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ORIGINAL PAPER

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Designing a microarray experiment to estimate dominance in maize (*Zea mays* L.)

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Abstract Experiments using cDNA microarrays for the identification of genes with certain expression patterns require a thoughtfully planned design. This study was conducted to determine an optimal design for a microarray experiment to estimate differential gene expression between hybrids and their parental inbred lines in maize (i.e. dominance). It has two features: the contrasts of interest contain more than two genotypes and the procedure may be customised to other microarray experiments where different effects may influence hybridisation signals. A mixed model was used to include all important effects. Impacts during growth of the plant material were taken into consideration as well as those occurring during hybridisation. The results of a preliminary experiment were used to determine which effects were to be included in the model, and data from another microarray experiment were used to estimate variance components. In order to select good designs, an optimality criterion adapted to the problem of differential gene expression between hybrids and their parental inbred lines was defined. Two approaches were used to determine an optimal design: the first one simplifies the problem by dividing it into several subproblems, whereas the second is more sophisticated and uses a simulated annealing (SA) algorithm. We found that the first approach constitutes a useful means for designing microarray experiments to study this problem. Using the more sophisticated SA approach the design can be further improved.

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Introduction

The phenomenon that the cross of two genetically different inbred lines results in F_1 progenies showing an increase in performance, such as vigour or height, over the mean of their parental lines is called heterosis. Selfing of the F_1 in subsequent generations leads to a reduced mean performance due to inbreeding depression (Darwin 1876; East 1908; Shull 1908). Hybrid vigour has been utilised in plant breeding since the middle of the 18th century, but the underlying theory was formulated much later by Shull (1908). Even at the present time, underlying mechanisms of heterosis are still not fully understood on the genetic and molecular levels. Several quantitative genetic explanations that make the combination of a considerable number of genes responsible for the phenomenon of heterosis have been discussed (Stuber et al. 1992; for review see Lamkey and Edwards 1998), but little consensus has emerged. Most hypotheses were formulated before the molecular concepts of genetics were discovered and are not related to molecular principles (Birchler et al. 2003).

This paper describes the development of an experimental design for a microarray study to determine differential gene expression between hybrids and their parental inbred lines in maize, denoted as dominance. Microarray technology is a promising new approach that allows the simultaneous transcriptome-wide expression profiling of thousands of different genes in a single experiment. Microarrays are small silica-coated glass slides that contain cDNA samples of several thousand different genes of a species to which cDNA of the sample of interest is hybridised (reviewed in Schnable et al. 2004). Thus, global patterns of gene expression can be analysed at a defined developmental stage

between different genotypes. A number of studies dealing with locating differentially expressed genes between inbred lines and reciprocal hybrid have been published (Ni et al. 2000; Kollipara et al. 2002; Guo et al. 2003), and the phenomenon of heterosis in maize is discussed in Auger et al. (2005), but without the aid of microarrays.

The design of microarray experiments has been the subject of various recent articles. For example, special designs, such as the common reference design and the loop design, have been proposed (Kerr and Churchill 2001; Kerr 2003). The objectives of these designs differ in one major aspect from the problem we address: in most of the earlier investigations, only contrasts between two mRNA populations were considered, whereas in our study the contrasts to be estimated include more than two genotypes. This complicates the problem since only two of the involved genotypes can be hybridised with one array as for economic reasons most experiments are performed with the fluorochromes Cy3 and Cy5. Classical microarray designs have been used for problems concerning expression between hybrids and parents; one example of this is the loop design applied by Gibson et al. (2004) to assess the degree of additivity in gene expression in *Drosophila melanogaster*. While these designs work, they are not usually optimal with respect to the specific contrasts of interest.

When estimating contrasts between inbred lines and hybrids, the choice of an optimality criterion is not obvious. In studies where pairs of treatments (genotypes) are compared, one often assumes that all pairwise comparisons are of equal interest, as in Kerr and Churchill (2001). If this premise is valid, then criteria such as A-optimality or E-optimality can be applied (John and Williams 1995; Yang et al. 2002). Both approaches do not apply to our problem, however, as we consider contrasts among several genotypes with unequal coefficients. John and Williams (1995) propose the minimization of the weighted mean of the efficiency factors of interest, yet they admit that the choice of the weights is very subjective.

The characteristic of the microarray design problem considered in the present paper is that information about three genotypes is combined with different weights, contrary to the main application of microarray analysis where two genotypes or treatments of equal interest are compared. This makes it necessary to have a newly defined optimality criterion and a tailor-made strategy to search the design space. We employ the mean standard error of dominance contrasts as optimality criterion and propose two different approaches to address the search problem—the first one is based on a full search for appropriately defined sub-designs and the second one is based on simulated annealing (SA). By using a flexible mixed model approach our procedure can easily be accommodated to microarray experiments with other experimental conditions, thereby allowing for the inclusion of different effects in the model.

Material and methods

The precise definition of the objectives of the study lead us to a definition of design optimality within the given context. The main steps of the planned experiment (experiment 1) are described in detail to account for all effects that might influence hybridisation signals. These effects are included in the model used for the design search. To determine the significance of these effects, we used data from a pre-experiment (experiment 2). Information on the variance components was derived by analysing a microarray experiment that had previously been conducted in the same laboratory (experiment 3). Finally, we explain two methods to find designs with the defined optimality properties. Without a doubt, other microarray studies are carried out in a different manner, and some of the effects we account for will not emerge. It should be stressed, however, that with the mixed-model approach other effects can easily be included in the model.

Outline of the planned experiment and optimality criterion

The planned experiment for which we develop a design will be performed in order to identify genes for which the expression level of the hybrid significantly exceeds the mean expression level of the parents. These genes will then be subjected to a subsequent detailed analysis. For the experiment, two local flint lines, A (UH002) and B (UH005), and two dent lines, C (UH250) and D (UH301), generated at the University of Hohenheim, Germany, were chosen. The experiments are to be performed with the inbred lines and the F_1 hybrids. The reciprocal of a hybrid is defined as a cross of the same parents, where the male and female parents are exchanged. The resulting hybrids are denoted as AB, AC, AD, BC, BD, CD and their reciprocals as BA, CA, DA, CB, DB, DC.

Let κ_A denote the expected value of a characteristic of line A, such as height or vigour; κ_B and κ_{AB} denote the same expectations for line B and hybrid AB, respectively. Heterosis is defined as the increase in performance of the hybrid compared to the mid-value parent value, or, in mathematical terms

$$\delta(\mathrm{AB}) = \kappa_{\mathrm{AB}} - \frac{\kappa_A + \kappa_B}{2} \qquad (1)$$

Carrying this definition to the molecular level, ''heterosis'' occurs when the expression level of a gene in a hybrid differs from the mean expression level of the parents. This phenomenon we denote by dominance. In Eq. 1, κ then is the expression level of a defined gene. We use the term dominance in place of heterosis because dominance commonly refers to gene effects, while heterosis is usually defined in terms of phenotypic means. Dominance may occur at various intensities: the

expression level of the hybrid may lie between the expression levels of the parents (partial dominance), or the expression level of the hybrid may exceed that of both parents (overdominance). If the expression level of the hybrid is lower than the mid-parent level, we denote this as negative dominance.

Planned experiment (experiment 1)

To account for all of the effects that might influence cDNA samples, a knowledge of their origin is of utmost significance. In the planned experiment, 20 maize seeds are laid out in a row on a piece of filter paper. In a second step, these filter papers are rolled up in a direction perpendicular to the row of seeds, and several paper rolls are placed upright (with the seeds on the upper end) into a water-filled beaker. In order to harvest all seedlings at approximately the same time of day and thus avoid circadian effects, the number of paper rolls in one beaker is limited to 16. After 3.5 days (84 h), mRNA is extracted from the roots of the germinated seedlings. Total RNA is first isolated with the Trizol (Invitrogen, Carlsbad, Calif.) method according to the manufacturer's instructions, and then mRNA is purified with Oligotex mRNA columns (Qiagen, Hilden, Germany). Approximately 20 maize roots are required to isolate sufficient mRNA for the hybridisation experiments. Reverse transcription of mRNA into cDNA and the incorporation of aminoallyl dUTPs and coupling of the Cy3 and Cy5 esters are performed as described in Nakazono et al. (2003). After 45 min of pre-hybridisation, the hybridisation solution containing the Cy3- and Cy5-labelled cDNAs are applied to the microarray chips and hybridised overnight as described in Nakazono et al. (2003). Maize 12-k cDNA microarray slides containing 12,160 different genes (Generation II, Version A) from the Iowa State MicroArray Facility (Ames, Iowa) are used for the experiments (http://www.zmdb.iastate.edu/zmdb/microarray/). Following hybridisation, the slides are washed and immediately scanned with a GMS 418 Array Scanner (Genetic Micro Systems, Woburn, Mass.). IMAGENE software (Biodiscovery, Marina Del Rey, Calif.) is used to quantify the spot intensities on the slides. We assume that there is a roughly log-linear relationship between the amount of expression product and the signal detected by the scanner for spots that do not show saturated hybridisation signals. The experiment was planned for a total of 72 microarray chips.

Effects that occur during this procedure and which might influence hybridisation signals are included in the following model:

$$y_{ijkl} = \mu + g_i + d_j + (gd)_{ij} + b_k + c_l + e_{ijkl}. \qquad (2)$$

Here, for $i = 1,...,n_i$, $j = 1,2$, $k = 1,...,n_k$ and $l = 1,...,n_l$, y_{ijkl} is the log signal intensity for genotype i on array l, marked with dye j. Plant material for this sample was cultivated in beaker k. Further definitions are:

μ, the overall mean;
g_i, the fixed effect of genotype i;
d_j, the fixed effect of dye j;
$(gd)_{ij}$, the interaction between genotype i and dye j;
b_k, the fixed effect of beaker k;
c_l, the random effect of array l;
e_{ijkl}, the random residual error associated with y_{ijkl};
n_i, n_k and n_l, the numbers of levels of the corresponding effect.

A filter paper effect can be taken into account as well, but we found in experiment 2 described below that this effect is not significant. When the array effect is treated as random, the recovery of inter-array information becomes possible. This may result in more accurate estimates of contrasts between inbred lines and hybrids, depending on the magnitude of the variance component involved and the associated degrees of freedom. Contrary to the present study, the recovery of inter-array information (analogous to inter-block information in incomplete block designs; see John and Williams 1995) is not an issue in experiments studying only two treatments, where arrays constitute complete blocks.

To study design efficiency, we computed standard errors of contrasts (1) and (2) for each potential design. The array variance/residual variance-ratio was provided by an earlier microarray experiment, which will be reported in a following section (experiment 3).

In our case it is not possible to perform the experiment with biological replicates in the sense that each sample consists of RNA of one single maize plant. As field design is not known, we only have RNA from a pool of plants with a certain genotype. However, biological replicates allow the investigator to make an inference on the population from which the replicates derive and should be used whenever possible. One would then include a replicate effect in the model to account for variance between individual biological replicates.

Pre-experiment for significance testing of possible effects (experiment 2)

A pre-experiment was performed to assay the influence of filter paper and beaker, which may arise during the germination of the seedlings. Filter papers with maize seeds were cultivated, and after 4 days root length was determined. To keep genotype-environment interactions low, a hybrid (UH005 × UH301) was chosen instead of an inbred line. Effects of filter paper and beaker were incorporated in a mixed model. We assume that results from the pre-experiment, which are based on phenotypic data, also apply to the gene expression level. As reported in the Results section, the pre-experiment revealed no significant effect of the filter paper, whereas the influence of the beakers was confirmed in the pre-experiment. Therefore, with respect to the experimental design for the planned microarray experiment, we did not account for a filter paper effect.

Estimating variance components from an earlier microarray experiment (experiment 3)

To collect information about the variances between and within arrays, we analysed data from a microarray experiment where two maize genotypes [wild type and the mutant *rtcs* (Hetz et al. 1996)] had been examined for differentially expressed genes. The experiment was carried out according to the same protocol and in the same laboratory as will be experiment 1. The analysis was performed for every spot according to a mixed model, including effects for genotype, dye, array and genotype-by-dye interaction, with the array being the only random effect. We thus obtained estimates for array variance as well as for residual (within-array) variance. Medians of both estimates were used for later design considerations where we used the so-determined ratio of variance components.

Finding optimal designs

In experiment 2, we showed that the filter papers, in which the maize seeds were germinated, have no major influence on root length. Thus, it is reasonable to germinate only one genotype per filter paper, instead of using filter paper as a blocking variable. This simplifies the experimental design considerably. With the filter paper having no significant influence, the design problem is the following: how should genotypes be allocated to the cDNA samples, how should the two dyes be allocated to genotypes and how should the filter papers be assigned to the beakers to achieve low standard errors for the contrasts?

We addressed the design problem in two steps. As we have six pairs of hybrids and reciprocal hybrids, we formed six groups containing hybrid, reciprocal hybrid and parents. For example, the first group would comprise A, B, AB and BA; the second, A, C, AC and CA; and so on. The groups were denoted as "A–B", "A–C", etc. For estimating the dominance contrasts of a certain hybrid and its reciprocal, one group is sufficient. For example, to estimate δ(AB) and δ(BA), only the first group is necessary. Also, each hybridisation of two genotypes can be uniquely allocated to a certain group; for example, an array with genotypes A and AC is said to be in the second group.

With a total of 72 arrays, we have 12 arrays available for every hybrid-reciprocal group. To have similar experimental conditions for all samples, it would be preferable to germinate all seeds in the same beaker. However, for lack of space, the number of filter papers per beaker is limited and two beakers per group are needed.

To find a good design one approach is to search for an optimal design for one group, i.e. indicate the optimal number of replicates of the six combinations of the four genotypes (A-AB, B-AB, A-BA, B-BA, AB-BA, A-B) as well as the optimal allocation to beakers and dyes. To reduce the number of possibilities, we imposed a restriction: one-half of the replicates with a certain genotype pair, e.g. A–AB, should be grown in each beaker and, accordingly, with one-half of the replicates of a certain genotype pair the dyes should be swapped. This restriction excludes highly unbalanced designs, which are expected to be inferior with respect to the optimality criterion, and the number of possible designs is computationally feasible. We generated and evaluated all possible designs in this restricted set and chose the best. This optimal design was then adapted to the other groups by inserting the appropriate genotype identifiers. Finally, all generated design matrices were composed to a matrix including all genotypes. The resulting design will in future reference be denoted as the "compound design".

The compound design neglects the fact that a parent does not only occur in one group but in three. Combining information of groups will increase the information on the parents and, therefore, the dominance contrast. Hence, the compound design might not be optimal for the whole problem. As the computing and evaluating of all possible full designs (72 microarrays, four effects) is very time-consuming, we performed the search with a SA algorithm. Providing a start design, the algorithm performs a random change in the design matrix, i.e. an array and an effect (of either genotype, dye or beaker) to be changed is randomly chosen. If the beaker effect is chosen, then a second array currently allocated to the other beaker is picked and swapped with the first array. This ensures that there is the same number of filter papers in both beakers. If the design is improved, the new design is retained. If not, it is accepted with a certain probability p. Otherwise, the design is rejected. In the next step, either the new design or, in case of rejection, the old design is altered, and so on. Accepting a design in some cases, even if it is worse, allows moving away from a local minimum. The acceptance probability $p = p(\theta)$ is dependent on the difference θ between the optimality criteria of the design before and after the variation. For details, see Kirkpatrick et al. (1983) and Angelis et al. (2001). The idea of forming groups of hybrid, reciprocal and parents is kept in the sense that, when altering the genotypes hybridised to an array, the "new" genotypes must be of the same group as the former genotypes. But unlike the first approach, optimisation is done for all genotypes simultaneously.

To analyse the usefulness of our optimality criterion we compared the design satisfying this criterion with an A-optimal and D-optimal design for genotype effects. We included the three effects of genotype, array and dye and searched for the optimal design for one group (i.e. for 12 arrays) in each case. Both cases of fixed and random error effects were evaluated. Furthermore, we varied model (1) underlying both SA-design and compound design and considered the consequences for the complete design. We assumed the array effect to be fixed or random with different variance components and omitted the beaker effect.

All computations were done using Version 8 of the SAS System for Windows (SAS Institute, Cary, N.C.).

Results

Analysis of pre-experiment data showed significance of the fixed effect for beaker (p-value 0.0218). Evaluating the random effect for filter paper, we found no significance. This had the important consequence that we could choose the simplest way of cultivating plants for one sample, i.e. cultivate plants on the same piece of filter paper. If the filter paper effect had been significant, it would have been worthwhile to use the filter paper as a blocking variable.

We obtained estimates for array and residual variance. After computing medians for both variance components, we took the relation array variance $\approx 0.48 \times$ residual variance for further calculations.

The results of these preliminary analyses were used to parameterise the model with which the design was optimised. The first solution is a design generated by optimising the sub-design for each group and then piecing together sub-designs. Therefore, designs for every group have the same number of replicates of hybrid-parent, reciprocal-parent, hybrid-reciprocal and parent-parent hybridisations. The second solution, optimised for the full design, was obtained by an SA-algorithm. Again, the design has the same number of replicates for every group, although here it is not pre-determined as in the first solution.

We first note that with both approaches, the selected design has no parent-parent arrays (Fig. 1). The reason is that this pair does not provide any information on the dominance contrast. However, the parent-parent contrast can be estimated with good accuracy because the designs provide many indirect comparisons among the parents via the hybrids. For example, the contrast A-B can be estimated from the difference of the contrasts A-AB and B-AB or from contrasts A-AC, C-AC, B-BC and C-BC.

It is striking that with the compound design we do not have any hybrid-reciprocal hybridisations, while in the SA-design there are two per group. The explanation for this is that in the SA-approach we also exploit information about the parents that is available from other groups in which the same parents occur. Thus, fewer parents need to be hybridised and hybrids are hybridised instead. As a certain hybrid only appears in one group, it makes sense to increase the number of hybrid hybridisations. A closer look at one group of the SA-design (Table 1) reveals that there is a dye swap across all beakers except those in the third row where the parent changes. Due to this change the number of both parents is balanced.

We also see that in the SA-approach, we have unequal numbers of replicates for hybrid-parent and reciprocal-parent hybridisations. Consequently, with this design the dominance contrast for a hybrid cannot be estimated with the same accuracy as the dominance contrast for the reciprocal. Of course, the hybrid and reciprocal hybrid are interchangeable. Therefore, it is possible to estimate the favoured dominance contrast with greater accuracy. Parental contrasts are estimated with varying accuracy depending on the genotypes. The variations may be caused by different dye-allocations and beaker-allocations. These allocations do not show any systematic pattern as can be seen from the allocation of genotypes to the arrays. Standard errors for hybrid-reciprocal contrasts are the same for every group, as we always have within each group one hybrid hybridised six times and one hybridised four times.

Considering the effectiveness of both approaches (Table 2), it is not astonishing that the value of the optimality criterion is worse with the compound-design approach, because this design was optimised for only one group and not the problem as a whole. As the optimality is worse, standard errors for dominance contrasts are higher than the mean of standard errors for the SA-approach. Only parental contrasts are estimated better in the first approach, which seems plausible as the parents are hybridised more often.

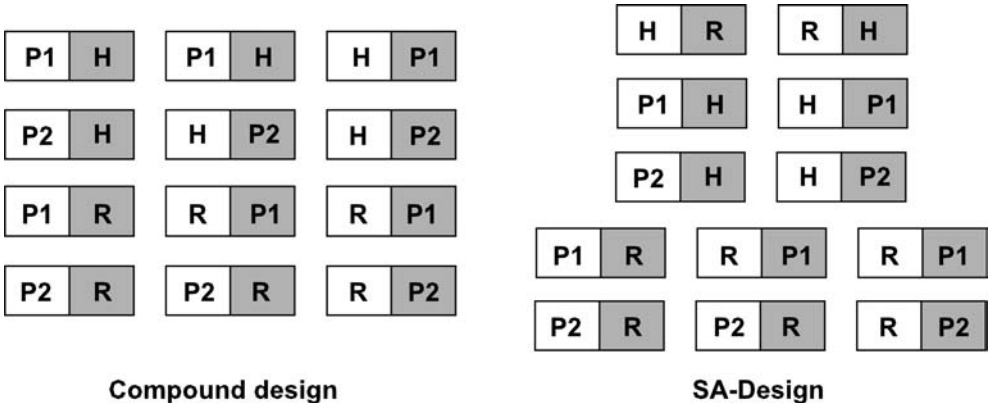

Fig. 1 Diagram indicating hybridisations and labelling directions for the compound-approach and SA-approach for one group (*white*≙ Cy3, *grey*≙ Cy5)

Table 1 Allocation of genotypes[a], beakers and dyes exemplary for one group of the SA-design

Beaker 1		Beaker 2	
Cy3	Cy5	Cy3	Cy5
P1	H	H	P1
P2	H	H	P2
H	P1	P2	H
R	P1	P1	R
R	P2	P2	R
H	R	R	H

[a]P1, P2, Parents; H, R hybrid and reciprocal cross

Table 2 Effectiveness of the two approaches (complete design)

	Standard error of contrast	
	Compound design	SA-design
Dominance contrast		
Mean (optimality criterion)	0.5268	0.5256
Range	0.5268	0.4979 or 0.5534
Parental contrasts (range)	0.4802	Between 0.5308 and 0.5814
Hybrid-reciprocal contrasts	0.6658	0.5948

Table 3 Comparison of one-group- and complete SA-design

	Range of standard errors of contrast	
	One group of SA-design	Complete SA-design
Dominance contrast	0.5027 or 0.5555	0.4979 or 0.5534
Parental contrast	0.7478	0.5469
Hybrid-reciprocal contrast	0.5952	0.5948

The increase in the accuracy of estimation when joining information of several groups can be seen when comparing standard errors of a reduced design containing genotypes of only one group with standard errors of the complete SA-solution (Table 3). By combining all groups, the gain in accuracy of estimation for the dominance contrasts is rather small. The parental contrasts are estimated more accurately when the complete design is taken, as we have altogether three groups which provide information about one certain parent. The accuracy of contrasts between hybrid and reciprocal differs only slightly between designs because no other hybridisations are of interest than those with the parents of the according group.

The increase in accuracy achieved with the SA-approach is relatively small (Table 2). Also, not all contrasts are estimated with the same accuracy. Therefore, the gain from using the SA-algorithm was not dramatic for this experiment. Generally, the gain from the SA-method strongly depends on the factors and their levels included in the model and can hardly be evaluated in advance.

For the evaluation of our optimality criterion, we developed an A-optimal and D-optimal design, which has two replicates of each genotype-combination (A-B, A-AB, A-BA, B-AB, B-BA, AB-BA) with the dyes swapped. The design optimum for the heterosis contrasts contains two additional hybrid-parent-replicates instead of the parent-parent replicates. Standard errors for the heterosis contrast are 0.5276 (heterosis-optimal) and 0.5466 (A-optimal/D-optimal). This means that the variance of the heterosis-optimal design is approximately 93% of the variance of the A-optimal/D-optimal design. If the chip effect is taken as being fixed, the design optimised for heterosis performs even better than the A-optimal/D-optimal design: its variance then is only 87% of the A-optimal/D-optimal design for the heterosis contrast.

Considering the complete design with the array effect taken as fixed changes the compound design (Fig. 2) but not the design obtained by simulated annealing. A fixed array effect corresponds to a random array effect with infinite variance. Therefore, if the array variance is high compared to the residual variance, this makes a difference only for the compound design but not the SA-design. The extreme case of fixed chip effects suggests that along with other variance ratios, a change in the optimal

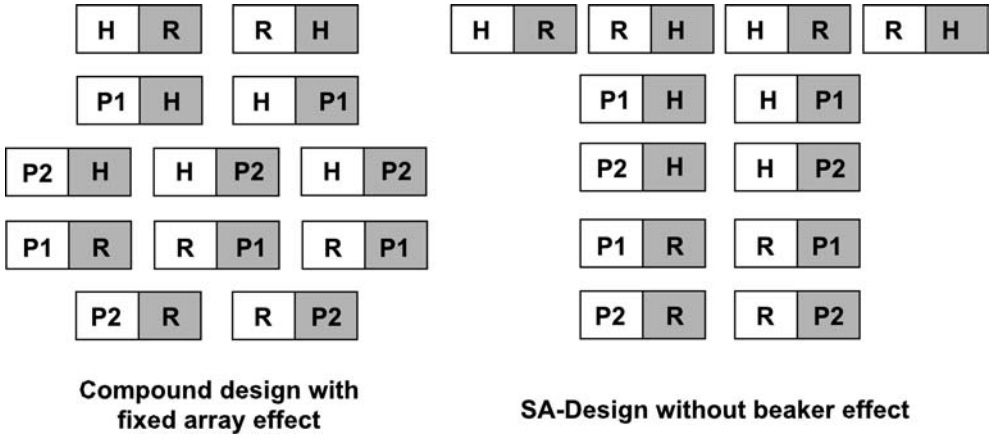

Fig. 2 Diagram indicating hybridisations for variations of model (1) (*white*≙ Cy3, *grey*≙ Cy5)

design is more likely with the compound design than with the SA design. If the beaker effect is omitted, the compound design is not affected, but the SA-approach results in increasing the number of hybrid-reciprocal arrays at the cost of hybrid-parent arrays (Fig. 2). Therefore, it seems justified to account for this effect.

Discussion

We sought for a microarray design with minimum standard errors for the desired contrasts. As a first method, we computed a solution for a simplified version of the problem. A simulated annealing algorithm was used for optimisation in the second method, and a design adapted to the specific problem was provided.

As the optimality criterion, the mean standard errors of all dominance contrasts was chosen. Other solutions would be possible depending on the research objectives. John and Williams (1995) proposed choosing a criterion weighting the contrasts according to their importance. In our case, we have given zero weight to all contrasts except the dominance contrasts, because this conformed to the main objective of the planned experiment. Other weighting schemes comprising standard errors for other contrasts, for example parental contrasts, are imaginable. For example, in order to study the dominance and the over-dominance hypotheses, it is useful to consider the comparison of a hybrid with one of its parents. These contrasts were not of primary interest for the planned experiment since the main objective was to identify genes showing dominance effects.

This discussion shows that choice of an optimal design depends on a number of factors. In addition, the common optimality criteria (D-optimality and A-optimality, average pairwise variance) are not generally helpful. Thus, standard packages for experimental design do not usually give the most useful answer, and a tailor-made approach is needed. Further details regarding this aspect can be found in Pearce (1974) and Freeman (1976).

With the analysis of a pre-experiment as well as of a subsequent microarray experiment, we gained useful knowledge on the significance and magnitude of error effects. Because a significant effect of filter paper could not be proved for phenotypic data, we neglected this effect. Yet it is not clear if this is satisfactory proof that this effect does not show up in mRNA. If so, the filter paper effect will be confounded with the residual intra-array variance and then will increase the error term. Our analysis of microarray data showed that array variance is about one-half of the residual variance. This, however, is an estimate based on another experiment, and in the planned experiment the variance ratio may possibly change.

In this study, we applied some basic principles that can generally be used when designing microarray experiments. First of all, a mixed model underlies all design considerations. Effects for array, dye, and genotype will probably be incorporated in every microarray design. Depending on the way in which plant material is obtained, the inclusion of other effects will be necessary. If one is doubtful which of these factors are significant, a separate experiment can be performed to check these factors. If information on the variance components of the random effects is available from other sources, these can be utilised. Then, after defining an appropriate optimality criterion, the search for the optimal design can be carried out. One approach is to simplify the design problem and choose the best one among all possible designs that satisfy some reasonable restrictions. This simple strategy provides fairly good results compared to a more complex design solution.

The article is the outcome of collaborative efforts within a research network "Heterosis in Plants" addressing the microarray analysis of young seedling roots in maize. Naturally, other research groups will face different design problems, mainly in the early stages of their projects (e.g. during the cultivation of plant material used for hybridisation), but some of the concepts elaborated here still hold, and, with some modifications, results can be applied to similar problems.

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