

Randomized, replicated, staggered clonal-row (R^2 SCR) seed orchard design

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Abstract Spatial randomization of clones across a seed orchard's grid is commonly applied to promote cross-fertilization and minimize selfing. The high selection differential attained from advanced-generation breeding programs sets high premier on the genetic gain and diversity delivery from seed orchards, thus clonal allocation is important and even more challenging when clones share common ancestry. Evidences of low selfing in many conifers' seed orchards, as a result of their high genetic load, inbreeding depression, and polyembryony are abundant and call for orchards' design re-evaluation, specifically when randomization is associated with added managerial burden. Clonal-rows represent a viable option for simplifying orchards management; however, they are often associated with elevated correlated matings between adjacent clones. Here, we propose a modified clonal-row design that replicates, staggers, and randomizes the rows, thus doubling the number of adjacent clones and providing different set of neighboring clones at each replication, thus allowing accommodating related parents more readily than any single-tree arrangement. We present a novel algorithm packaged in user-friendly software for executing various seed orchards' designs. The developed program is interactive and suitable for

any orchard size and configuration, accommodates any number of clones that are allocated to rows with variable length (ranging from a single tree to any even number) and pre-set separation zone between ramets of the same clone. The program offers three deployment modes (equal, linear, and custom) each with multiple layouts determined by the number of iterations requested. The resulting layouts are ranked based on four criteria including: (1) the number of empty positions, (2) deviation between expected and observed clone size, (3) minimum inbreeding, and (4) a neighborhood index that expresses the efficiency of clonal distribution.

Keywords Seed orchard design · Randomization · Clonal-row · Separation zone · Clonal deployment

Introduction

The genealogical relationship among a seed orchard' parents/ clones determines the type of matings while their spatial arrangement drastically affects the magnitude and frequency of each mating type (El-Kassaby et al. 1986; El-Kassaby 1989; El-Kassaby and Askew 1998). Randomization of parents across an orchard's grid is sternly practiced to minimize selfing and maximize inter-parents mating. In theory, pure randomization is not associated with any mathematical objective and therefore leads to local aggregations without systematic dispersion of genetic entries along the orchard's grid, thus it is not suitable. Seed orchards designs are developed to optimize the spatial allocation of parents with various goals and constraints (see Giertych 1975; Hodge and White 1993, for an extensive review); however, the permuted neighborhood design of La Bastide (1967) is the most commonly used specifically following the development of the Computer Organized Orchard Layouts (COOL) program (Bell and Fletcher 1978). The COOL program was further modified to

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accommodate related and/or unequal parental representation (see Vanclay 1991; “Seed Orchard Designer” (SOD)). The main premise of all seed orchard designs, including COOL and SOD, is focused on minimizing mating among members of the same parent (ramets of the same clone) through deliberate spatial separation.

While selfing, in general and for conifers in particular, is considered detrimental, the extensive body of conifers’ mating system literature highlights its rare occurrence and when it is present, the observed rate is low in natural populations and even lower in seed orchards (see Mitton 1992; El-Kassaby 1995; O’Connell 2003); however, recent studies utilizing DNA markers in seed orchards revealed varying selfing rates ranging from 1.0 to 15 % (Slavov et al. 2005; Lai et al. 2010; Doerksen et al. 2011). It should be stated that exceptions exist and are well documented (e.g., western red cedar: El-Kassaby et al. 1994; O’Connell et al. 2004 and eastern white cedar: Perry and Knowles 1990; Xie et al. 1991; Lamy et al. 1999). The low observed selfing rate is not surprising considering conifers’ high genetic load, severe inbreeding depression, and their unique polyembryony reproductive biology (Buchholz 1962; Sorensen 1982; Griffin and Lindgren 1985; Williams and Savolainen 1996). These factors, individually and/or in concert, act as effective barriers to selfing and directly favor outcrossing. Furthermore, it is worth noting that the commonly detected low selfing rate is an indication that most selfing events do not develop to viable seed (Woods and Heaman 1989). In light of these biological facts and the added crop management burden caused by randomization, the development of new seed orchard designs that maximize the genetic efficiency (gain and diversity) and optimize crop management activities is worth considering. Seed orchards are established for seed production over many years and sometimes decades, their design should be optimized with respect to their entire lifespan and this highlights the importance of accommodating all relevant factors at the establishment phase.

The clonal-row orchard design has the potential to optimize crop management activities (e.g., individual clone management such as cone harvest, pollination, and pest control will be done on fewer locations) and maximize orchard’s genetic efficiency. However, mating system studies evaluating clonal-row versus randomization indicated that the former consistently produced higher correlated matings (i.e., correlated paternity) than the latter while differences in selfing rates were negligible (El-Kassaby 2003; El-Kassaby et al. 2007). The observed disparities in correlated matings estimates between the clonal-row vs. random design were pronounced reaching orders of magnitude (western hemlock: 34.9 vs. 7.7 (El-Kassaby 2003); interior spruce: 9.3 vs. 3.4 (El-Kassaby et al. 2007)). In the classical clonal-row design; each clone is present in a single-row that is spatially contiguous to two other clones/rows (Giertych 1975). This arrangement of a complete side-by-side clonal flanking, creates a localized

“neighborhood effect” promoting between-rows matings. To overcome this neighborhood effect, El-Kassaby (2003) and El-Kassaby et al. (2007) proposed either staggering of rows or replicating and randomizing the staggered rows throughout the orchard. The staggering of rows will effectively flank each individual clone by four different clones and the randomization secures flanking by four different clones at each replication. It is expected that the staggering and randomization of rows would promote outcrossing among clones and reduce correlated matings. It should be stated that the previous description did not include the two neighboring clones present at the beginning and end of each row.

Modern seed orchard designs must be flexible to deal with many constraints that are either associated with the use of related parents, specifically in advanced generations; the desire to deploy parents linearly proportionate to their breeding values (Lindgren and Matheson 1986); site physical constraints; and the availability of parental stock for planting (i.e., unbalanced clonal size). Here, we present the “randomized, replicated, staggered clonal-row (R^2 SCR)” seed orchard design as an alternative to existing designs. The proposed R^2 SCR design is a flexible, interactive computer program capable of addressing the often encountered seed orchards’ biological and logistical/managerial constraints. It is not limited to a “row” layout as the name suggests, but the row size could be either one (i.e., single-tree-plot) or any even number of ramets (i.e., row plot). The program produces multiple orchard layouts considering many parameters that include: (1) number of clones, (2) clones genealogical relationship (coancestry), (3) seed orchard size, (4) row length (single or any even number), (5) number of positions separating ramets or rows of the same clone, and (6) site configuration (presence of barriers or non-plantable spots). These parameters are collectively considered for three deployment modes; namely, equal (same clone size), linear (clone size is a linear function of their breeding value), and custom (based on the available number of ramets per clone).

R^2 SCR developed application

The R^2 SCR is a user-friendly desktop application designed for Microsoft Windows environment (Online Resource 1) and is coded using Microsoft® Visual Studio 10 Ultimate edition and utilizes a graphical user interface (e.g., toolbar and dropdown menus) that eases navigating (Online Resource 2). The application produces an orchard design for the desired deployment option (equal, linear, and custom (see below)), displays the resulting solutions (layout(s)) in different formats, and saves them for further design changes. The resulting solutions could be exported to Microsoft Excel file in two modes: (a) export all solutions and (b) export the current solution in a format suitable for handheld data loggers.

Furthermore, the application has the capability to: (1) generate additional solutions, (2) modify existing project, (3) abort current calculations, (4) display the orchard's grid, specific clone's spatial arrangement, and four ranking criteria to compare the resulting solutions (# of empty positions, the difference between expected and observed clone size, minimum inbreeding, and neighborhood index that expresses the efficiency of clonal distribution), (5) print the resulting solutions, and (6) include a zoom function for improved visualization.

Detailed information regarding the program information such as seed orchard's grid specifications (e.g., length, width, location of non-plantable spots), number of parents and their genealogical relationships, the extent of separation among ramets/rows of the same clone, deployment modes (equal, linear, and custom), and the number of layouts requested and their evaluation/comparison is available on Online Resource 3.

Orchard's layout comparison/ranking

After the completion of a particular orchard design, multiple layouts are produced as determined by the number of iterations invoked. These layouts are automatically and sequentially compared and ranked according to four criteria:

1. The number of empty spots: to meet the seed orchard collective design requirements, sometimes the presence of empty spots is necessary. Thus, a layout without empty spots is better than others with vacant position and a layout with fewer is more desirable than others with higher number,
2. How close the resultant layouts meeting the specified input parameters: this is evaluated using the method of least squares algorithm (Wolberg 2005) which estimates the deviation between each layout's observed and expected number of ramets within each clone. Layouts are ranked in a decreasing order, thus those with lower deviation are better than their counterparts with higher deviation,
3. Minimum inbreeding: following the "minimum inbreeding distance" concept of Lstiburek and El-Kassaby (2010) which is a proxy to potential inbreeding (selfing in this case) and estimated as the sum of the inverse square distances among all possible combinations of ramets within clone and summed over all clones. This distance is estimated for each layout and the resultant layouts are ranked in a decreasing order, so a layout with the lowest "minimum inbreeding distance" is best. For clonal-row design, this distance is estimated among rows within clone and each row is considered as a single entity, and
4. Coefficient of variation (CV): estimated as a ratio between standard deviation and mean, where, standard deviation

and mean are estimated based on the number of all possible clonal-pair occurrences. Thus, it is a measure of local clonal-pair dispersal across the orchard's grid which is determined by counting the number of times any two specific clones are planted side-by-side. Theoretically, if all clonal-pair combinations shared the same number of occurrences (i.e., equal distribution across all neighboring clones), the CV value would be very close to zero and other values would indicate the degree of deviation from equal occurrences. The same relationship also will hold for row plots as the entire row is considered as a one entry.

Case study 1 (Alpha): single-tree-plot, equal distribution

Orchard's specifications Grid dimension (30×40), non-plantable spots (NP: 50), separation radius (3), row size (1; single-tree-plot), parents (40), related parents (1, 2), equal distribution, least square deviation (3), empty spots (4), and iterations (10) (Fig. 1a).

Solution (Fig. 1b–d) Results for this case study are presented as follows:

- 1- Nine solutions with empty spots ranging between 1 and 4 were generated (solution #4 is highlighted (Fig. 1b)).
- 2- Parents' information with their respective ID and clone size including *Target* and *Observed Clone Size* (solution #4 (Fig. 1b)).
- 3- Orchard's grid layout with the allocation of parents as follows:
 - (i) The 50 NP Spots are shown (gray squares with NP) along with three empty spots (light yellow centered with E) and the locations of all clones (Fig. 1b).
 - (ii) The spatial arrangement of the *Related Parents* #1 and 2 is presented in red and blue, respectively (see Fig. 1c).
 - (iii) Graphical illustration of parents' *Target* and *Observed Clone Size* and the magnitude of deviation as determined by randomization and least square technique (Fig. 1d).

Case study 2 (Beta): four tree-row-plot, equal distribution

Orchard's specifications Grid dimension (29×40), non-plantable spots (NP: 50), separation radius (3), row size (four tree-row-plot), parents (40), related parents (1, 2 and 4, 6, 7,

New Project Specifications

Orchard's Name:

Orchard Length: Orchard Width:

Separation Radius: Row Size: Parents:

Parents Distribution:

- ☒ Equal
- ☐ Linear Deployment
- ☐ Custom

Maximum Deviation: Iterations:

Maximum Empty Spots: ☐ Extend Row

Leftover: ☐ Relax Radius

Available Spots: NP Spots:

#	Parent Code	Target
1	PC998877001	29
2	PC998877002	29
3	PC998877003	29
4	PC998877004	29
5	PC998877005	29
6	PC998877006	29
7	PC998877007	29
8	PC998877008	29
9	PC998877009	29
10	PC998877010	29
11	PC998877011	29
12	PC998877012	29
13	PC998877013	29
14	PC998877014	29

Start Cancel

a

Alpha [Solution = 4, Parent = 1]

Solution	Empty Spots	Maximum	Minimum	Average	Deviation	MI	CV
2	2	29	27	28	0	118	2
3	3	29	27	28	0	116	2
4	3	29	27	28	0	117.2	2.01
5	3	29	27	28	0	118	2

Parent	1	2	3	4	PC
Parent Code	PC998877001	PC998877002	PC998877003	PC998877004	
Target	29	29	29	29	
Observed	29	28	29	29	

E: Empty Spot NP: Non Plantable Spot

b

Fig. 1 Case study 1 (Alpha) showing: **a** seed orchard's input specification: single-tree-plot, equal clonal representation, two related clones (1 and 2), orchard size (length:width—40:40), and a grid with 50 non-plantable spots (NP), **b** solution #4 showing the distribution of clones

and three empty positions highlighted with yellow, **c** distribution of the related parents (1 and 2), and **c** chart showing the expected (*Target*) and *Observed* clone size

9), equal distribution, least square deviation (5), empty spots (4), extend row, and iterations (10).

Solution (Fig. 2a–c)

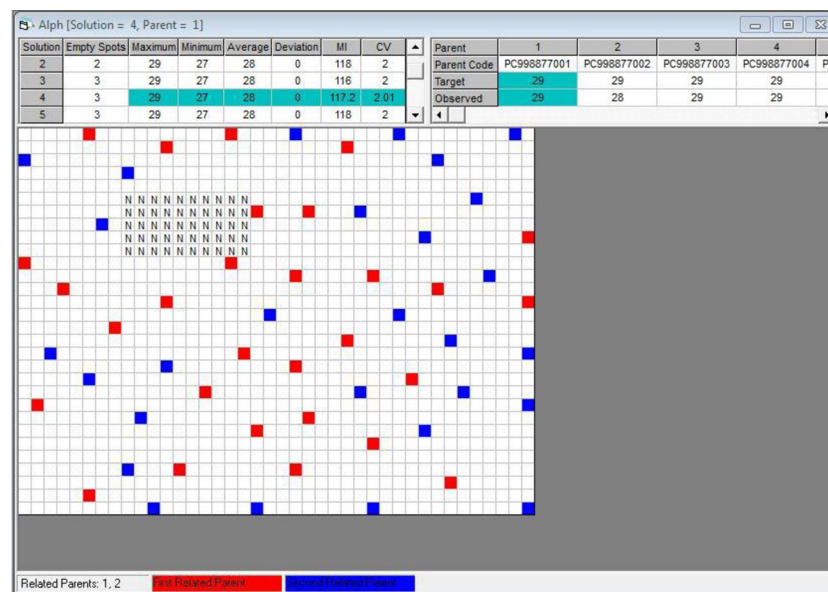
Results for this case study are presented as follows:

1- Ten solutions with no empty spots were generated (solution #9 is highlighted; Fig. 2a).

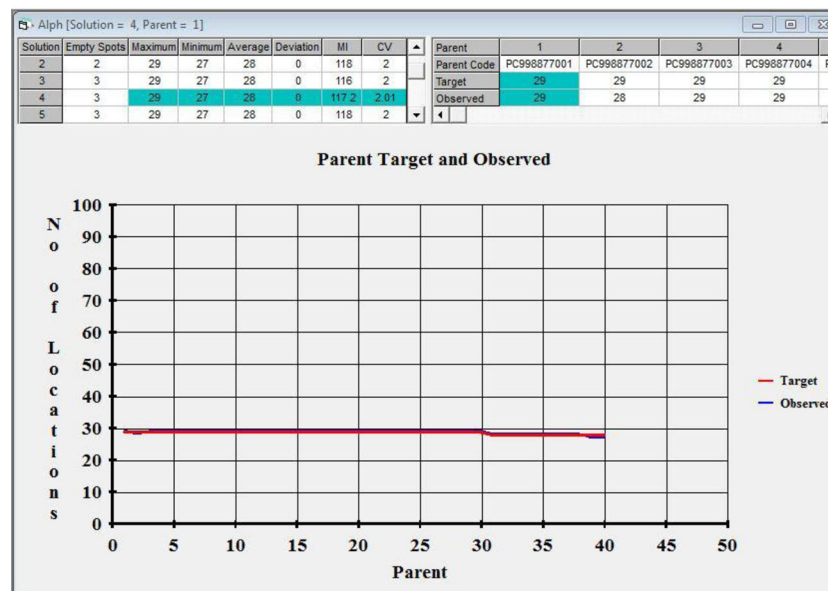
2- Parents' information with their respective ID and clone size including *Target* and *Observed Clone Size* (solution #9; Fig. 2a).

3- Orchard's grid layout with the allocation of parents' clonal-rows across the grid is as follow:

(i) The 50 *NP Spots* (gray squares with NP) and a special case of row extension are illustrated (position 5I):



c



d

Fig. 1 (continued)

- Fig. 2a). This row extension occurred at the end of a row which is adjacent to NP and was manually filled by a ramet of the adjacent clone #27. This situation can also be repeated manually for positions 5K, 5M, 5O and 5Q with ramets from clones #s 38, 36, 14 and 17, respectively) (Fig. 2a).
- (ii) The spatial arrangement of the *Related Parents* #4, 6, 7 and 9 is presented in red, blue, green and brown, respectively (Fig. 2b). Note that each parent is assigned a specific color which was highlighted in the status bar of application.

- (iii) Graphical illustration of parents' *Target* and *Observed Clone Size* and the magnitude of deviation as determined by randomization and least square technique (Fig. 2c).

Case study 3 (Gamma): four tree-row-plot, linear deployment

Orchard's specifications Grid dimension (29×40), non-plantable spots (NP: 50), separation radius (3), row size (four

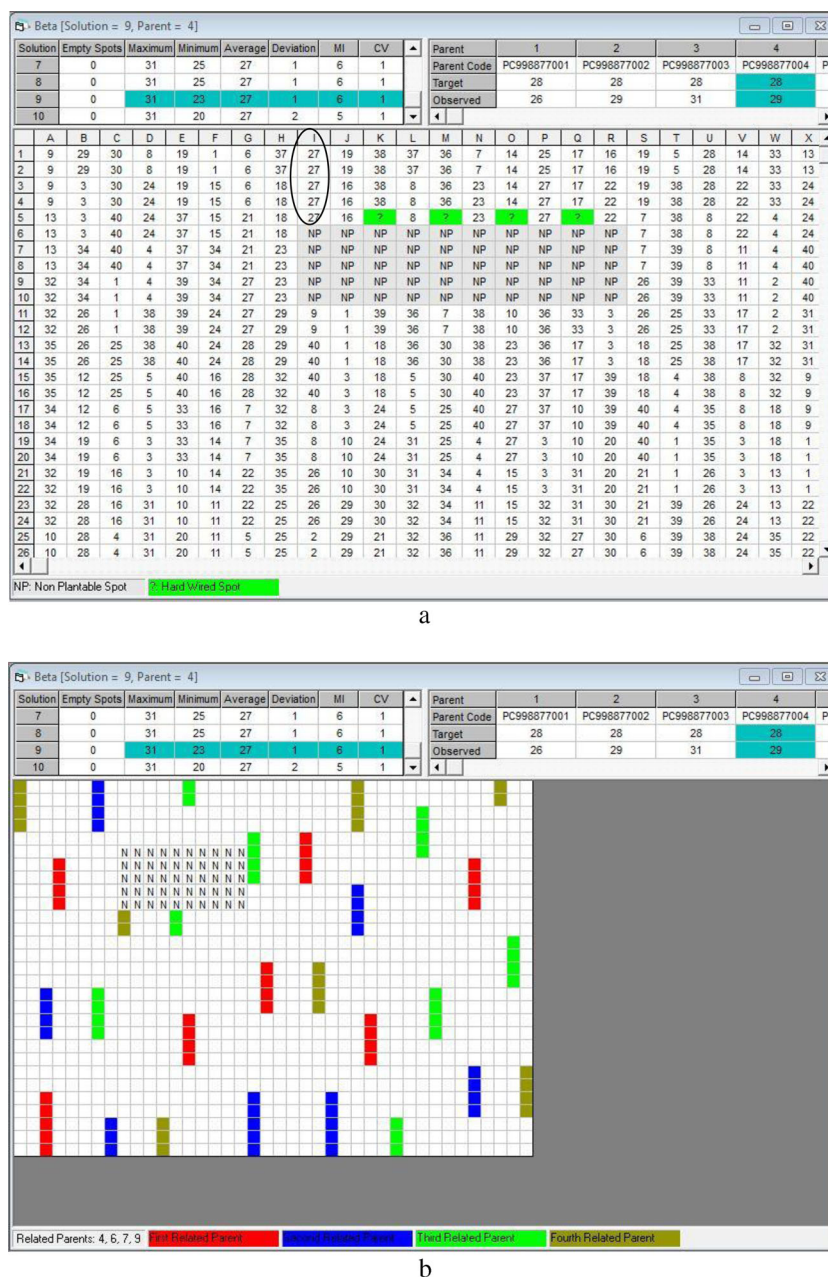


Fig. 2 Solution #9 showing: **a** the distribution of clones in a randomized, replicated, staggered 4-tree-row plots, 50 non-plantable spots, four unallocated spots adjacent to the block of non-plantable spots ("?" in green highlighted squares), and corresponding results, **b** distribution of the

related parents (4, 6, 7, and 9 depicted as red, blue, green, and brown rows, respectively), and **c** chart showing the expected (*Target*) and *Observed* clone size

tree-row-plot), parents (40), linear deployment (slope is determined by the range 2 to 7, where 2 and 7 corresponding to the parents with the lowest and highest parents' breeding value, respectively), least square deviation (5), possible empty spots (4), extend row, and iterations (10). Note: when linear deployment option is selected, parents should be ranked from the lowest to the highest and the slope parameters (lowest and highest values) can be iteratively changed until the desired distribution is reached.

Solution (Fig. 3)

In this case, the demonstration is restricted to graphical illustration of parents' *Target* and *Observed Clone Size* and the magnitude of deviation as determined by randomization and least square technique (Fig. 3). Note: the use of row-plot makes it difficult to reach an exact allocation, so deviation between *Target* and *Observed* often occurs specifically as the clone size increases (Fig. 3).

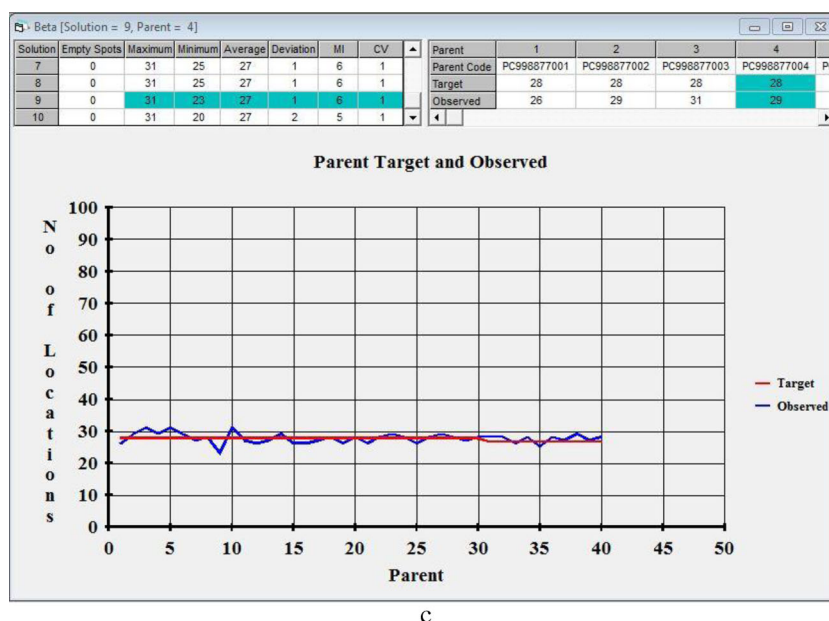


Fig. 2 (continued)

Case study 4 (Delta): custom single-tree-plot, unequal clone size

Orchard's specifications Grid dimension (29×40), two non-contiguous non-plantable spots (NP: 50; Fig. 4a), separation radius (3), single-tree-plot with custom design for 40 parents with unequal clone size (Online Resource 5), least square deviation (5), possible empty spots (10), and iterations (10).

Solution (Fig. 4b) In the case of the variable clone size, the demonstration is restricted to graphical illustration of parents' Target and Observed Clone Size and the location of the ten non-plantable spots (Fig. 4a–b).

The above case studies were provided to demonstrate the various deployment modes (equal, linear, and custom) with different layouts options (related and unrelated and single-tree-plot, clonal-row). It should be noted that in cases where the selected design parameters could not be met, a “No solution is found, use wider scope” message will appear (see solution evaluation in Online Resource 3) and change(s) of the design parameters are needed to reach solutions. These include, the use of smaller separation radius, invoking *Relax Radius* option (see orchard's specifications in Online Resource 3) or changing the layout from single-tree-plot to row-plot. The difference between smaller separation radius and *Relax Radius* is significant as the former reduces the

Fig. 3 Solution #10 showing a chart of the expected (Target) and Observed clone size for a linear deployment

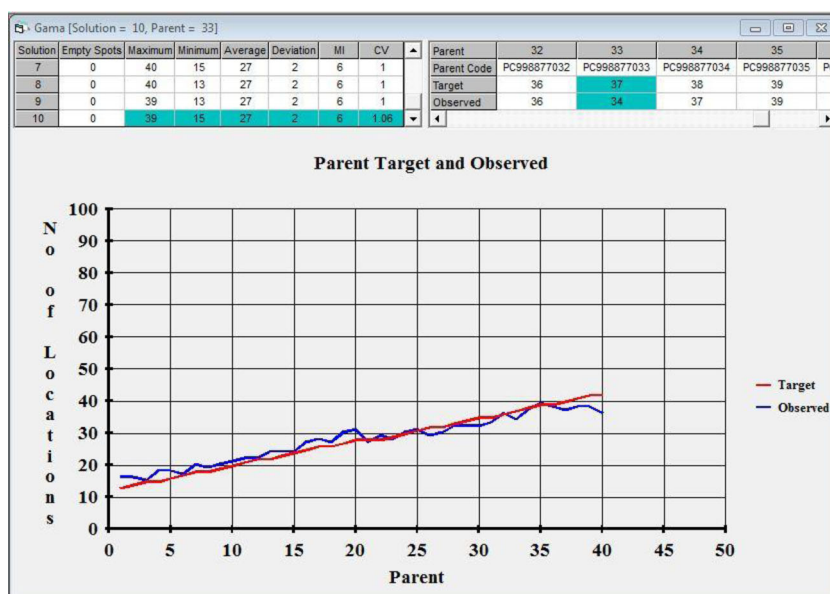
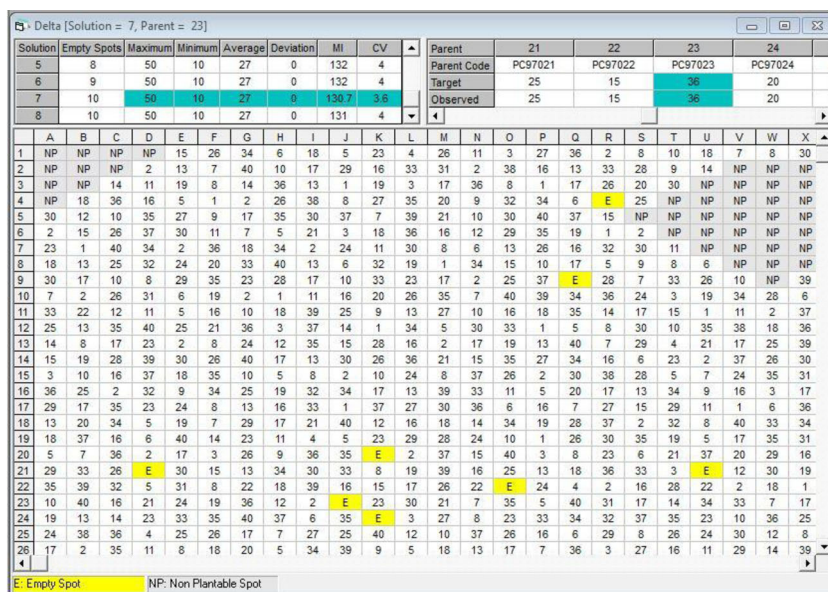
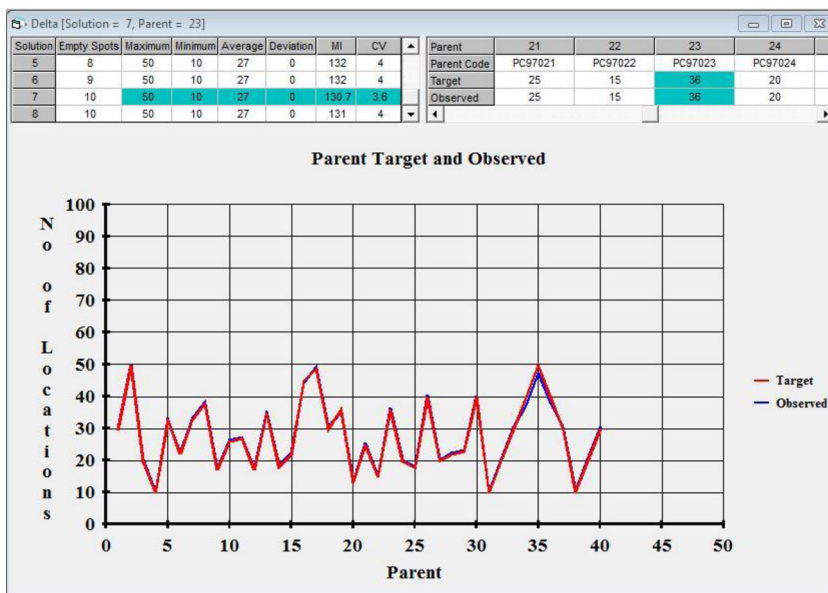


Fig. 4 Solution #7 showing: **a** the distribution of clones in a randomized single-tree-row plot with custom, unequal clone size, two non-contiguous 50 non-plantable spots, 8 out of the 10 non-plantable spots, and corresponding results and **b** chart showing the expected (*Target*) and *Observed* clone size



a



b

distance and hence smaller separation zones around any ramet or a row of ramets with its clonal members while the latter uses the designated separation zone radius while allowing some of the unallocated ramets to be placed closer to other members of the same clone.

The above examples demonstrate the utility of the $R^2\text{SCR}$ design and its effectiveness in meeting seed orchardists' needs. It is important to highlight the fact that while clonal-row deployment is advantageous in accommodating multiple related clones, the potential increase is selfing within rows could be associated with some reduction in potential seed production. Additionally, this design separates ramets and/or rows of ramets of a particular clones as well as randomization

of clones across the orchard's grid, thus it would not be effective if assortative mating is desired for capturing specific combining ability (SCA) between pairs of clones.

Conclusion

The developed $R^2\text{SCR}$ orchard program is versatile offering three deployment modes (equal, linear, and custom) each with unlimited number of layouts for either related or unrelated parental population. The program is exceedingly flexible to accommodate any orchard size and configuration no matter

what the number of non-plantable spots is. Clonal allocation can be either single-tree or even number row-plot with pre-set separation zone between members of the same or related clones. The summary statistics provided (number of empty spots, least square deviation, minimum inbreeding distance, and coefficient of variation) permit ranking the resultant layouts. The built-in options such as extend row and hard-wiring of entering specific clones, all make it easy for layout comparison and selection. Finally, the capability to change the input parameters and save outputs in various formats increases orchards' data utilization facilitating multiple year clonal fecundity, phenology, and health monitoring (Reynolds and El-Kassaby 1990; El-Kassaby and Barclay 1992). Enquiries regarding the program or designing orchards should be directed to YAE.

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Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards The experiments presented in this manuscript comply with the current law of the country in which they were performed.

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