/sE}^2$) and the male×location ($\sigma_{m/sE}^2$) variances of both designs were similar (0.003 and 0.017, respectively). The genetic analysis of these designs (Table 4) showed that the additive variance (σ_A^2) was in excess of a quarter of the total phenotypic variation as determined by h^2 (0.291 for design I and 0.260 for design II, respectively). However the estimates of the digenic variance (σ_D^2) and its interaction with locations (σ_{DE}^2) were negative in design I.

The mean squares for the respective items of the analysis of variance for tuber yield in design-I mating schemes with different number of parents are given in

Table 2 Full- and half-sib covariances (covar) and associations with components of variance (var) of design I and design II in polysomic tetraploid species with non-inbred parents and assuming that higher-order variances (σ_T^2 and σ_Q^2) are nil. m=male, f=female, s=set

Associations between covariances and genetic variances

$$\text{Covar (half-sib)} = (1/4) \sigma_A^2 + (1/36) \sigma_D^2$$

$$\text{Covar (full-sib)} = (1/2) \sigma_A^2 + (2/9) \sigma_D^2$$

Design I

$$\sigma_{m/s}^2 = \text{Covar (half-sib}_m)$$

$$\sigma_{f/m/s}^2 = \text{Covar (full-sib)} - \text{Covar (half-sib}_m)$$

Thus,

$$\sigma_A^2 = (14/3) \sigma_{m/s}^2 - (2/3) \sigma_{f/m/s}^2$$

$$\sigma_D^2 = 6[\sigma_{f/m/s}^2 - \sigma_{m/s}^2]$$

Design II (when m=f)

$$\sigma_A^2 = 2(\sigma_{m/s}^2 + \sigma_{f/s}^2) - (2/3)\sigma_{fm/s}^2$$

$$\sigma_D^2 = 6\sigma_{fm/s}^2$$

Heritability on a plot-mean basis unbiased by genotype by environment interaction in multi-site experiments

$$h^2 = \sigma_A^2 / (\sigma_A^2 + \sigma_D^2 + \sigma_{AE}^2/E + \sigma_{DE}^2/E + \sigma_e^2/rE)$$

$$\text{Standard error of } h^2 = 4SE(\sigma_A^2) / (\sigma_A^2 + \sigma_D^2 + \sigma_{AE}^2/E + \sigma_{DE}^2/E + \sigma_e^2/rE)$$

Table 4 Components of variance and heritability for tuber yield from design I and design II

Design I		Design II	
Item	Variance component	Item	Variance component
Analysis of variance			
$\sigma_{m/s}^2$	0.004	$\sigma_{m/s}^2$	0.008
		$\sigma_{f/s}^2$	0.0003
$\sigma_{f/m/s}^2$	0.003	$\sigma_{fm/s}^2$	0.003
$\sigma_{m/sE}^2$	0.017	$\sigma_{m/sE}^2$	0.017
		$\sigma_{f/sE}^2$	0.004
$\sigma_{f/m/sE}^2$	0.013	$\sigma_{fm/sE}^2$	0.001
σ_e^2	0.016	σ_e^2	0.019
Genetic analysis			
σ_A^2	0.017	σ_A^2	0.015
σ_D^2	-0.008	σ_D^2	0.017
σ_{AE}^2	0.071	σ_{AE}^2	0.042
σ_{DE}^2	-0.001	σ_{DE}^2	0.004
h^2	0.291	h^2	0.260

Table 3 Items of the analysis of variance for tuber yield combined across two locations (L) in design I [100 crosses from five females (F) each with four males (M) in five sets (S)], and in design II (64 crosses from four females each with four males in four sets)

Design I			Design II		
Item	Degrees of freedom	Mean square	Item	Degrees of freedom	Mean square
M/S	16	0.437	M/S	12	0.445
			F/S	12	0.091
F/M/S	80	0.058	F×M/S	36	0.038
M/S × L	16	0.301**	M/S×L	12	0.230
			F/S×L	12	0.067
F/M/S×L	80	0.042**	F×M/S×L	36	0.021
Pooled error	384	0.016	Pooled error	240	0.019
Mean (kg plant ⁻¹)		0.562	Mean (kg plant ⁻¹)		0.575
Coefficient of variation (%)		22.507	Coefficient of variation (%)		23.972

** $P \leq 0.01$

Table 5 Items of the analysis of variance for tuber yield (kg plant⁻¹) for designs I with two and four females per each of six or three males per set, respectively, across two locations

Item	Two females per six males, mean square	four females per three males, mean square
Males (M)/S	0.0017	0.1431
Females (F)/M/S	0.0009	0.1271
M/S×E	0.0016	0.0626
F/M/S×E	0.0007	0.1289**
Pooler Error	0.0008	0.0399
Mean	0.630	0.546

** $P \leq 0.01$

Table 6 Components of variance and heritability for tuber yield from designs I with two and four females per each six or three males, respectively

Item	Two females per six males	Four females per three males
$\sigma^2_{m/s}$	-0.00001	0.0034
$\sigma^2_{f/m/s}$	0.00005	-0.0003
$\sigma^2_{m/sE}$	0.0002	-0.0055
$\sigma^2_{f/m/sE}$	-0.00005	0.0297
σ^2_e	0.0008	0.0399
σ^2_A	0	0.0159
σ^2_D	0.0004	0
σ^2_{AE}	0.0009	0
σ^2_{DE}	0	0.1782
h^2	0	0.1421

Table 5. The estimate for $\sigma^2_{m/s}$ was negative in design I that includes two distinct females per each of the six males within sets (Table 6). Hence, the σ^2_A was zero for this trial with the lowest number of females but the highest number of males per set. The heritability estimate for design I with four distinct females for each of three males within sets was about half of that calculated in the early experiment involving designs I and II. Perhaps, the available genetic variation of the respective reference populations could explain these distinct h^2 for tuber yield as reported in this article.

Discussion

The North Carolina Mating designs (Cockerham 1963) are meant to provide plant breeders with information regarding the trait(s) being investigated for a reference population. This knowledge allows plant breeders to determine whether selection aiming at cultivar development will be feasible from this source population and what breeding method could be the best for such a goal.

Wricke and Weber (1986), among other authors, indicated that though the estimates of σ^2_A are not as precise as those from parent-offspring covariance analysis or design II, they can be still tolerated. However, the very low precision of σ^2_D by design I makes this mating scheme less accepted than others if this kind of genetic variation

is important in the crop species under research. The variance of the estimated σ^2_D may be quite large because design I does not allow a direct estimate of this genetic variance. Nonetheless, the individual analyses of designs I and II in the first experiment showed that, although design I provides a negative estimate of σ^2_D , h^2 was only slightly larger when determined by this mating scheme.

An advantage of design I with respect to design II is the operational easiness to generate the offspring to be included in the former design because each male will be crossed with a random sample of females. Design II requires a systematic crossing scheme in which each female must be crossed several times with the same sample of males within the set, which sometimes may be difficult to achieve in potato. Furthermore, a large sample of parents from the reference population will be ensured by design I, e.g. in the first experiment 120 parents from the CIP heterogeneous breeding population were crossed for design I versus only 32 for design II. According to the results of first experiment and above considerations, we recommend the hierarchical mating design for the genetic analysis of quantitative variation in tetrasomic potato.

There were negative estimates for σ^2_D and σ^2_{DE} in the design I of the first experiment and for $\sigma^2_{m/s}$, $\sigma^2_{f/m/sE}$ (3 females×6 males per set) or $\sigma^2_{f/m/s}$, $\sigma^2_{m/sE}$ (4 females×3 males per set) in design-I mating schemes of the second experiment. Although variance components are positive by definition, some estimates ensuing from the analysis of variance method may be negative due to an inadequate model, inadequate sampling or inadequate experimental techniques. The negative estimate of σ^2_D in design I of the first experiment could result from the shortcomings of design I to estimate epistatic variance in tetrasomic potato, a crop species in which non-allelic interactions are important (Ortiz 1998). As indicated early in this article, σ^2_D in design I will be only obtained after solving the expectations of other variance components (Table 2), while in design II the mean square between males and females yields a direct estimate of σ^2_D (Hill et al. 1998). Hence, design I will be appropriate for estimating σ^2_A in the reference population and useful for selection methods capitalizing on this genetic variation. However, the negative estimates of $\sigma^2_{m/s}$, $\sigma^2_{f/m/sE}$ in design I obtained by crossing two females with six males per set demonstrated the inadequate sampling due to the small number of female parents included in the experiment. This indirect negative calculation of the σ^2_A (regarded as zero in Table 6) could result from the sampling variance rather than assortative mating, linkage effects, genotype by environment interaction or deficiency of the genetic model, which are other factors associated to a negative component of variance in genetic mating designs (Obilana et al. 1979).

The smaller h^2 recorded in the second experiment (design I obtained by crossing four females with three males per set) suggest that although the higher number of females provided a better estimate of h^2 than in design I with two females per each of the six males within sets, the smaller number of parents (than in design I of the

first experiment) could account for half the h^2 for tuber yield observed in the second experiment (versus that of the first experiment). It seems that at least four females should be crossed to each male in design I for a genetic analysis of quantitative variation in potato.

The range of h^2 for tuber yield (0.14–0.29) fits within those reported for general combining ability/specific combining ability ratios for tuber yield by other authors in potato materials adapted to the northern hemisphere: 0.13 by Killick (1977) in UK, 0.22 by Plaisted et al. (1962) in USA, 0.26 by Tai (1976) in Canada. Similarly, Bradshaw et al. (2000) indicated that offspring-midparent regression was low for tuber yield due to a significant specific combining ability (SCA). Therefore, progeny testing for tuber yield should be considered by potato breeders due to the importance of SCA for this characteristic.

References

- Bradshaw JE, Todd D, Wilson RN (2000) Use of tuber progeny tests for genetic studies as part of a potato (*Solanum tuberosum* subsp. *tuberosum*) breeding program. *Theor Appl Genet* 100:772–781
- Buso JA, Boiteux LS, Tai GCC, Peloquin SJ (1999) Chromosome regions between centromere and proximal crossovers are the physical sites of major effect loci for yield in potato: genetic analysis employing meiotic mutants. *Proc Natl Acad Sci USA* 96:1773–1778
- Cockerham CC (1963) Estimation of genetic variances. In: Hanson WD, Robinson HF (eds) *Statistical genetics and plant breeding*, NAS – NRC Pub 982, Washington DC
- Comstock RE, Robinson HF (1948) The components of genetic variance in populations of biparental progenies and their use in estimating the average degree of dominance. *Biometrics* 4:254–266
- Comstock RE, Robinson HF (1952) Estimation of average dominance of genes. In: Gowen JW (ed) *Heterosis*. Iowa State University Press, Ames, pp 494–516
- Dabholkar AR (1999) *Elements of bio-metrical genetics*. Concept Publishing Company, New Delhi
- Dudley JW, Moll RH (1969) Interpretation and use of estimates of heritability and genetic variances in plant breeding. *Crop Sci* 9:257–262
- Hallauer AR, Miranda JB (1981) *Quantitative genetics in maize breeding*. Iowa State University Press, Ames
- Hill J, Becker HC, Tigerstedt PMA (1998) *Quantitative and ecological aspects of plant breeding*. Chapman and Hall, London
- Killick RJ (1977) Genetic analysis of several traits in potato by means of a diallel cross. *Ann Appl Biol* 86:279–289
- Mendoza HA (1983) Breeding of potato populations at the International Potato Center. *CIP Circular* 11:1–6
- Mendoza HA (1989) Population breeding as a tool for germplasm enhancement. *Am Potato J* 66:639–653
- Obilana AB, Hallauer AR, Smith OS (1979) Estimated genetic variability in maize interpopulation improvement. *J Hered* 70:127–132
- Ortiz R (1998) Potato breeding via ploidy manipulations. *Plant Breed Rev* 16:15–86
- Peloquin SJ, Boiteux LS, Carputo D (1999) Meiotic mutants in potato: valuable variants. *Genetics* 153:1493–1499
- Plaisted RL, Sanford L, Federer WT, Kehr AE, Peterson LC (1962) Specific and general combining ability for yield in potato. *Am Potato J* 61:395–403
- Searle SR (1971) Topics in variance component estimation. *Biometrics* 27:1–74
- Tai GCC (1976) Estimation of general and specific combining ability in potato. *Can J Genet Cytol* 18:463–470
- Tai GCC, de Jong H (1997) A comparison of performance of tetraploid progenies produced by diploids and their vegetatively doubled (tetraploid) counterpart parents. *Theor Appl Genet* 94:303–308
- Tarn TR, Tai GCC, de Jong H (1992) Breeding potatoes for long-day, temperate climates. *Plant Breed Rev* 9:217–332
- Wricke G, Weber WE (1986) *Quantitative genetics and selection in plant breeding*. Walter de Gruyter, Berlin, New York