Tutorial on managing hybrid crossing and Line by Tester analysis

This tutorial assumes that the user has a good knowledge of the basic functions and operation of BMS and shows how these functions can be used to facilitate a hybrid breeding program. The user is expected to obtain their own input lists and follow the steps with their own germplasm.

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Introduction

In a typical hybrid breeding program line development follows a traditional pedigree or DH program and when lines are considered reasonable candidates to be tested as hybrids, they are marked as 'fixed' and may be given a code name. In fact, in a pedigree breeding program for lines they may not be genetically fixed at this stage but subsequent inbred generations will be grouped with the first 'fixed' line. These lines are crossed to testers, or to other candidate lines so that the lines and their combining ability can be evaluated through the performance of those candidate hybrids. As the 'fixed' lines are further selected based on the results of the hybrid tests and on other evaluations, the selected descendants are grouped with their earliest fixed ancestor. When crosses are made with hybrid crossing methods between 'fixed' (grouped) lines BMS checks to see if a previous hybrid cross was made between members of the same parental groups and if so, the new cross is grouped with the previous ones. So, all crosses between lines having the same male and female parental groups from a group of hybrids. These groups of hybrids can also be given a code name.

BMS has several methods assigned for producing candidate or more advanced hybrids, and the method IDs of these hybrid crossing methods is customized in the file C:\BMS4\infrastructure\tomcat\webapps\Fieldbook\WEB-INF\classes\crossing.properties. These are set by default to the following methods.

| | | | Hybrid methods |
|-----|------|------------------------------|---|
| ID | Code | Name | Description |
| 321 | VHY | Hybrid formation | Forming a hybrid cultivar |
| 416 | TNL | Narrow based tester, line | Test (Top) cross between a known plant and a narrow based (1 or few plants) population. For practical reasons the tester population or line is used as the male which can be stored pollen. |
| 417 | TNP | Narrow based tester, pop | Test (Top) cross between a known population and a narrow based tester population. For practical reasons the tester population or line is used as the male which can be stored pollen. |
| 418 | TBL | Broad based tester, line | Test (Top) cross between a known plant and a broad based (many plants) tester. For practical reasons the tester population is used as the male which can be stored pollen. |
| 419 | ТВР | Broad based tester, pop | Test (Top) between a known population and a broad based tester. For practical reasons the tester population is used as the male which can be stored pollen. |
| 426 | TCR | Test cross | Crosses made for generating hybrids for early generation testing. The females can be lines in any generation of inbreeding or DH lines, and the male is usually a fixed inbred line, or a F1 single cross between two fixed inbred lines. |

More methods could be added and then added to the configuration file.

Fixing and coding some inbred lines

For this tutorial, pick any list with five inbred lines and mark then as fixed.

| Lis | st De | tails | | | | Show List Build |
|------|---------------------------------|---|--|---|------|---|
| vse | or sear | ch for a list to wor | k with. | | | Close All |
| VT5 | 00 × | | | | | |
| L | ist e | ntries | | | 1 | Inventory view |
| otal | Entrie | s: 5 Selected: : | 5 CROSS | | LOTS | List editing options Export list |
| otal | Entrie # | s: 5 Selected: . DESIGNATION CL02101-RR1 | 5 CROSS Pob21C5HC57-1 | Assign codes | LOTS | List editing options Export list Coding and Fixing Options |
| otal | Entrie # 1 2 | s: 5 Selected: : DESIGNATION CL02101-RR1 CL02102-RR1 | 5 CROSS Pob21C5HC57-1 Pob21C5HC78-3 | Assign codes Mark lines as fixed | LOTS | List editing options Export list Coding and Fixing Options Remove selected germplasm |
| otal | Entrie # 1 2 3 | s: 5 Selected: : DESIGNATION CL02101-RR1 CL02102-RR1 CL02103-RR1 | 5 CROSS Pob21C5HC57-1 Pob21C5HC78-3 Pob21C5HC84-2-1 | Assign codes Mark lines as fixed -8-3-B | LOTS | List editing options Export list Coding and Fixing Options Remove selected germplasm |
| otal | Entrie # 1 2 3 4 | s: 5 Selected: 3 DESIGNATION CL02101-RR1 CL02102-RR1 CL02103-RR1 CL02104-RR1 | 5 CROSS Pob21C5HC57-1 Pob21C5HC78-3 Pob21C5HC84-2-1 Pob21C5HC109-3- | Assign codes Mark lines as fixed -B-3-B 1-5-4-B-1-#-BB-1-B | LOTS | List editing options Export list Coding and Fixing Options Remove selected germplasm - E0003 - E0004 |

Check the options "Preserve existing group" and "Include descendants".

Preserve existing Group means that if the line you are fixing is already fixed don't start a new group but leave the line in the group it currently has. If this is unchecked the line would start a new group. This

could be desirable if you decided that the original line had not been ready to be fixed and you had to do some purification and start the grouping again.

Include Descendants is simply an option in case the line you are grouping has already been advanced (not usual) in which case you have the choice to bring those advances into the group as well. Normally you would want to, so again the default seems the less usual case.

| SS 21C5HC57-1-2- 21C5HC78-3-4- 21C5HC84-2-1- 21C5HC109-3-1-5-4-B-1-#-BB-1-B | | Mark lines as | fixed | × | | |
|--|---------------|---|----------|---|------|----|
| SS 21C5HC57-1-2- 21C5HC78-3-4- 21C5HC84-2-1- 21C5HC109-3-1-5-4-B-1-#-BB-1-B | | Preserve existing Preserve e | ng group | | L | |
| Cancel Continue 21C5HC78-3-4- 21C5HC84-2-1- 21C5HC109-3-1-5-4-B-1-#-BB-1-B | SS | and include descen | udints. | | LOTS | AV |
| 21C5HC78-3-4- 21C5HC84-2-1- 21C5HC109-3-1-5-4-B-1-#-BB-1-B | 21C5HC57-1-2- | Cancel | Continue | | | |
| 21C5HC84-2-1- 21C5HC109-3-1-5-4-B-1-#-BB-1-B | 21C5HC78-3-4- | | | | | |
| 21C5HC109-3-1-5-4-B-1-#-BB-1-B | 21C5HC84-2-1- | | | | | |
| | 21C5HC109-3-1 | -5-4-B-1-#-BB-1-B | _ | | - | |
| 21C5HC133-1-B-##-B | 21C5HC133-1-B | -##-B | | | | |

Next assign codes by going to the Assign code item in the Coding and Fixing options sub menu.

Codes are names assigned to lines at a certain level of development. These become the preferred name for the lines and are inherited as the preferred names of descendants in the same group. There are three levels of codes which can be assigned at different stages of development and each level supersedes the other as the preferred name for the group as it is assigned to any member of the group.

Enter a prefix which reflects the project producing the germplasm (CGM) and the type of germplasm (L for line). A sequence number reflecting the next code with the specified prefix will be added to produce the code name.

| Please specify | codes | × |
|-----------------------|-----------------------------------|---|
| Coding Level: | Level 1 Level 2 Level 3 | |
| Prefix CGML | SEQ | |
| Example: CGML[SEQ] | I | |
| | Cancel Apply Codes | |

You can see the codes applied by filling the ENTRY CODE column with preferred name (right-click on the ENTRY CODE header).

🕄 List Details

Browse or search for a list to work with.

| | | | 1 / | ENTRY_CODE | GID GROUD ID STOCKID SEE |
|---|-------------|----------------------------------|-----|------------|--------------------------|
| 1 | CL02101-RR1 | Pob21C5HC57-1-2-B-## | | CGML1 | Make Column Empty |
| 4 | CL02104-RR1 | Pob21C5HC109-3-1-5-4-B-1-#-BB-1- | | CGML2 | Fill With Preferred ID |
| 5 | CL02105-RR1 | Pob21C5HC133-1-B-##-B | | CGML3 | Fill With Germplasm Date |
| 2 | CL02102-RR1 | Pob21C5HC78-3-4-B-#-B | | CGML4 | Fill With Preferred Name |
| 3 | CL02103-RR1 | Pob21C5HC84-2-1-B-3-B | | CGML5 | Fill With Attribute |

< Show List Buil

For this tutorial, you also need a set of tester lines which should also be 'Fixed' (grouped). For example, the CML lines.

Make some hybrid test crosses

Create a list for the crossing block with five testers (CML lines in this example) and our five inbreds. Notice that the inbreds appear with their codes in the new list since these are now the preferred names of these lines.

| Lis | t De | tails rch for a list to work | with. | > Hide List Builder Close All Tabs | Build or | ld a revise | New List your list by draggi | ng in entries from the left. | |
|-------|--------|---------------------------------|-----------------------------------|---------------------------------------|----------|----------------|---------------------------------|------------------------------|----------|
| CML | Lines | 2 × AVT500 × | | | | | | | |
| ΞL | ist e | ntries | | ¢ ACTIONS | iii u | ist e | ntries | | Edit Lis |
| Total | Entrie | s: 5 Selected: 5 | | 6 / 1 | Total | Entrie | s: 10 Selected: 0 | | ۰. |
| 1 | # | DESIGNATION | CROSS | II | 1 | # | DESIGNATION | CROSS | LOTS |
| | 1 | CL02101-RR1 | Pob21C5HC57-1-2-B-## | | | 1 | CML320 | P501c0F112-2-5-1-1-B*4 | 1 |
| | 2 | CL02102-RR1 | Pob21C5HC78-3-4-B-#-B | | | 2 | CML360 | SA4C2HC(21X26)-1-2-2-8 | 1 |
| | 3 | CL02103-RR1 | Pob21C5HC84-2-1-B-3-B | | | 3 | CML370 | MBR-ET(W)F2-14-3-1-3-B-B-B | 1 |
| | 4 | CL02104-RR1 | Pob21C5HC109-3-1-5-4-B-1-#-BB-1-B | | | 4 | CML432 | KTX3752F2-7-1-1-1-B-B-B | 1 |
| | 5 | CL02105-RR1 | Pob21C5HC133-1-B-##-B | | | 5 | CML444 | P43C9-1-1-1-1-BBBB | 1 |
| | | | | | | 6 | CGML1 | Pob21C5HC57-1-2-B-## | |
| | | | | | | 7 | CGML4 | Pob21C5HC78-3-4-B-#-B | |
| | | | | | | 8 | CGML5 | Pob21C5HC84-2-1-B-3-B | |
| | | | | | | 9 | CGML2 | Pob21C5HC109-3-1-5-4-B-1-i | |
| | | | | | | 10 | CGML3 | Pob21C5HC133-1-B-##-B | |

We are going to use a matrix crossing layout to facilitate the crossing as follows:

| Male Testers | Female Line 1 | Female Line 2 | Female Line 3 | Female Line 4 | Female Line 5 |
|-----------------|-----------------|-----------------|-----------------|-----------------|------------------|
| Plot 1 Entry 1 | Plot 2 Entry 6 | Plot 3 Entry 7 | Plot 4 Entry 8 | Plot 5 Entry 9 | Plot 6 Entry 10 |
| Plot 7 Entry 2 | Plot 8 Entry 6 | Plot 9 Entry 7 | Plot 10 Entry 8 | Plot 11 Entry 9 | Plot 12 Entry 10 |
| Plot 13 Entry 3 | Plot 14 Entry 6 | Plot 15 Entry 7 | Plot 16 Entry 8 | Plot 17 Entry 9 | Plot 18 Entry 10 |
| Plot 19 Entry 4 | Plot 20 Entry 6 | Plot 21Entry 7 | Plot 22 Entry 8 | Plot 23 Entry 9 | Plot 24 Entry 10 |
| Plot 25 Entry 5 | Plot 26 Entry 6 | Plot 27 Entry 7 | Plot 28Entry 8 | Plot 29 Entry 9 | Plot 30 Entry 10 |

Translate this table into a csv file as follows. This layout file can be used whenever the same layout is required (CGMTCLayout.csv). Any layout design can be accommodated in this way.



You should do seed preparation. Start by reserving seed by switching to inventory view and using the menu item Inventory management options > Reserve inventory. Save the reservations, Print labels, Export the seed preparation list, pack the seed into sufficient packets dictated by the layout and then Import the seed preparation list to update the inventory.

MANAGE GERMPLASM Ø

| GM1 | 7ТСВ | List × | | | | | | |
|------|-------|-------------|-------------|---------|--------------------|------|-----------------------------------|------------------|
| otal | Lots: | Selected: | 0 | | | | Return to List List editing op | entries view |
| 1 | # | DESIGNATION | LOCATIO | Rese | erve inventory | | Inventory ma | nagement options |
| | 1 | CML320 | Default S | Save | changes | | Export Seed p | reparation list |
| | 2 | CML360 | Default S | Cano | cel Reservations | | Import Seed p | preparation list |
| | 3 | CML370 | Default S | Clos | e lots | | Print Labels | |
| | 4 | CML432 | Default See | d Store | 53.19999999999998g | 0.0g | 0.0g | From Li |
| | 5 | CML444 | Default See | d Store | 54.39999999999998g | 0.0g | 0.0g | From Li |

Create a crossing block nursery with the Nursery Manager. Add the Management Details (Project_prefix, Target_Region, LOCATION_ABBR, Crop_season_code) that you want to be part of your cross codes. Add the list you created. Import the design from file CGMTCLayout.csv you made above and save the

Nursery. (You must do the whole process without saving to avoid a bug in importing the design in v6 - fixed in V6.2).

| Create Nurse | ery Save | | | | Return to Manage Nur | |
|--|---|---------------|--|---|---------------------------------|--|
| ASIC DETAILS indicates a mandator | v field | | | | Act | |
| ursery name: * | cription: * CGM17TCB 2017 Test cross production for project CG | | Save in: * | | Import Crosses Import Design | |
| escription: * | | | Created by: * | Christopher Mo | Laren | |
| bjective: | 2017 Test cross production for proj | ject | Creation date: * | 2017-07-28 | | |
| | CGM | | Completion date: | yyyy-mm-dd | III bt | |
| Use a previously cr | eated nursery as a template | nts | | | | |
| Use a previously cr | eated nursery as a template Germplasm & Checks Measureme DETAILS @ | Add | 🍝 NURSERY CONI | | | |
| Use a previously or ursery Settings | eated nursery as a template Sermplasm & Checks Measureme DETAILS C Christopher McLaren | Add | NURSERY CONI | DITIONS 🕝 | ord in this section. | |
| Use a previously cr ursery Settings MANAGEMENT PLNAME: Project_Prefix: | eated nursery as a template Sermplasm & Checks Measureme DETAILS Christopher McLaren Project Prefix 2 | Add v | NURSERY CONI Click Add to begin sele Click Add to Begin Sele | DITIONS 🕢 | ord in this section. | |
| Use a previously cr rrsery Settings (MANAGEMENT PI_NAME: Project_Prefix: Target_Region: | eated nursery as a template Germplasm & Checks Measureme DETAILS Christopher McLaren Project Prefix 2 Region 3 | Add | NURSERY CONI Click Add to begin sele TRAITS @ Trait | DITIONS @ ecting items to reco Descri | ord in this section. | |
| Use a previously cr ursery Settings (MANAGEMENT PI_NAME: Project_Prefix: Target_Region: LOCATION_ABBI | eated nursery as a template Germplasm & Checks Measureme DETAILS Christopher McLaren Project Prefix 2 Region 3 H | Add v v | NURSERY CONI Click Add to begin sele TRAITS @ Trait | DITIONS 🕢 | ord in this section. | |

Now export the crossing template. Fill in the description sheet:

| UL | | J. | CUTI | 10000100000101 | project com |
|----|------|--------|------|----------------|-------------|

| | A | В | С |
|---|------------------|-----------------------------------|---|
| 1 | LIST NAME | CGM17TC List | |
| 2 | LIST DESCRIPTION | 2017 Test crosses for project CGM | |
| 3 | LIST DATE | 20170728 | |

And add the crossing plan to the observation sheet:

| 1 | A | В | С | D | E | F |
|----|-------------|--------------|-----------|-----------------|---------------|-------|
| 1 | FEMALE PLOT | MALE NURSERY | MALE PLOT | BREEDING METHOD | CROSSING DATE | NOTES |
| 2 | 2 | | 1 | TCR | 20170728 | |
| 3 | 3 | | 1 | TCR | 20170728 | |
| 4 | 4 | | 1 | TCR | 20170728 | |
| 5 | 5 | | 1 | TCR | 20170728 | |
| 6 | 6 | | 1 | TCR | 20170728 | |
| 7 | 8 | | 7 | TCR | 20170728 | |
| 8 | 9 | | 7 | TCR | 20170728 | |
| 9 | 10 | | 7 | TCR | 20170728 | |
| 10 | 11 | | 7 | TCR | 20170728 | |
| 11 | 12 | | 7 | TCR | 20170728 | |
| 12 | 14 | | 13 | TCR | 20170728 | |
| 13 | 15 | | 13 | TCR | 20170728 | |
| 14 | 16 | | 13 | TCR | 20170728 | |
| 15 | 17 | | 13 | TCR | 20170728 | |
| 16 | 18 | | 13 | TCR | 20170728 | |
| 17 | 20 | | 19 | TCR | 20170728 | |
| 18 | 21 | | 19 | TCR | 20170728 | |
| 19 | 22 | | 19 | TCR | 20170728 | |
| 20 | 23 | | 19 | TCR | 20170728 | |
| 21 | 24 | | 19 | TCR | 20170728 | |
| 22 | 26 | | 25 | TCR | 20170728 | |
| 23 | 27 | | 25 | TCR | 20170728 | |
| 24 | 28 | | 25 | TCR | 20170728 | |
| 25 | 29 | | 25 | TCR | 20170728 | |
| 26 | 30 | | 25 | TCR | 20170728 | |

This crossing plan can be copied into the crossing template of future test cross nurseries which use the same layout. Fill in the date that the crosses are made and any notes before Importing the crosses. Use the method TCR (Test cross) specified in the template, but check 'Apply grouping to new crosses only' as in the screenshot below. This means that if the cross has been made before with a hybrid crossing method between lines in the same parental inbred groups and therefore grouped then that group will be preserved and the new cross added to it. Only new crosses which have not been made before will start new groups.

Specify Breeding Method

By default, the breeding method for new crosses will be based on the status of their parental lines. As an alternative, you can select a method to use for all crosses.

- Use the breeding methods specified in the import file
- Use parental status
- Select a method to use for all crosses :

| | ٣ | 0 |
|------------------------------------|----|--------------------|
| All methods | ۲ | Generative methods |
| Show only favorite methods | Ma | anage Methods |
| Apply grouping to new crosses only | | |
| | | |

| Back | Continue |
|------|----------|
| | |

You can see the crosses which have been imported and that they are grouped. The cross names have been constructed following the [CIMCRS] rules as Projecrt_Prefix + Target_Region + Crop_season_Code + LOCATION_ABBR+[SEQUENCE] and this is customizable.

| Vursery | : CGM17TCB | Save | | | | | | | R | eturn to Manage N | Jurserie |
|------------------|---------------------|---------------|------------------|--------------|-------------|--------------|-------------|---------------|----------|-------------------|----------|
| BASIC DET | AILS | | | | | | | | | | Actions |
| Nursery Settin | gs Germplasm | & Checks Expe | rimental Design | Measurements | Imported Cr | osses: [CGM1 | 7TC List] 🗙 | | | | |
| Cross List: CG | M17TC List | N | lotes: | | | | | | | View Lis | st Detai |
| Total Entries: 2 | 5 Selected: 0 | | | | | | | | | Cross List | Actions |
| 100 × s | howing 1 to 25 of 2 | 5 entries | | | | | | | | | |
| ENTRY_NO | DESIGNATION | CROSS | FEMALE PARENT | FGID | MALE PARENT | MGID | GID | SEED_SOURCE | GROUP ID | DUPLICATE | |
| 1 | PBR317MH1 | CGML1/CML320 | CGML1 | 13543 | CML320 | 16 | 13667 | CGM17TCB:2/CG | 13667 | | |
| 2 | PBR317MH2 | CGML4/CML320 | CGML4 | 13544 | CML320 | 16 | 13668 | CGM17TCB:3/CG | 13668 | | |
| 3 | PBR317MH3 | CGML5/CML320 | CGML5 | 13545 | CML320 | 16 | 13669 | CGM17TCB:4/CG | 13669 | | |
| 4 | PBR317MH4 | CGML2/CML320 | CGML2 | 13546 | CML320 | 16 | 13670 | CGM17TCB:5/CG | 13670 | | |
| 5 | PBR317MH5 | CGML3/CML320 | CGML3 | 13547 | CML320 | 16 | 13671 | CGM17TCB:6/CG | 13671 | | |
| 6 | PBR317MH6 | CGML1/CML360 | CGML1 | 13543 | CML360 | 1480 | 13672 | CGM17TCB:8/CG | 13672 | | |
| 7 | PBR317MH7 | CGML4/CML360 | CGML4 | 13544 | CML360 | 1480 | 13673 | CGM17TCB:9/CG | 13673 | | |
| | | | | | | | | | | | |

You should continue to add inventory by one of the available methods for the new test hybrids.

Make a Line x Tester Trial

First you must add the line and tester names to the list containing the test cross germplasm from our nursery. To do this in the GERMPLASM MANAGER, right-click on the ENTRY CODE column header and fill it with Cross-Female Preferred Name. In the same way fill the SEED SOURCE column with the Cross-Male Preferred Name.

×

MANAGE GERMPLASM @

View Lists View Germplasm

🕄 List Details

Browse or search for a list to work with.

| CG | M1 | 71 | C | Lis | t | × |
|----|----|----|---|-----|---|---|
| | | | | | | |

List entries

Total Entries: 25 Selected: 0

| 1 | # | DESIGNATION | CROSS | LOTS | AVAILABLE | ENTRY_CODE | CID | CROUPID | | D SOURCE |
|---|----|-------------|--------------|------|---------------------|---------------------|---------------------------------------|------------------------------------|----------|-------------|
| | 1 | PBR317MH1 | CGML1/CML320 | - | | 1 | Mak | e Column Empty | | CB:2/CO |
| | 2 | PBR317MH2 | CGML4/CML320 | - | | 2 | Fill V | Vith Preferred ID | | CB:3/CG |
| | 3 | PBR317MH3 | CGML5/CML320 | - | - | 3 | Fill V | Vith Germplasm Da | ate | CB:4/CO |
| | 4 | PBR317MH4 | CGML2/CML320 | | - | 4 | Fill With Preferred Name | | CB:5/CG | |
| | 5 | PBR317MH5 | CGML3/CML320 | - | | 5 | Fill V | Vith Attribute | | CB:6/CC |
| | 6 | PBR317MH6 | CGML1/CML360 | c | Take sector income | 6 | Fill With Breeding Method Information | | | CB:8/CG |
| | 7 | PBR317MH7 | CGML4/CML360 | | Fill with Cross-Fem | nale GID | Fill v | Fill with Cross-Female Information | | CB:9/CG |
| | 8 | PBR317MH8 | CGML5/CML360 | | Fill with Cross-Fem | nale Preferred Name | Fill v | vith Cross-Male Inf | ormation | CB:10/C |
| | 9 | PBR317MH9 | CGML2/CML360 | - | - | 9 | Fill v | vith Sequence Nun | nber | CB:11/C |
| | 10 | PBR317MH10 | CGML3/CML360 | - | - | 10 | 13676 | 13676 | CGI | M17TCB:12/C |
| | 11 | PBR317MH11 | CGML1/CML370 | - | | 11 | 13677 | 13677 | CGI | M17TCB:14/C |
| | 12 | PBR317MH12 | CGML4/CML370 | | | 12 | 13678 | 13678 | CGI | M17TCB:15/C |
| | 13 | PBR317MH13 | CGML5/CML370 | - | 100 | 13 | 13679 | 13679 | CGI | M17TCB:16/C |
| | 14 | PBR317MH14 | CGML2/CML370 | - | - | 14 | 13680 | 13680 | CGI | M17TCB:17/C |

The list now looks like this:

🕄 List Details

Browse or search for a list to work with.

| CGM | 17TC L | ist × | | | | | | | |
|---------------------|----------------------|-----------------|--------------|----|------------|-------|----------|-------------|---------|
| i I | ist e | ntries | | | | | | • | ACTIONS |
| <mark>Fot</mark> al | <mark>Entr</mark> ie | s: 25 Selected: | 0 | | | | | | 6 / 🕯 |
| 1 | # | DESIGNATION | CROSS | 17 | ENTRY_CODE | GID | GROUP ID | SEED_SOURCE | Ш |
| | 1 | PBR317MH1 | CGML1/CML320 | | CGML1 | 13667 | 13667 | CML320 | * |
| | 2 | PBR317MH2 | CGML4/CML320 | | CGML4 | 13668 | 13668 | CML320 | |
| | 3 | PBR317MH3 | CGML5/CML320 | | CGML5 | 13669 | 13669 | CML320 | |
| | 4 | PBR317MH4 | CGML2/CML320 | | CGML2 | 13670 | 13670 | CML320 | |
| | 5 | PBR317MH5 | CGML3/CML320 | | CGML3 | 13671 | 13671 | CML320 | |
| | 6 | PBR317MH6 | CGML1/CML360 | | CGML1 | 13672 | 13672 | CML360 | |
| | 7 | PBR317MH7 | CGML4/CML360 | | CGML4 | 13673 | 13673 | CML360 | |
| 1000 | 0 | 0000471440 | | | 00111 | 1000 | 40074 | 010000 | |

< Show List Builder

In the TRIAL MANAGER, start a new trial (CGM17LXT) add the same Trial Settings as for the Nursery. On the GERMPLASM tab add ENTRY CODE (change its name to LINE) and SEED SOURCE (change its name to TESTER) variables to the germplasm variable list. Add the list of test crosses.

| Settings | Germplasm | Environments | Experimental Design | Measurements | | | |
|------------|-------------------|--------------|-------------------------|---------------------|----|----------|---------------|
| ▼ Defin | ne Germplasm | Details | | A | dd | | |
| GERM | IPLASM DESCR | IPTORS 🕜 | | | | - | - |
| Nam | | | Description | | | | |
| INdf | | | Entry type (test/chec | k) accigned (type) | | | |
| CID | KI_IIPC | | Cormplasm identifie | r, assigned (DPID) | | | |
| DES | | | Germplasm identifie | r - assigned (DBCV) | | | |
| ENT | RY NO | | Germplasm entry - e | numerated (number) | | | |
| PLO | T ID | | Field plot id - assigne | d (text) | | | |
| | | | Germplasm ID - Assig | gned (Code) | | | |
| TEST | TER | | Seed source - Selecte | ed (Code) | | | |
| Select | All Remove | | | | | | |
| Tria | lliet | | | | | | |
| Browse a | list to work with | | | | | | |
| Fotal Entr | ies: 25 View | Header | | | | | |
| ENTRY_T | YPE | GID | DES | GIGNATION | | ENTRY_NO | ENTRY_NO LINE |
| Test entr | у | 13697 | PB | R317MH26 | | 1 | 1 CGML1 |
| Test entr | у | 13698 | PB | R317MH27 | | 2 | 2 CGML4 |
| Test entr | у | 13699 | PB | R317MH28 | | 3 | 3 CGML5 |
| Test entr | у | 13700 | PB | R317MH29 | | 4 | 4 CGML2 |
| Test entr | у | 13701 | PB | R317MH30 | | 5 | 5 CGML3 |
| Test entr | У | 13702 | PB | R317MH31 | | 6 | 6 CGML1 |

Add one environment with a LOCATION_NAME and whatever Trial Conditions you usually record (irrigation, rainfall, fertilizer etc.).

Generate an appropriate design and add traits you want to measure. Collect the data.

Analyse the Line x Tester Trial

Prepare the standard SSA analysis for the design you chose. You can analyse this preparation and it will do the usual analysis ignoring the Line x Tester decomposition. The obtain the Line x Tester analysis you must edit the Breeding View project xml file. Simply insert the text in red into the xml file with a text editor. The run the Breeding View analysis again.

```
<?xml version="1.0" encoding="UTF-8" standalone="yes"?>
<Session>
    <IBWS InputDataSetId="25470" OutputDataSetId="0"

<
             <Pipeline type="SEA">
                 <DataConfiguration>
                      <Design type="Resolvable incomplete block
design">
                          <Blocks name="BLOCK_NO"/>
                          <PlotNo name="PLOT NO"/>
                          <Replicates name="REP NO"/>
                      </Design>
                      <Environments name="LOCATION_NAME">
                          <Environment active="true" name="ART
FARM; HARARE"/>
                     </Environments>
<Traits>
                           <Trait active="true" name="GY_Adj_tha"/>
                     </Traits>
                 </DataConfiguration>
             </Pipeline>
        </Pipelines>
    </BreedingViewProject>
</session>
```

I generated data with the following parameters:

| LINES | LValue | TESTERS | Tvalue | CROSSES | Gvalue |
|-------|--------|----------|--------|---------|--------|
| CGML1 | 1 | CML320 | 1 | 1 | 3 |
| CGML2 | 2 | CML360 | 2 | 2 | 0 |
| CGML3 | 3 | CML370 | 3 | 3 | 0 |
| CGML4 | 4 | CML432 | 4 | 4 | 0 |
| CGML5 | 5 | CZL99029 | 5 | 5 | -3 |
| | | | | 6 | 0 |
| | | | | 7 | 3 |
| | | | | 8 | 0 |
| | | | | 9 | -3 |
| | | | | 10 | 0 |
| | | | | 11 | 0 |
| | | | | 12 | 0 |
| | | | | 13 | 0 |
| | | | | 14 | 0 |
| | | | | 15 | 0 |
| | | | | 16 | 0 |
| | | | | 17 | -3 |
| | | | | 18 | 0 |
| | | | | 19 | 3 |
| | | | | 20 | 0 |
| | | | | 21 | -3 |
| | | | | 22 | 0 |
| | | | | 23 | 0 |
| | | | | 24 | 0 |
| | | | | 25 | 3 |

The standard analysis report from Breeding View is not very different from the usual SSA analysis, except that is shows the significance of the LINE, TESTER and LINExTESTER effects:

Estimated generalized heritability

| Term | Heritability |
|-------------|--------------|
| LINE | 0.4012 |
| LINE.TESTER | 0.6679 |

Tests for combinability effects

| | Deviance | d.f. | Chi pr. |
|--------------|----------|------|---------|
| | | | |
| LINE | 8.04 | 1 | 0.005 |
| LINE.TESTER: | 29.35 | 1: | <0.001 |

Wald/F tests when genotypes modelled as fixed effects

| Term | Wald statistic | n.d.f. | F statistic | d.d.f. | F pr. |
|-------------|----------------|--------|-------------|--------|---------|
| REP_NO | 0.2 | 2 | 0.12 | 48 | 0.887 |
| TESTER | 58.5 | 4 | 14.62 | 48 | < 0.001 |
| LINE | 55.0 | 4 | 13.76 | 48 | < 0.001 |
| TESTER.LINE | 121.0 | 16 | 7.56 | 48 | <0.001 |

However the report on the Output tab is more informative and has estimates of GCA:

LINE BLUPs (GCA effects)

| | GCA | s.e. |
|-------|---------|--------|
| LINE | | |
| CGML3 | -0.1173 | 0.8648 |
| CGML4 | 0.4748 | 0.8655 |
| CGML1 | -0.6611 | 0.8652 |
| CGML2 | -0.5714 | 0.8651 |
| CGML5 | 0.8750 | 0.8648 |

and SCA:

LINE.TESTER BLUPs (SCA effects)

| TESTER | CML370 | | CML320 | |
|--------|--------|-------|--------|-------|
| | SCA | s.e. | SCA | s.e. |
| LINE | | | | |
| CGML3 | -0.440 | 1.605 | -3.221 | 1.607 |
| CGML4 | 0.072 | 1.609 | 1.196 | 1.603 |
| CGML1 | -2.982 | 1.608 | 1.952 | 1.608 |
| CGML2 | 0.426 | 1.604 | -0.810 | 1.602 |
| CGML5 | 2.925 | 1.615 | 0.884 | 1.608 |
| TESTER | CML360 | | CML432 | |
| | SCA | s.e. | SCA | S.e. |
| LINE | | | | |
| CGML3 | 0.770 | 1.607 | -1.652 | 1.609 |
| CGML4 | 2.862 | 1.605 | -3.429 | 1.604 |

... table continued.

If you rearrange the SCA table to match the crossing layout you can see that the SCA values used in generating the data were reasonably well recovered (except for cross 13 in the middle).

| TESTER | | LINE | CGML1 | CGML4 | CGML5 | CGML2 | CGML3 |
|----------|------|------|--------|--------|--------|--------|--------|
| CML320 | SCA | | 1.952 | 1.196 | 0.884 | -0.81 | -3.221 |
| | s.e. | | 1.608 | 1.603 | 1.608 | 1.602 | 1.607 |
| CML360 | SCA | | -1.01 | 2.862 | 0.507 | -3.129 | 0.77 |
| | s.e. | | 1.607 | 1.605 | 1.604 | 1.605 | 1.607 |
| CML370 | SCA | | -2.982 | 0.072 | 2.925 | 0.426 | -0.44 |
| | s.e. | | 1.608 | 1.609 | 1.615 | 1.604 | 1.605 |
| CML432 | SCA | | 1.354 | -3.429 | 2.057 | 1.67 | -1.652 |
| | s.e. | | 1.602 | 1.604 | 1.608 | 1.607 | 1.609 |
| CZL99029 | SCA | | -3.626 | 2.397 | -0.665 | -1.885 | 3.779 |
| | s.e. | | 1.602 | 1.607 | 1.604 | 1.605 | 1.605 |

Make a second generation of test crosses using the Cross Design Tool

Advance the selected lines and the testers

Although the GCA values are not significantly different they have been estimated in the same order that they were supplied as parameters, so deciding to keep CGML4 and CGML5 would not have been a bad decision. Although normally you would have separate nurseries to advance lines we can illustrate the process using the crossing block. You might use this nursery to bulk new seed for the tester lines (which seems reasonable). We will also use it do demonstrate the advance of the lines CGML4 and CGML5 (which is less practical). To do this add the variables NPSEL and BM_CODE_VTE to the Selection group on

the Nursery Settings Page. Add 1 to the variable NPSEL for the male plots (CML lines) and for the first plots containing CGML4 and CGML5 which we wish to advance. Put the method code Seed Increase Bulk (NBK) in the variate BM_CODE_VTE next to the selected male plots, and Selected Bulk (DSB) next to the plots for CGML4 and CGML5 using the pull down list.

| ► B | ASIC DETAILS | SIVIT/TCB Save | | | | | |
|-------|--------------|----------------------------|----------------------------|--------|--------------|--------|----------------------------|
| lurse | ry Settings | Germplasm & Checks Exp | e <mark>rime</mark> ntal [| Design | Measurements | Import | ed Crosses: [CGM17TCx List |
| ÎE M | leasurements | 5 | | | | | |
| 100 | * Showi | ing 1 to 30 of 30 entries | | | | | |
| Actio | ENTRY_NO | CROSS | PLOT_NO | GID | DESIGNATION | NPSEL | BM_CODE_VTE |
| 5 | 1 | P501c0F112-2-5-1-1-B*4 | 1 | 16 | CML320 | 1 | Seed increase bulk - NBK |
| 5 | 6 | Pob21C5HC57-1-2-B-## | 2 | 13543 | CGML1 | | |
| 2 | 7 | Pob21C5HC78-3-4-B-#-B | 3 | 13544 | CGML4 | 1 | Selected bulk - DSB |
| 5 | 8 | Pob21C5HC84-2-1-B-3-B | 4 | 13545 | CGML5 | 1 | Selected bulk - DSB |
| 5 | 9 | Pob21C5HC109-3-1-5-4-B-1- | 5 | 13546 | CGML2 | | |
| 2 | 10 | Pob21C5HC133-1-B-##-B | 6 | 13547 | CGML3 | | |
| Ð | 2 | SA4C2HC(21X26)-1-2-2-2-B | 7 | 1480 | CML360 | 1 | Seed increase bulk - NBK |
| Ð | 6 | Pob21C5HC57-1-2-B-## | 8 | 13543 | CGML1 | | |
| | 7 | Pob21C5HC78-3-4-B-#-B | 9 | 13544 | CGML4 | | |
| 5 | 8 | Pob21C5HC84-2-1-B-3-B | 10 | 13545 | CGML5 | | |
| 2 | 9 | Pob21C5HC109-3-1-5-4-B-1- | 11 | 13546 | CGML2 | | |
| 2 | 10 | Pob21C5HC133-1-B-##-B | 12 | 13547 | CGML3 | | |
| 5 | 3 | MBR-ET(W)F2-14-3-1-3-B-B-E | 13 | 1490 | CML370 | 1 | Seed increase bulk - NBK |
| DN. | 120 | | | | 001114 | | |

Save the data, then use nursery advance from the Actions menu. Uncheck the box for 'Breeding method is the same for each advance' and for 'All plots are selected' and use BM_CODE-VTE to define the breeding method and NPSL to define the plots for advancement.

| METHODS | |
|---|---|
| Breeding Method is the same for each advance | • 0 |
| | Derivative and Maintenance methods |
| | All methods |
| | Show only favorite methods Manage Methods |
| Choose a variate that defines the breeding method for each advance: | BM_CODE_VTE |
| LINES | |
| Same number of lines is selected for each plot | |
| Choose a variate that defines the number of lines selected from each plot | NPSEL |
| BULKS | |
| ✓ All plots are selected | |
| HARVEST DETAILS | |
| Harvest Location: | ART FARM, HARARE |
| | Breeding locations All locations types |
| | Show only favorite locations Manage Locations |
| Harvest Date: | 2017 🔻 07 💌 |
| _ | |
| Cancel | Finish |

The resulting bulks will be grouped with the source material. You will see the seed increase list displayed as in the screenshot below. The names have been constructed as source name + Crop_season_Code for the method Seed Increase Bulk and as Source Name+'-SB' for the Selected bulks as customized in the methods table for the seed increase method. Note that the original source name has been used for CGML4, CGML5 and CML5 because these are derivative names and the naming convention specifies these. They also have the group names and to see these you can fill eht ENTRSY_CODE column with preferred name in the List Manager.

| Nursery BASIC DET | E NURSERIE CGM17TCB | Save | | | Return to Manage Nurseries |
|---|--------------------------|----------------------------|-------|-------------|----------------------------|
| lursery Settin | gs Germplasm & C | hecks Experimental Design | Measu | rements 🔳 🕇 | |
| Advance List: Advance List Fotal Entries: 7 | CGM17CD N Selected: 0 | otes: Ies | | | View List Details |
| ENTRY_NO | DESIGNATION | CROSS | GID | SEED_SOURCE | GROUP ID |
| 1 | CML320:17M | P501c0F112-2-5-1-1-B*4 | 13722 | CGM17TCB:1 | 16 |
| 2 | CL02102-RR1-SB | Pob21C5HC78-3-4-B-#-B | 13723 | CGM17TCB:3 | 13544 |
| 3 | CL02103-RR1-SB | Pob21C5HC84-2-1-B-3-B | 13724 | CGM17TCB:4 | 13545 |
| 4 | CML360:17M | SA4C2HC(21X26)-1-2-2-2-B | 13725 | CGM17TCB:7 | 1480 |
| 5 | CML370:17M | MBR-ET(W)F2-14-3-1-3-B-B-B | 13726 | CGM17TCB:13 | 1490 |
| 5 | CML432:17M | KTX3752F2-7-1-1-1-B-B-B | 13727 | CGM17TCB:19 | 1551 |
| 7 | CZL99029:17M | P43C9-1-1-1-1-BBBB | 13728 | CGM17TCB:25 | 13 |
| _ | | | | | |

Again add inventory for the new batches.

Make a new Crossing nursery

CGML4 combined best with CML360 (Cross PBR317MH7) and CGML5 combines best with CML370 (Cross PBR317MH13) so for example we might decide to make these crosses again. Similarly, as an example we might make three-way test hybrids CGML4/CML360// CGML5 (PBR317MH7/CGML5) and CGML5/CML370//CGML4 (PBR317MH13/CGML4). We can assume we will use the new batches of tester lines and the new generation of inbred lines. We will use the Crossing tool to make these crosses and illustrate the grouping of hybrids although normally such crosses would be made in larger nurseries and would use the layout and crossing template features illustrated above.

Make a new nursery and the lines CGML4, CGML5, PBR317MH7, PBR317MH13 from appropriate lists as females. Then add CML360, CML370, CGML5 and CGML4 as male parents.

| | # | DESIGNATION | CROSS | ENTRY_CODE | GID | GROUP ID | LOTS | AVAILABLE | |
|---------------------|---|--|---|---------------|---|---|--|--|-----------------------|
| D | 1 | CML320:17M | P501c0F112-2-5-1-1-B | CML320:17M | 13722 | 16 | | - | |
| 0 | 2 | CL02102-RR1-SB | Pob21C5HC78-3-4-B-# | CGML4 | 13723 | 13544 | | | |
| þ | 3 | CL02103-RR1-SB | Pob21C5HC84-2-1-B-: | CGML5 | 13724 | 13545 | | - | |
| 0 | 4 | CML360:17M | SA4C2HC(21X26)-1-2 | CML360:17M | 13725 | 1480 | | - | |
| D | 5 | CML370:17M | MBR-ET(W)F2-14-3-1-: | CML370:17M | 13726 | 1490 | | - | |
| ar an ma | ent Id dra | Lists g entries from a list o rents | n the left to modify a paren | t list. | Male Pare | ents | | | |
| ar an ma | ent Id dra Ile Pa | Lists g entries from a list o rents | n the left to modify a paren | t list. | Male Pare | ents | | | |
| ar an ma | ent Id dra Ile Pa Ist e Entrie | Lists g entries from a list o rents entries es: 2 Selected: 2 | n the left to modify a paren | t list. | Male Pare | entries es: 4 Sele | cted: 4 | ¢ AC | TION |
| ar an ma | ent Id dra Ile Pa Ist e Entrie # | Lists g entries from a list o rents entries es: 2 Selected: 2 DESIGNATION | n the left to modify a paren מ אכדוסא CROSS | t list. IS | Male Pare List Total Entri | entries es: 4 Sele DESIGNATIO | cted: 4 DN CR | ¢ AC oss | CTION |
| ar an na L | ent id dra ile Pa ist e Entrie # 1 | Lists g entries from a list o rents entries es: 2 Selected: 2 DESIGNATION CLO2102-RR1-5B | n the left to modify a paren С Асттом cRoss s Pob21C5HC78-3-4-B#-B | t list. IS | Male Pare | entries es: 4 Sele DESIGNATIO CML360:171 | cted: 4 DN CR | रे AC oss 4C2HC(21X26)-1-2-2- | 2-2-B |
| ar ma al | ent id dra ile Pa ist e Entrie # 1 2 | Lists g entries from a list o rents :s: 2 Selected: 2 DESIGNATION CL02102-RR1-SB CL02103-RR1-SB | n the left to modify a paren С АСПОМ СROSS S Pob21C5HC78-3-4-В#-В Pob21C5HC84-2-1-В-3-В | t list. IS | Male Pare List of Total Entri V # Z 1 V 2 | entries es: 4 Sele DESIGNATIO CML360-171 CML370-171 | cted: 4 DN CR И SA И Mt | <mark>Ф АС</mark> oss 4C2HC(21X26)-1-2-2 3R-ET(W)F2-14-3-1-3 | 2-2-B i-B-B |
| ar an L al | ent ad dra ile Pa ist e Entrie # 1 2 | Lists g entries from a list o rents entries es: 2 Selected: 2 DESIGNATION CL02102-RR1-SB CL02103-RR1-SB | CROSS Pob21C5HC78-3-4-B-#-B Pob21C5HC84-2-1-B-3-B | t list. S | Male Pare List of Total Entri V # V 1 V 2 V 3 | ents entries es: 4 Sele DESIGNATIO CML360:171 CML370:171 CL02103-RR | cted: 4 DN CR M SA M Mt 1-SB Po | © AC oss 4c2HC(21X26)-1-2-2 8R-ET(W)F2-14-3-1-3 b21C5HC84-2-1-B-3 b21C5HC84-2-1-B-3 | 2-2-B 3-B-B 1-B |

Now browse the CGM17TC List and open it for review. Add PBR317MH7 and PBR317MH13 from this list to the female parent list. So that the parent lists look like this:

Parent Lists

Select and drag entries from a list on the left to modify a parent list.

| Fema | emale Parents | | | | | Pare | nts | | |
|-------------------|--------------------------|----------------------------|-----------------------|-----|-------|------------------------|-----------------------------|--------------------------|-----------|
| L Fotal | . ist e Entrie | ntries s: 4 Selected: 4 | ACTIO | DNS | total | ist e Entrie | entries s: 4 Selected: 4 | Ø ACTIO | NS |
| 1 | # | DESIGNATION | CROSS | STO | 5 | # | DESIGNATION | CROSS | S |
| • | 1 | CL02102-RR1-SB | Pob21C5HC78-3-4-B-#-B | | | 1 | CML360:17M | SA4C2HC(21X26)-1-2-2-2-B | 3 |
| • | 2 | CL02103-RR1-SB | Pob21C5HC84-2-1-B-3-B | | | 2 | CML370:17M | MBR-ET(W)F2-14-3-1-3-B-B | <u>}.</u> |
| - | 3 | PBR317MH7 | CGML4/CML360 | | | 3 | CL02103-RR1-SB | Pob21C5HC84-2-1-B-3-B | |
| | 4 | PBR317MH13 | CGML5/CML370 | | | 4 | CL02102-RR1-SB | Pob21C5HC78-3-4-B-#-B | |

Specify the crossing of matched pairs and generate the crosses:

Crossing Method



V Preview Crosses

| / | # | FEMALE PARENT | MALE PARENT | FEMALE CROSS | MALE CROSS | |
|---|---|---------------|-------------|--------------|------------|--|
| 0 | 1 | CGML4 | CML360:17M | CL02102 | CML360 | |
| | 2 | CGML5 | CML370:17M | CL02103 | CML370 | |
| | 3 | PBR317MH7 | CGML5 | CGML4/CML360 | CL02103 | |
| | 4 | PBR317MH13 | CGML4 | CGML5/CML370 | CL02102 | |

Specify a hybrid breeding method such as Test Cross and select the option to 'Apply grouping to new crosses only'.

| y default, the breeding method for new s an alternative, you can select a metho | crosses will be based on the status of their parental lines. d to use for all crosses. |
|--|---|
| Use parental status | |
| Select a method to use for all crosses | : |
| Test cross - TCR | • 🕜 |
| All methods | Generative methods |
| Show only favorite methods | Manage Methods |
| 1990 C | |

Save the germplasm list and add inventory by one of the available methods.

| CGM1 | 18TC L | .ist × | | | | | | |
|-------|--------|----------------|------------------|------|-----------|------------|-------|----------|
| ΠL | ist e | ntries | | | | | | Ф АСТІО |
| Total | Entrie | s: 4 Selected: | 0 | | | | | 6 / |
| 1 | # | DESIGNATION | CROSS | LOTS | AVAILABLE | ENTRY_CODE | GID | GROUP ID |
| | 1 | PBR318MH1 | CGML4/CML360:17M | 1 | 120.0 g | 1 | 13729 | 13673 |
| | 2 | PBR318MH2 | CGML5/CML370:17M | 1 | 120.0 g | 2 | 13730 | 13679 |
| | 3 | PBR318MH3 | PBR317MH7/CGML5 | 1 | 120.0 g | 3 | 13731 | 13731 |
| | 4 | PBR318MH4 | PBR317MH13/CGML4 | 1 | 120.0 g | 4 | 13732 | 13732 |

Note that the re-made crosses PBR317MH7 and PBR317MH13 have been given their own cross names PBR318MH1 and PBR318MH2 but have been grouped with the original test crosses even though they were made from the next generation of inbred lines, and they have inherited the names of those crosses with name type SELHISFIX.

| • | BASIC DETA | ILS | | | | | | | |
|---------------------------|----------------|----------|---------------------------------------|-------------------------|-----------|--------|--------------------|---------|--|
| Preferred Name: PBR317MH7 | | | | Creation Date: 20180300 | | | GID: 13729 | | |
| | Creation Metho | Loc | Location: ART FARM, HARARE Reference: | | | | | | |
| | Fixed Line | Gro | oup <mark>Id</mark> (M | IGID): 13673 | | | | | |
| • | ATTRIBUTE | s | | | | | | | |
| | PEDIGREE T | REE | | | | | | | |
| • | NAMES | | | | | | | | |
| | NAME | DATE | LOCATION | | ТҮРЕ | TYPE | DESC | | |
| | PBR317MH7 | 20170731 | ART FARM, HARA | RE | SELHISFIX | Select | tion history at fi | ixation | |
| | PBR318MH1 | 20180300 | ART FARM, HARA | RE | CRSNM | CROS | S NAME | | |

If we had not checked the option 'Apply grouping to new crosses only' they would have started new groups and not inherited the original test cross name.

On the other hand, the three-way crosses are first time combinations so they have been assigned as founding members. IF they are made again form subsequent version of the single crosses and subsequent versions of the inbred lines, these new versions will be grouped with the original three-way crosses.

You can also assign codes to test hybrids, as was done in section 1 for inbred lines. These codes will be inherited as the preferred name by all hybrid group members.

Annex 1. Review of coding functionality as specified for CIMMYT GMP

Since the idea of including coding in to BMS tools came from CIMMYT's maize breeding needs, it would be appropriate to discuss and summarize what we understood from CIMMYT's documentation on the subject and compare them with the tutorial.

CIMMYT assigns three different sets of codes to maize germplasm based on the stages of testing.

Code 1 – Every entry that goes in to Stage 1 testing – i.e. preliminary yield trials that are usually made by crossing S2 (or F3) ears to a tester. This is the first time that a breeder is able to test the germplasm generated by crossing two (or more) parents to generate hybrids for testing. If this is done with DH lines, obviously, those lines are fixed while if they are done with S2/S3 lines they are not fixed.

Code 2 – In preliminary yield trials, usually about 10-15% of the best performing entries are retained for stage 2 and stage 3 testing. These entries are assigned a Code 2. Basically, Code 2 superimposes Code 1. The difference between Stage 1 testing and Stage 2 testing is that entries that get to stage 2 testing are tested with more testers and in more environments. The S2/S3 lines that were used for crossing for stage 1 testing are now at S4/S5 stage – (while the hybrid combinations are evaluated in yield trials, in the nurseries, breeders grow the lines, self them to make them more genetically uniform). I believe that entries get a Code 2 assignment when they are ready to be tested outside a specific breeding program – i.e. in regional trials where multiple breeders may have entries contributing to the regional trial.

Code 3 - A code 3 is given to advanced inbreds (such as CMLs) or hybrids or OPVs when they get ready for commercialization in a country.

What is not very clear is that how many of breeders give code 1 and code 2 to hybrids instead of inbreds. Practically, it is obvious that a breeder would have a large number of Code 1s a subset of which will also get Code 2 and a small subset of them (Code 2s) get a Code 3. If a breeder selects a few entries originating from a specific cross (eg. AxB) to enter into Stage 2 trials, these few entries are related since they come from the same original cross and can be considered as 'sister lines' or as a group.