A Computer Program for Permutated Neighbourhood Seed Orchard Design

By G. N. Chakravarty and S. K. Bagchi

B-188, Lok Vihar, Delhi — 110034, India

(Received 7th October 1991)

Abstract

A computer program for permutated neighbourhood seed orchard design was developed. The program contains such facilities by which rectangular, triangular and hexagonal plots can be prepared as per desire or necessity. The design is meant for establishment of seed orchards for cross-fertilising species with minimum possibilities for inbreeding.

Key words: Computer program, permutated neighbourhood design, cross-fertilising species, seed orchard.

Introduction

In recent times, in Indian Forestry awareness about the need for tree improvement, seed improvement etc. has come to the fore. It has been particularly felt that increase in per hectare yield of wood will not be possible unless genetically superior seeds are available for regular plantation purposes. Attempts are constantly being made to select plus trees, make grafts from these trees and establish seed orchards. Gathering in seed orchards superior genotypes which subsequently cross-fertilise to produce superior seeds in large quantities may help raising forest trees with increased per hectare yield. Producing superior seeds thus depends on optimum cross-fertilisation among the grafted plants to maximise the genetic diversity.

The majority of seed orchards which have been established or which are being established for tropical tree species in India follow mainly the Randomised Block Design (BRD) method. The reason being that given the number of clones(1) and ramets per clone (2) the number of rows required to design an orchard could be made by almost anbody with some basic knowledge of the subject.

In the RBD a higher degree of panmixis is not possible at least theoretically, in comparison with the Permutated Neighbour-hood Design (PND) (Giertych, 1975). Therefore, from the theoretical point of view PND is far superior than RBD. PND's offer maximum permutation of neighbourhood combinations and minimum production of full-sibs in the orchard progeny. A version of PND (double ring isolation) with 30 clones and 10 ramets was used for establishing a Santalum Album seed orchard by the Sandal Research Center, Bangalore, India. Most probably that is the only example of the use of the PND method in India.

The major reason for not using the PND method may be due to the non-availability of the design in small and big versions. It may be clear from the foregoing that while RBD can be prepared easily, even manually, such a design does not guarantee minimum inbreeding, nor does it afford equal chances of producing all cross-combinations by participating clones. The PND method on the other hand offers such design sophistication to guarantee, at least, theoretically minimal inbreeding and to offer equal chances to produce cross-combinations by participating clones. The only handicap is the requirement of a computer and a computer program to take care of the various stringent parameters of the design.

The object of this article is to describe a program developed by the authors for a PND (double ring isolation). It was observed that even though the postulates given by Bastide (1965, 1967) in his article describing such a program were reasonable and valid, his design did not strictly adhere to these, particularly where the wind direction at the site is variable, which is the case in most areas. In India such a program for the PND is not available. PNDs are better than the RBDs providing higher degree of panmixis in the seed orchard population.

Materials and Methods

Bastide (1965) laid down 2 conditions for the arrangement of ramets of different clones in a clonal seed orchard. These are,

1. to avoid as much self-pollination as possible, and
2. to provide an equal opportunity for all cross combinations to occur with the same frequency.

Technical translation of these 2 requirements led him to the following,

1. each ramet of a clone should be isolated by two rings of dissimilar clones, and
2. two adjacent clone combinations should occur in that specific direction only once.

With these two design parameters Bastide (1965) succeeded in preparing a computer program. In this article he has given an example of a seed orchard layout for 30 clones, 10 ramets and 20 rows. The article, however, does not mention if it can produce designs of other types such as rectangular or hexagonal layouts. The design example is also restricted to specifying non repetition of one orientation of a pair of clones in a single direction with the result that this pair can reappear in diametrically opposite orientation in the layout. For example, the combination of 9—1 in the eighth row reappears as 1—9 in the 17th row. Similar is the case of 10—1 in first row and 1—10 in 19th row. These are not only contradictions of the basic postulates but allow certain combinations to produce more seed than others. The greater number of offspring so obtained may affect the overall superiority of the seeds produced.

Where wind is likely to blow in various directions, the requirements should be more stringent to assure that in this type of layout two clones do not appear not only in the opposite direction but also in adjacent positions a second time. Bastide's 2 postulates are valid and reasonable, the second one with the above modification, the program developed by him, however, does not truly meet the above requirements even without the further demands suggested by us. The program also does not provide for group-layouts (Giertych, 1965 and 1975; Klaehn, 1960; Bastide, 1965 and 1967).

Description of the Program

A computer program was prepared by the authors to run on the IBM PC machines and compatibles. With the program it is possible to produce the following type of designs:

1. Rectangular, (*Tab. 1, 2*)
2. Triangular, and, (*Tab. 3)*
3. Hexangonal (*Tab. 4, 5*).

Within these three types it is also possible to produce designs with adjacency restrictions on one side, as in BASTIDE's program, with similar restrictions for diametrically opposite directions, and with restrictions on repetition within the inner ring a second time.
Provision also exists for producing group layouts, where a layout can be repeated on one or more of the four adjacent sides without violating any of the original specifications of equal opportunity to all clones.

The program is of the interactive type. The variables accepted by the program are the number of clones, the number of ramets per clone and the number of rows. The program prompts the user to choose the type of layout he desires. After selection of the type, the program again prompts the user to choose · group (repeatable) layout or not by yes/no type response, and so on.

As success is dependant on the random selection from among the clones with under-utilized ramets at any stage during the progress of the analysis, eight different ways of controlling the process have been included in the program. These are termed *solution modes.* When the computer reports a failure of any mode, the next mode can be specified. In fact the trials can be doubled for a combination of clones, ramets per clone and rows by interchanging the rows and columns where these are not equal. If a set of data fails to produce any successful result after trying all the models, it may well be assumed that no solution is possible.

In order to prevent waste of computer stationary the user is given the option of printing or otherwise of any outcome whether successful or unsuccessful at any stage of the run.

The program may be available from Mr. G. N. CHAKRAVARTY, B-188, Lok Vihar, Delhi-110034, India.

Table 1. — Rectangular layout of Seed Orchard with 60 clones. 4 ramets per clone in 20 rows.

59	60	58	54	46	30	3	49	36	2	17	13
44	45	55	16	33	24	4	52	9	38	22	12
37	32	5	43	47	48	35	57	11	31	7	23
57	17	10	39	8	28	27	42	51	34	56	26
6	14	50	25	1	40	19	18	15	53	29	21
16	7	18	2	53	14	59	58	6	54	60	51
49	41	20	13	46	23	24	38	33	48	17	39
55	1	9	29	3	28	25	47	26	12	42	56
21	27	32	33	19	22	30	36	50	15	10	4
37	52	11	35	8	45	5	57	31	40	34	43
13	48	25	54	40	52	58	10	37	2	49	46
56	59	21	18	16	12	47	6	22	24	17	11
20	8	31	53	36	29	50	23	44	34	45	19
51	55	33	42	45	30	4	9	39	27	26	57
23	60	7	5	38	14	15	28	16	41	54	43
32	3	48	37	28	60	+2	26	10	3	59	52
47	14	9	58	46	42	32	8	18	38	15	20
1	12	25	34	41	31	43	6	49	21	36	40
56	19	29	5	4	27	13	51	50	22	39	11
44	7	35	20	55	30	44	53	35	1	24	41

Adjacency check in inner isolation ring: in all directions.
Solution mode adopted = 2.

Clones used:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
	49	50	51	52	53	54	55	56	57	58	59	60				
Ramet Usage:	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	4	4	4	4	4	4	4	4	4	4	4					

Table 2. — Rectangular layout of Seed Orchard with 50 clones. 4 ramets per clone in 20 rows.

49	50	48	3	36	20	13	25	15	36
41	11	30	14	17	29	3	1	28	27
42	33	8	32	40	37	2	4	21	22
24	38	46	43	45	47	39	45	16	23
6	26	12	31	34	18	25	32	5	9
4	10	28	22	1	8	19	36	6	30
31	2	33	32	21	40	42	45	29	41
24	15	13	46	3	20	23	48	12	27
39	14	9	16	18	11	7	35	49	25
50	37	26	43	17	44	5	47	38	34
43	22	29	38	45	13	12	1	50	19
48	9	42	32	11	6	25	10	44	31
40	3	49	34	14	20	30	21	26	47
28	17	8	7	41	35	15	5	36	4
16	37	12	24	46	40	38	18	33	14
27	30	23	44	+2	16	10	9	1	35
5	50	6	22	8	42	47	11	31	17
24	18	7	13	39	43	15	29	7	39
19	37	21	41	19	20	44	33	10	49
46	48	2	26	28	34	35	27	23	4

Adjacency check in inner isolation ring: in all directions. Isolation check made in octagon formed by ignoring outer corners of square.
Solution mode adopted = 2.

Clones used:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	
Ramet Usage:	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	

Results and Discussion

We present a few sample layouts produced by the program. These are as follows:

1. Rectangular type, with 60 clones, 4 ramets per clone in 20 rows where the corner members in outer ring are not ignored in adjacency checks (*Tab. 1*).
2. Rectangular type, with 50 clones, 4 ramets per clone in 20 rows where the corner members in the outer ring have been ignored for reasons given in the Annexure (*Tab. 2*).
3. Triangular type, with 36 clones, 4 ramets per clone in 16 rows (*Tab. 3*).
4. Hexagonal type group-layout, with 20 clones, 4 ramets per clone in 10 rows, the design repeatable on all sides any number of times (*Tab. 4*).
5. Hexagonal type layout, with 16 clones, 4 ramets per clone in 8 rows (*Tab. 5*).

In each type of design it was observed that there is a minimum number clones for the specified number of ramets, below which a design is not possible. Such number can be found out only by trial and error. It has been found that the rectangular design is most restrictive and the hexagonal type easiest to get because for a specified number of clones the highest number of ramets could be accommodated even with the more stringent requirements of a group-layout.

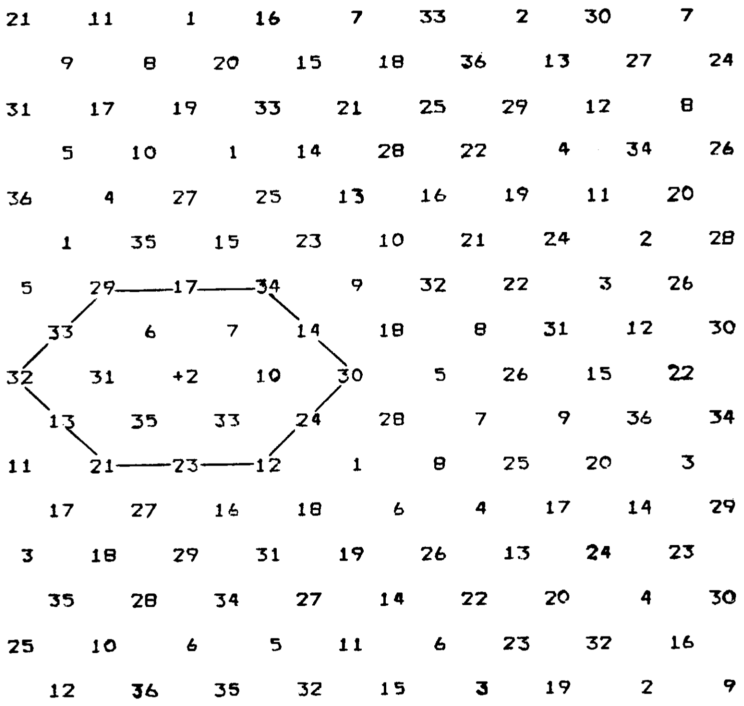
For example, with 4 ramets a comparison of the minimum number of clones that a layout could accommodate is given in *table 6*.

Conclusion

A computer program for seed orchard designs was prepared. This program is capable of producing rectangular, triangular and hexagonal designs. These are again capable of producing designs with or without adjacency restrictions. A group-layout system was developed where a layout can be repeated on one or more of the 4 adjacent sides without violating any of the original conditions for the design. However this design ignores pollinations by birds/insects/ants etc.

The program is meant for cross-fertilising species, as in a tropical country like India, where a large number of tree species are either cross-fertilising or predominantly cross — fertilising. Progenies obtained from seed

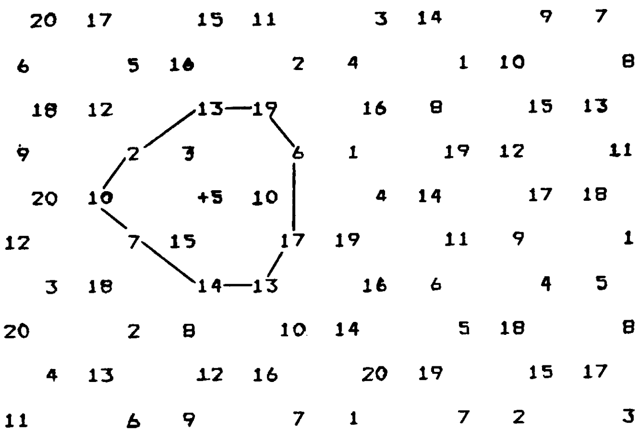
Table 3. — Triangular layout of Seed Orchard with 36 clones. 4 ramets per clone in 16 rows.



21	11	1	16	7	33	2	30	7
9	8	20	15	18	36	13	27	24
31	17	19	33	21	25	29	12	8
5	10	1	14	28	22	4	34	26
36	4	27	25	13	16	19	11	20
1	35	15	23	10	21	24	2	28
5	29	17	34	9	32	22	3	26
33	6	7	14	18	8	31	12	30
32	31	+2	10	30	5	26	15	22
13	35	33	24	28	7	9	36	34
11	21	23	12	1	8	25	20	3
17	27	16	18	6	4	17	14	29
3	18	29	31	19	26	13	24	23
35	28	34	27	14	22	20	4	30
25	10	6	5	11	6	23	32	16
12	36	35	32	15	3	19	2	9

Adjacency check in inner isolation ring: in all directions.
Solution mode adopted = 1.
Clones used: 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36.
Ramet Usage: 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4

Table 4. — Hexagonal layout of Seed Orchard with 20 clones. 4 ramets per clone in 10 rows.

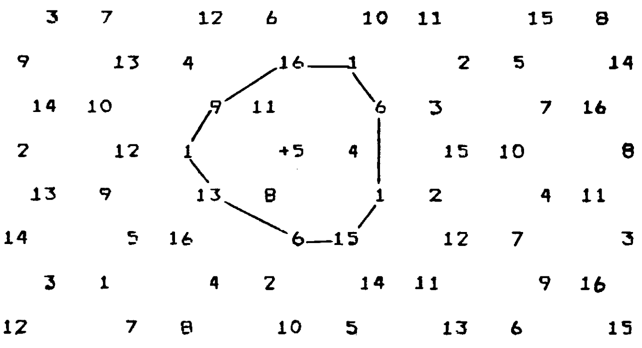


20	17	15	11	3	14	9	7
6	5	16	2	4	1	10	8
18	12	13	19	16	8	15	13
9	2	3	6	1	19	12	11
20	10	+5	10	4	14	17	18
12	7	15	17	19	11	9	1
3	18	14	13	16	6	4	5
20	2	8	10	14	5	18	8
4	13	12	16	20	19	15	17
11	6	9	7	1	7	2	3

Adjacency check in inner isolation ring: in all directions. Layout suitable for repetition on all sides.
Solution mode adopted = 2.
Clones used: 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Ramet Usage: 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4

orchards established with these designs are expected to exhibit greater genetic diversity.

Table 5. — Hexagonal layout of Seed Orchard with 16 clones. 4 ramets per clone in 8 rows.



3	7	12	6	10	11	15	8
9	13	4	16	1	2	5	14
14	10	9	11	6	3	7	16
2	12	1	+5	4	15	10	8
13	9	13	8	1	2	4	11
14	5	16	6	15	12	7	3
3	1	4	2	14	11	9	16
12	7	8	10	5	13	6	15

Adjacency check in inner isolation ring: in all directions.
Solution mode adopted = 1.
Clones used: 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Ramet Usage: 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4

Annexure

In a square layout, with the space between plants as 'a', (*fig. 1*), the distance AB and the distance AC are not equal although both points B and C are on the outer ring of plant at A. The distance between A and B is $2\sqrt{2}.a$ i. e. 2.83a approximately and is comparable to the distance 3a between A and G, a point outside the ring. In fact the intersection of the arc with radius AB and centre at

Table 6. — Comparison of different layouts for minimum number of clones with 4 ramets in each case.

Type of design	Number of clones	Repeatable design
Rectangular	60	No
Rectangular	50	No (Outer ring corners ignored)
Triangular	36	No
Hexagonal	20	Yes
Hexagonal	16	No

point A could in certain cases end up within the crown area of the plant at point G. Point B and similarly located points D, E, F on the outer ring could, therefore, be ignored in adjacency tests the same way as B.

In square layout it is not possible to give equal chance to all clones in the inner and outer rings due to the inherent inequality of the distances between centre and corners, and centre and midpoints.

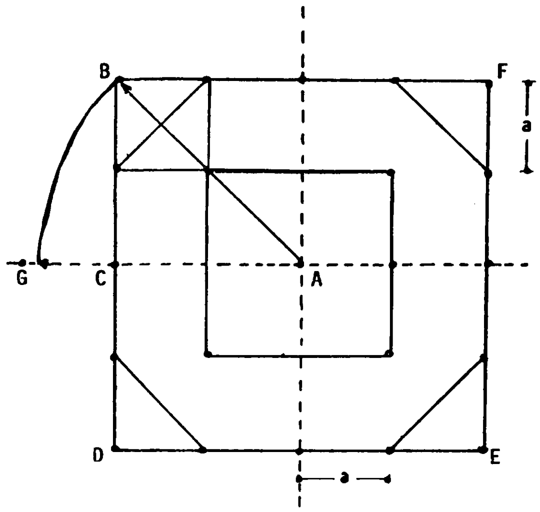


Fig. 1. — Schematic diagram showing corner plants on outer ring.

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Interpretation of Isozyme Patterns of Malate Dehydrogenase in Scots Pine Using Two Different Staining Methods

By R. Thormann and B. R. Stephan*)

Bundesforschungsanstalt für Forst- und Holzwirtschaft,
Institut für Forstgenetik,
Sieker Landstraße 2, DW-2070 Großhansdorf

(Received 21st April 1992)

Summary

The isozyme system malate dehydrogenase was analysed by means of horizontal starch gel electrophoresis using crude extracts of megagametophytes and embryos from Scots pine seeds. Two different staining methods were used to distinguish the loci coding for the enzyme system. It was shown that genetics of malate dehydrogenase in Scots pine can be explained with the presence of 4 coding loci. It is proposed to use both staining methods in studies of the enzyme system.

Key words: *Pinus sylvestris*, malate dehydrogenase, gene duplication, enzyme gene marker.

Zusammenfassung

Das Isoenzymsystem Malat-Dehydrogenase wurde bei Kiefer (*Pinus sylvestris* L.) mittels Stärkegelelektrophorese von Rohextrakten aus Megagametophyten und Embryonen untersucht. Zur Unterscheidung der einzelnen Genloci wurden 2 verschiedene Färbemethoden verwendet. Es wird gezeigt, daß die Genetik der Malat-Dehydrogenase mit 4 kodierenden Genloci erklärt werden kann. Es wird vorgeschlagen bei Untersuchungen des Enzymsystems beide Färbemethoden einzusetzen.

Introduction

Isozyme studies have become a useful tool in several areas of genetics. They possess practical importance for studies of population genetics and phylogenetic traits (e. g. papers in Tanksley and Orton, 1983). The basis for a correct interpretation of isozyme patterns obtained from electrophoretical studies is a complete knowledge of the modes of inheritance (Hattemer, 1991).

In Scots pine (*Pinus sysvestris* L.) the enzyme system of malate dehydrogenase is coded by genes at more than one locus. Due to the difficulty of interpreting many bands the number of loci considered to be responsible for the genetic control of the enzyme system varies between 3 (Rudin and Ekberg, 1978; Yazdani *et al.*, 1985; Muona and Harju, 1989; Prus-Glowacki and Siwecki, 1990) and 4 (Müller-Starck, 1985; Szmidt and Muona, 1989). Although the same isozyme patterns were observed by the different authors, they were interpreted in different ways.

The present paper reports an interpretation of the genetics of malate dehydrogenase in Scots pine using two different staining methods.

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*) Requests for reprints should be sent to B. R. Stephan.