## How to use MACRO for Generation of Polycross Designs

A polycross is the pollination by natural hybridization of a group of genotypes, generally selected, grown in isolation from other compatible genotypes in such a way to promote random open-pollination.

Polycross designs were devised originally for agricultural plant improvement. Larsen (1956) stated a systematic square pattern to be useful for forestry. For a systematic polycross design, Wright (1962) claimed the following pattern property: 'The plantlets are so arranged that any clone has every other clone as its immediate neighbour i.e., in the North, South, East, or West positions an equal number (four) of times. Later Wright (1965) gave ready-to-use field plans for a systematically designed polycross trial of any possible size from $6 \times 6$ to $46 \times 46$. In these plans involving $v$ replicates of $v$ genotypes, each genotype has each other genotype as its immediate neighbour once each in the North, South, East and West positions. If a design without borders is adopted, all plants may be used either as seed parents or as pollinators. In bordered designs, only the interior plants can be seed parents, the outside plants being pollinators only. Olesen and Olesen (1973) introduced a polycross pattern formula. They deduced that (a) the pattern was Latin square and (b) that every clone had any other clone as a nearest neighbour just once in each of the four main directions. The first property implies that no clone has itself as a nearest neighbour in any of the four main directions. Olesen (1976) obtained formula for polycrosses in $n(n \times n)$ Latin squares, where $n$ is any even integer such that $n+1$ is a prime. In this design, each clone has every other clone as nearest neighbour $n$ times in each of the primary directions and $n$ 2 times in each of the intermediate directions. Also each clone has itself as neighbour $n-1$ times in each intermediate direction. It was shown by Morgan (1988) that a completely balanced polycross design in $n$ Latin squares of side $n$ may be obtained for any even $n$ (dropping the condition that $n+1$ be prime), and same balance properties were obtained for odd $n$ in $n(n \times n)$ squares which are not Latin. Morgan has given a list of designs for $n \leq 12$.

A particular practical application of the polycross method occurs in the production of a synthetic variety resulting from cross-pollinated plants. Laying out these experiments in appropriate designs, known as polycross designs, would not only save experimental resources but also gather more information from the experiment. Different situations may arise in polycross nurseries where accordingly different polycross designs may be used. For situations in which some genotypes interfere in the growth or production of other genotypes, but have to be grown together, neighbour restricted design is a better option. Further, when the topography of the nursery is such that a known wind system in a certain direction may prevail, then designs balanced for neighbour effects of genotypes only in the direction of wind are appropriate which may help in saving experimental resources to a great extent. Also, when genotypes are planted in a small area without leaving much space between rows, designs balanced for neighbour effects from all possible eight directions are useful to have equal chance of pollinating and being pollinated by every other genotype.
Polycross designs balanced for all possible neighbours
Polycross designs for plant breeding are arrangements of $n$ replicates of $n$ clones in a rectangular pattern so that each clone is an immediate neighbour of each other clone an approximately equal
number of times. Such an arrangement is an attempt to ensure that all pairs of distinct clones have an equal chance of crossing. The vagaries of wind direction and other environmental factors make guaranteeing this equality of crosses impossible; the polycross method simply attempts to make conditions as favourable as possible.

In a square or rectangular pattern with adequate border plants, each genotype will have eight neighbours in eight possible directions adjacent to it (North, South, East, West, North-East, North-West, South-East, South-West).


A polycross design for 3 genotypes balanced for neighbours in all the directions is given below:

| 1 | 2 | 1 | 2 | 3 | 2 | 3 | 1 | 3 |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| 2 | 3 | 2 | 3 | 1 | 3 | 1 | 2 | 1 |
| 1 | 2 | 1 | 2 | 3 | 2 | 3 | 1 | 3 |

An alternative approach is usage of a honeycomb design in which each plant is at the centre of a regular hexagon having six nearest neighbours.


Polycross designs balanced for all possible neighbours are not available for all number of genotypes. Such designs are to be constructed ensuring that no clone/ genotype has itself as a nearest neighbour in all possible directions surrounding it. Further, every genotype should have every other genotype as a nearest neighbour in all the directions equally frequently. General methods for constructing such designs are also not available. Hence, there is a need to develop methods for constructing efficient polycross designs to suit different experimental situations.

Some places are known to have regular prevailing wind in certain direction and the wind system is highly predictable. Polycross designs for directional seed orchards like the one given below (for 4 genotypes) are appropriate in such situations.


## Neighbour restricted polycross designs

Neighbour restricted designs restrict randomization of entries in such a way that certain groups of entries do not occur together. They can be advantageously used in situations where some genotypes interfere the growth or production of other genotypes due to different maturity or plant height. Generally, for easy pollination, male (female) genotypes are note to be kept as neighbours to other male (female) genotypes. In such designs, a set of genotypes will never appear in the neighbouring positions of an identified genotype.

Here, SAS Macros have been developed to generate three series of designs (viz., Neighbour Restricted Block Designs, Polycross designs for directional wind system and Neighbour balanced polycross designs for v genotypes. User need to enter only the Series Number (1, 2 and 3) and the Number of Genotypes ( $\mathbf{v}$ ) in the program.

Series 1: SAS Macro for generation of neighbour restricted block designs with parameters v (Number of Genotypes) $=2 \mathrm{~m}$ ( m should be a prime number), b (Number of Blocks) $=\mathrm{m}, \mathrm{k}$ (Block Size) $=2 \mathrm{~m}$ and r (Number of Replications) $=\mathrm{m}$. Here, 1, $2, \ldots, \mathrm{~m}$ belong to the first group of genotypes and $\mathrm{m}+1, \mathrm{~m}+2, \ldots, 2 \mathrm{~m}$ belong to the second group of genotypes.

SAS Output
Neighbour Restricted Block Designs

| Design |  |  |  |  |  |  |  |  |
| :--- | ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: |
|  | COL1 | COL2 | COL3 | COL4 | COL5 | COL6 | COL7 | COL8 |
| ROW1 | 6 | 1 | 4 | 2 | 5 | 3 | 6 | 1 |
| ROW2 | 5 | 2 | 6 | 3 | 4 | 1 | 5 | 2 |
| ROW3 | 4 | 3 | 5 | 1 | 6 | 2 | 4 | 3 |

Consider rows as blocks and treat first and last columns are border plots

Series 2: SAS Macro for generation of neighbour balanced Polycross designs for directional wind system with parameters v (Number of Genotypes) $=$ a prime number with $(v-1)$ a multiple of 3, b (Number of Blocks) $=(\mathrm{v}-1) / 3, \mathrm{k}($ Block Size $)=\mathrm{v}$ and $\mathrm{r}($ Number of Replications $)=(\mathrm{v}-$ $1) / 3$.

## SAS Output

Polycross designs for directional wind system

Array_number
1

| Array |  |  |  |  |  |  |  |  |
| :--- | ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: |
|  | COL1 | COL2 | COL3 | COL4 | COL5 | COL6 | COL7 | COL8 |
| ROW1 | 1 | 4 | 7 | 3 | 6 | 2 | 5 | 1 |
| ROW2 | 3 | 6 | 2 | 5 | 1 | 4 | 7 | 3 |
| ROW3 | 5 | 1 | 4 | 7 | 3 | 6 | 2 | 5 |

Treat first and 3rd rows and last column as border plots

Array_number
2

| Array |  |  |  |  |  |  |  |  |
| :--- | ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: |
|  | COL1 | COL2 | COL3 | COL4 | COL5 | COL6 | COL7 | COL8 |
| ROW1 | 1 | 5 | 2 | 6 | 3 | 7 | 4 | 1 |
| ROW2 | 3 | 7 | 4 | 1 | 5 | 2 | 6 | 3 |
| ROW3 | 5 | 2 | 6 | 3 | 7 | 4 | 1 | 5 |

Treat first and 3rd rows and last column as border plots

Series 3: SAS Macro for generation of neighbour balanced polycross designs with parameters $v$ (Number of Genotypes) with ( $\mathrm{v}+1$ ) a prime number, s (Number of Arrays) $=\mathrm{v} / 2$, p (Number of Rows $)=\mathrm{q}$ (Number of Columns) $=\mathrm{v}$ and r (Number of Replications) $=v^{2} / 2$.

## SAS Output

Square_number
1

| Square |  |  |  |  |  |  |  |  |  |  |
| :--- | ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: |
|  | COL1 COL2 | COL3 | COL4 | COL5 | COL6 | COL7 | COL8 | COL9 | COL10 |  |
| ROW1 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| ROW2 | 2 | 4 | 6 | 8 | 10 | 1 | 3 | 5 | 7 | 9 |
| ROW3 | 3 | 6 | 9 | 1 | 4 | 7 | 10 | 2 | 5 | 8 |
| ROW4 | 4 | 8 | 1 | 5 | 9 | 2 | 6 | 10 | 3 | 7 |
| ROW5 | 5 | 10 | 4 | 9 | 3 | 8 | 2 | 7 | 1 | 6 |
| ROW6 | 6 | 1 | 7 | 2 | 8 | 3 | 9 | 4 | 10 | 5 |
| ROW7 | 7 | 3 | 10 | 6 | 2 | 9 | 5 | 1 | 8 | 4 |
| ROW8 | 8 | 5 | 2 | 10 | 7 | 4 | 1 | 9 | 6 | 3 |
| ROW9 | 9 | 7 | 5 | 3 | 1 | 10 | 8 | 6 | 4 | 2 |
| ROW10 | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 |

## Square_number

$$
2
$$

| Square |  |  |  |  |  |  |  |  |  |  |
| :--- | ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: |
|  | COL1 | COL2 | COL3 | COL4 | COL5 | COL6 | COL7 | COL8 | COL9 | COL10 |
| ROW1 | 3 | 6 | 9 | 1 | 4 | 7 | 10 | 2 | 5 | 8 |
| ROW2 | 6 | 1 | 7 | 2 | 8 | 3 | 9 | 4 | 10 | 5 |
| ROW3 | 9 | 7 | 5 | 3 | 1 | 10 | 8 | 6 | 4 | 2 |
| ROW4 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| ROW5 | 4 | 8 | 1 | 5 | 9 | 2 | 6 | 10 | 3 | 7 |
| ROW6 | 7 | 3 | 10 | 6 | 2 | 9 | 5 | 1 | 8 | 4 |
| ROW7 | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 |
| ROW8 | 2 | 4 | 6 | 8 | 10 | 1 | 3 | 5 | 7 | 9 |



Square_number

Square
COL1 COL2 COL3 COL4 COL5 COL6 COL7 COL8 COL9 COL10

| ROW1 | 5 | 10 | 4 | 9 | 3 | 8 | 2 | 7 | 1 | 6 |
| :--- | ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: |
| ROW2 | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 |
| ROW3 | 4 | 8 | 1 | 5 | 9 | 2 | 6 | 10 | 3 | 7 |
| ROW4 | 9 | 7 | 5 | 3 | 1 | 10 | 8 | 6 | 4 | 2 |
| ROW5 | 3 | 6 | 9 | 1 | 4 | 7 | 10 | 2 | 5 | 8 |
| ROW6 | 8 | 5 | 2 | 10 | 7 | 4 | 1 | 9 | 6 | 3 |
| ROW7 | 2 | 4 | 6 | 8 | 10 | 1 | 3 | 5 | 7 | 9 |
| ROW8 | 7 | 3 | 10 | 6 | 2 | 9 | 5 | 1 | 8 | 4 |
| ROW9 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| ROW10 | 6 | 1 | 7 | 2 | 8 | 3 | 9 | 4 | 10 | 5 |

Square_number
4

## Square

COL1 COL2 COL3 COL4 COL5 COL6 COL7 COL8 COL9 COL10

| ROW1 | 7 | 3 | 10 | 6 | 2 | 9 | 5 | 1 | 8 | 4 |
| :--- | ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: |
| ROW2 | 3 | 6 | 9 | 1 | 4 | 7 | 10 | 2 | 5 | 8 |
| ROW3 | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 |
| ROW4 | 6 | 1 | 7 | 2 | 8 | 3 | 9 | 4 | 10 | 5 |
| ROW5 | 2 | 4 | 6 | 8 | 10 | 1 | 3 | 5 | 7 | 9 |
| ROW6 | 9 | 7 | 5 | 3 | 1 | 10 | 8 | 6 | 4 | 2 |


| Square |  |  |  |  |  |  |  |  |  |  |
| :--- | ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: |
|  | COL1 | COL2 | COL3 | COL4 | COL5 | COL6 COL7 | COL8 | COL9 | COL10 |  |
| ROW7 | 5 | 10 | 4 | 9 | 3 | 8 | 2 | 7 | 1 | 6 |
| ROW8 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| ROW9 | 8 | 5 | 2 | 10 | 7 | 4 | 1 | 9 | 6 | 3 |
| ROW10 | 4 | 8 | 1 | 5 | 9 | 2 | 6 | 10 | 3 | 7 |

## Square_number

| Square |  |  |  |  |  |  |  |  |  |  |
| :--- | ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: | ---: |
|  | COL1 COL2 | COL3 | COL4 | COL5 | COL6 COL7 | COL8 | COL9 | COL10 |  |  |
| ROW1 | 9 | 7 | 5 | 3 | 1 | 10 | 8 | 6 | 4 | 2 |
| ROW2 | 7 | 3 | 10 | 6 | 2 | 9 | 5 | 1 | 8 | 4 |
| ROW3 | 5 | 10 | 4 | 9 | 3 | 8 | 2 | 7 | 1 | 6 |
| ROW4 | 3 | 6 | 9 | 1 | 4 | 7 | 10 | 2 | 5 | 8 |
| ROW5 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| ROW6 | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 |
| ROW7 | 8 | 5 | 2 | 10 | 7 | 4 | 1 | 9 | 6 | 3 |
| ROW8 | 6 | 1 | 7 | 2 | 8 | 3 | 9 | 4 | 10 | 5 |
| ROW9 | 4 | 8 | 1 | 5 | 9 | 2 | 6 | 10 | 3 | 7 |
| ROW10 | 2 | 4 | 6 | 8 | 10 | 1 | 3 | 5 | 7 | 9 |

## Prepared By

Cini Varghese, Seema Jaggi and Eldho Varghese
Indian Agricultural Statistics Research Institute
Cini_v@iasri.res,in, seema@iasri.res.in, Eldho@iasri.res.in

## References

Larsen, C.S. (1956). Genetics in Silviculture, p. 15, (Translated by Anderson, M.L.), Oliver and Boyd, Edinburgh.
Olesen, K. and Olesen, O.J. (1973). A polycross pattern formula. Euphytica, 22, 500-502.
Olesen, K. (1976). A completely balanced polycross design. Euphytica, 25, 485-488.
Morgan, J.P. (1988). Polycross designs with complete neighbour balance. Euphytica, 39, 59-63.
Varghese, Cini, Jaggi, Seema and Varghese, Eldho (2014). Designs for polycross experiments, Unpublished Project Report, IASRI, New Delhi.
Wright, C.E. (1962). A systematic polycross design. Res. Exp. Rec. Min. Agric. N.I., 1, 7-8.
Wright, C.E. (1965). Field plans for a systematically designed polycross. Rec. Agric. Res.,14(1), 31-41.

