PROCEDURES FOR THE EVALUATION AND ANALYSIS OF SWEETPOTATO TRIALS

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Sweetpotato Research Guides (SPRGs)
Describe technologies that have been developed and used by CIP in cooperation with national agriculture research programs to promote research and the exchange of information among sweetpotato scientists.

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1. INTRODUCTION

Breeding programs involve large investments of time and money, but can pay very large returns on investment in the form of improved varieties which benefit farmers, societies and the environment. International breeding efforts involving multiple partners and targeting regionally important constraints have great potential for efficiently and rapidly achieving impact. Standardized information on the performance of progenies and selected clones across environments is necessary in order to assist breeders to efficiently make decisions about selection and variety release. Standardized methods also facilitate the reporting of breeding program results to the agencies that support us. This manual of procedures for the evaluation and analysis of sweetpotato trials provides standard methods for partners in CIP's global breeding efforts.

The manual is the result of an iterative process involving discussions among breeders at a series of meetings held over the past two years, starting with support from the HarvestPlus program of the CGIAR and continuing under the Sweetpotato for Profit and Health Initiative. The manual is a work in progress and will be refined further in the coming years in response to the needs of sweetpotato breeders and the producers and consumers we serve. We are excited about the application of new methods to sweetpotato breeding, including an accelerated breeding approach that will emphasize recurrent selection and may make systematic use of heterosis, and which will lead to the release new varieties in 3 years. Standardized methodology and reporting will help us to achieve our objectives.

This manual is divided into 6 sections. First, this introduction provides brief discussion of some of the key principles for our sweetpotato breeding effort, including the need for check varieties, and an overview of the Accelerated Breeding . In section 2 we describe and discuss the standard trialing stages used in the breeding program, and in section 3 we present the standard data forms to be used in the trials, providing examples of completed forms. In section 4, we provide a brief introduction to the analysis of data from selected trials using Plabstat, SAS and R statistical packages, along with some suggestions for using results of analysis to make selections. Section 5 presents references for further reading, and the Appendix provides sets of blank forms which partners may copy for use in their trials.

Check varieties. Breeding is a process for adapting a crop to human needs. An important component of breeding is the selection of new varieties. The selection of better varieties requires a good understanding of what is needed by farmers and societies, and it requires good biological and statistical knowledge.

A variety is always characterized by several traits. A better variety must have

good performance over all traits and at least in one important trait it must be clearly superior to all other varieties, which are so far available in a region. It is not possible to compare a set of new genotypes with all existing varieties across all target environments. Therefore we evaluate and compare new genotypes with important standard varieties (check varieties) in important environments (check environments). The check environments should be representative for the region we are aiming at.

Selection of new varieties for a region requires comparing new genotypes with check varieties. This is a very complex task, and may involve many partners working in different places throughout the region. Only what is comparable can be compared! If we evaluate different traits or if we use different procedures to evaluate these traits in different trials, we cannot compare the performance of genotypes across trials. The same is true if we use different sets of new genotypes and different check varieties. Only commonly measured genotypes and check varieties can be compared. We distinguish two designs which allow us to compare results of trials: (i) The complete design in which all genotypes are commonly tested across all check environments and (ii) the incomplete design in which only a fraction of all genotypes (at least 6) are tested across all check environments. If there are no or only very few genotypes (1 to 4 genotypes) commonly tested across check environments, it is not possible to make meaningful comparisons among trials. Hence there needs to be agreement among breeders about: (i) the most important traits to be evaluated, (ii) standardized procedures to record these traits, and (iii) commonly used genotypes and checks varieties (> 5 genotypes).

Accelerated sweetpotato breeding (ASPB). This method, which can be used for both pre-breeding (population improvement) and for varietal selection, is illustrated in figure 1, below. It can allow for completion of a selection and recombination cycle (in the case of prebreeding) in 1 to 2 years, or the selection and release of a new variety in a period of 3 to 4 years. The main features of the approach are to use multiple selection sites from the initial stage of selection, and to minimize replication (a maximum of two reps per trial are used) at each site so as to conserve resources while obtaining information on stability of genotypes being tested. The trial stages for which we present forms in this manual are the observational yield trial (OT), the preliminary yield trial (PT) and the advanced yield trial (AT). An important feature of our breeding and cultivar selection method is the use of farmer participation, which provides essential input to the breeding and selection process. Here, we detail the use of farmer input at the AT stage.

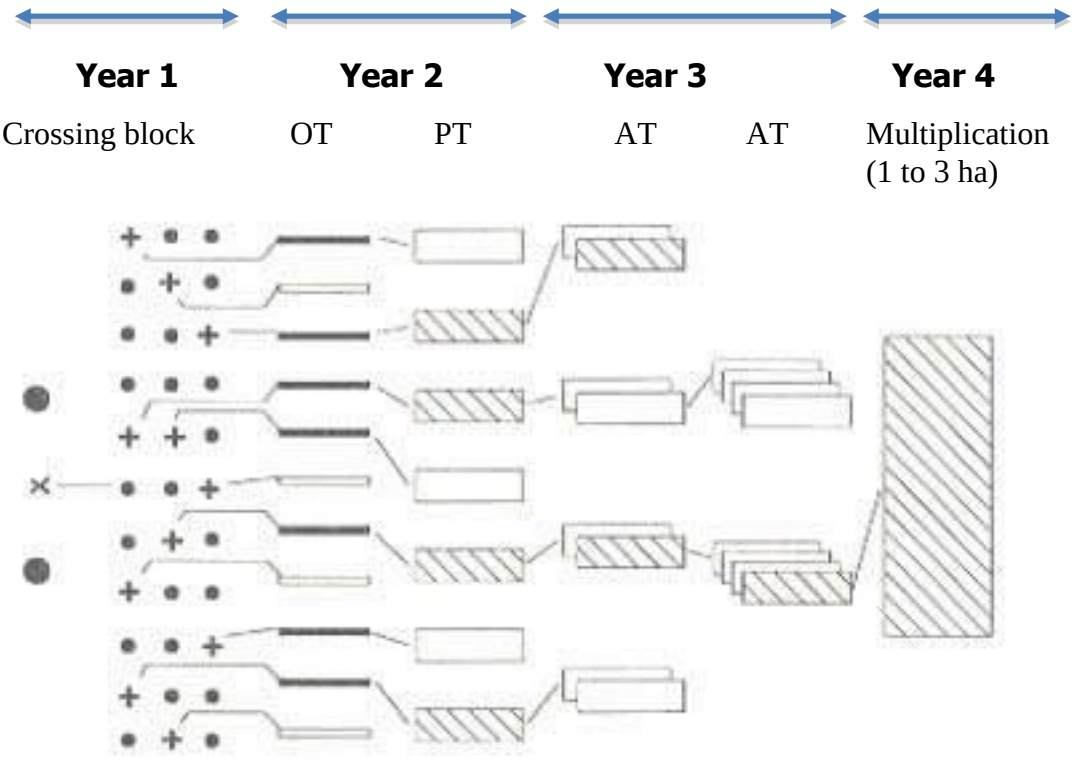

Figure 1. Accelerated Sweetpotato Breeding Scheme

2. PROCEDURES

2.1. Multiply clones for trial and maintain their identities

Sweetpotato clones for trialing may be newly derived from seedling populations or may be important varieties or promising selections from other breeding programs which have been introduced as pathogen-tested in vitro plantlets. Within regions sweetpotato clones may be moved as cuttings, following approved quarantine procedures. Quarantine procedures may slow down the exchange of breeding material, but are important. Who wants to become famous by introducing new pests or pathogens into environments where these have so far not been present?

Clones have to be multiplied to produce planting materials for initial trials. Locally important as well as standard check varieties should be included in multiplication plots to provide uniform planting materials for trials. Planting material of all the genotypes for any trial should to come from a single source, and the health status of genotypes in the trial should be similar. Often a common health status

among clones is difficult to achieve. For example, local clones might not be virus free, whereas introduced clones are obtained pathogen free. In such a case the effect of the genotype might be confounded with its health status. Similar problems might occur although you are not working with introduced clones. Standard check varieties might have been used for a long time, without renewing them from a source of pathogen-free planting material. In contrast newly developed clones are young. It should be noted that health status does not only affect yield related traits. The same clone can appear morphologically different if it is pathogen-free or infected. Therefore the multiplication of check varieties should, to the extent possible, routinely make use of pathogen-free mother plants. These mother plants are maintained in greenhouses and are routinely checked to be pathogen free. A mother plant in which a pathogen has been detected must be immediately removed from the greenhouse. Mother clones with pathogens have to be cleaned up or replaced from pathogen-free in vitro source.

Verify and maintain the identity of clones during the process of multiplication and evaluation. The most frequently asked question by a professor in plant breeding to his PhD students was: Is genotype number 1 still number 1 or is it perhaps already number 2? Many plant breeders have encountered surprising results, only to realize that a mistake in the labeling of the genotypes must have happened, leading to a mix-up in identities. Such mistakes can be drastically reduced by giving clones both numbers and names. Mistakes in numbers occur more frequently than mistakes in names. If a genotype has no name, give the clone a ”code name”. A code name can easily be formed by the family the clone is tracing back [six digits; three digits for the father and three digits for the mother – (in case the clone derived from polycrosses put 000 for the father)] and then – separated by a decimal point – the clone number within the family. It is very unlikely that a mistake in the clone number and in the code name would happen at the same time, so you can usually quickly identify mistakes. It should be noted that mistakes cannot completely avoided, however, the problem starts when you cannot identify mistakes or when you cannot identify them rapidly.

Finally we want to mention that the identity of clones can be confirmed by morphological characteristics and descriptor lists. However, these are usually only available for clones maintained in genebanks. Anyway, the finally selected clones must be morphologically described and distinguishable from other clones to allow a registration as a new variety. If published and observed descriptors (including pigmentation of foliage and roots, and especially leaf shape) do not coincide (in other words, if you observe for the same clone two different types) both clones can still be entered in trials. In this case it is required to rename the clones to distinguish them. You should use the original name with an extension where the morphologically different type has been observed for the first time e.g. Jonthan-L for the original clone described and maintained at the sweetpotato genebank in Lima and a Jonathan-M for a clearly distinguishable

Jonathan first observed in Maputo. Since sweetpotato has a tendency to mutate, it may well be that the new type is not the result of a mix-up, but could be a new Jonathan superior to the original Jonathan.

2.2 Trial types and selection schemes

Examples of sweetpotato selection schemes are provided by Hahn (1982), Martin (1983), Jones et al. (1986), Wilson et al. (1989), Kukimura et al. (1990), Saladaga et al. (1991), Tan et al. (2007) and Grüneberg et al. 2009a). Sweetpotato breeding as been reviewed by Martin and Jones (1986), Laurie and van den Berg (2002), Tan et al. (2007) and Grüneberg et al. (2009b). However, each sweetpotato variety is a highly heterozygous hybrid and we think that the use of more breeding methods from hybrid breeding applied to sweetpotato breeding has merit (for a discussion of this topic see Grüneberg et al. (2009b). This might lead to changes for sweetpotato population improvement in the future, but not to changes in variety development and selection.

In formal plant breeding we distinguish between observation yield trials (OTs), preliminary yield trials (PTs) and advanced yield trials (ATs). Note: ATs are occasionally designated as uniform as well as national trials, but the procedures used in both are usually the same. Therefore, we refer to both of these as ATs. Formal plant breeding (on station breeding) has been criticized for being slow to develop better varieties for resource poor farmers. For this reason CIP supports formal plant breeding programs which involve farmers by (i) farmer participatory variety selection and (ii) on-farm trials.

Early breeding stages: In the early breeding stages plants are raised from true seeds. Selection of single true seedling plants may not be advisable, because measurements on single plants have an extremely high error and plants grown from seeds are very different from those grown from cuttings with respect storage root formation. For this reason, evaluations of true seed plants are limited to a few highly heritable traits such as susceptibility to pathogens or storage root flesh color. Genotypes selected among true seed plants enter observation trials (OTs).

The **observation yield trial (OT)** belongs to the early breeding stages. The breeder has to evaluate many genotypes (several thousands) grown from cuttings of true seed plants and most of the genotypes grown in OTs clearly do not meet a lowest acceptable value in at least one character. OTs are also recommended for clones which are introduced from other regions of the world (i) to obtain a better understand how to handle foreign materials and (ii) to discard clones which clearly do not meet minimum acceptable values in the new region. In the sweetpotato breeding program at CIP we plant OTs without replication, in a single-row plot comprising 3 plants. However, each clone is planted at 4 locations (each location is treated as a replication of a randomized complete

block design). Each OT is bordered by planting guard rows on all four sides of the trial to provide competition to all entries. The name OT might seem to imply that only visual observations are made at this stage of selection. However, an OT grown across several locations merits to record and analyze highly heritable traits. Formerly CIP planted the OT in a single-row comprising 10 plants at only one location. Other breeders conduct the OT so that each genotype is evaluated on single plant basis (for example SPVD resistance or storage root flesh color). In the new OT design at CIP (OTs in one meter row plots across several locations) it is possible to observe the stability of genotypes across locations and to separate the genotype by environment (GxE) effect from the genotype effect. Heritability (h^2) estimates show that the harvest index and several storage root quality characteristics (i. e. dry matter, protein, starch, sugar(s) and pro-vitamin A concentrations) can be evaluated in such an OT design with sufficient precision for selection ($h^2 > 0.6$). To our surprise we observed significant low to medium heritabilities (h^2 about 0.4 to 0.5) for storage root yield. However, visual selection for storage root size, shape and form - recorded using a single rating scale from 1 to 5 - showed a significant correlation with yield measurements in kg per plot in the new OT design. This can be explained by the extremely large genetic variation for storage root yield in early breeding stages due to very high segregation in hexaploid sweetpotato. However, this allows us only to discard genotypes with poor yield performance since discrimination among clones with medium to high yield performance is only possible in larger plots (see PTs and ATs).

Note: The OTs in the sweetpotato breeding program of CIP are directly used to select parents for crosses in hybridization blocks. Hence the breeding program operates with very short recurrent selection cycles to improve breeding populations and allow for variety development. Each year about 250 genotypes are selected from OTs: (i) to be used for crosses for population development and (ii) to enter PTs for variety development and selection, respectively (see below). All crosses are carried out by controlled crossings. In contrast to polycross nurseries, this results in more balanced seed production per parent, since both parents can be controlled. Selection theory tells us that controlled crosses are more efficient than polycrosses. however, considerably fewer seeds can be produced using controlled crosses. [Current research aims to compare progress using both approaches.] Our crosses are carried out in a factorial design (the best with the rest), in which about 6 x (250-6) crosses are carried out. About 1/3 of all cross combinations results in no or low seed set so that each year about 900 families with about 20 to 40 seeds per family are developed for the OTs of the next recurrent selection cycle. To summarize: OTs are characterized by a very large number of genotypes evaluated in very small plots without replications. The OT can be carried out at one location or at several locations and environments, respectively. The design of the OTs depends on the priorities of specific regions. For example in high virus pressure regions the breeder has first to eliminate all genotypes which show insufficient virus resistance, whereas in a

drought prone region the breeder first has to eliminate all genotypes which show insufficient vine survival.

Here we do not prescribe traits to record in OTs, because the traits to be recorded depend on the region, the country and the major breeding objectives, respectively. However, we are aiming to obtain general OT trial information as well as information for the recorded traits in selected clones, which will allow us to group breeding programs into clusters to support appropriate true seed exchange among breeding programs. Moreover, we request to record the parents and clone numbers of selected clones. The reason for this is that it is common knowledge among breeders that often good clones trace back to a few cross combinations. The record of parents and clone numbers of selected clones will allow us to determine the frequency of selected clones among parental combinations and to determine which promising crosses should be repeated on a larger scale. For details on record keeping for OTs, see section 3. Data collection forms for all trials are provided in Appendix 1.

Later breeding stages: Clones selected in OTs enter into later breeding stages or into variety selection and development. These later breeding stages are comprised of preliminary yield trials (PTs) and advanced yield trials (ATs). In PTs and ATs the same characters are recorded, but on different plot size basis. Note: the ATs should be recorded on a plot size basis, which is identical with those required for official variety release - this is country specific. Moreover, the names uniform trials (UTs), national trials (NTs) and elite trials (ETs) are used for ATs. However, usually these have the same plot size and the same traits have to be recorded as for ATs. It should be noted that NTs and ETs are used for those finally selected clones to be tested against a group of check clones for variety release. In this case we distinguish between ATs and NTs or ETs, but all three have the same plot size and the same traits have to be recorded. They differ in the number of clones to be tested – usually in NTs or ETs a smaller number of clones is tested compared to ATs, so that the test precision / power is larger compared to ATs (see statistical analysis and multiple comparison procedures).

The **preliminary yield trial (PT)** is normally carried out in two row plots, 30-plants per plot (15 plants per row), and two plot replications. The PTs are planted in a randomized complete block design [**RCBD** (replications of clones are planted in blocks and in each block all genotypes are randomized)]. Single row plots should not be used because of the inter–plot competition (border effects due to neighbor plots within a block). Border effects are assumed to be large in sweetpotato, due to the large genetic variation for the upper biomass production among sweetpotato clones. The coefficient of variation (CV%) for the storage root yield error term in a single PT (two rows, 15 plants per row) is typically very large and ranges in our breeding programs from 28% and 52%. It would

probably be larger if we used single rows.

The PTs must be carried out in at least 3 locations and with plot replication for each genotype, or it should not be considered as a preliminary yield trial. The clear advantage to conducting the PTs at three or more locations is that this saves time (years), because in sweetpotato trials, temporal variation of test environments (years) can be replaced by spatial variation of test environments (locations). Conducting PTs across locations, with two or more replications, allows us to separate the effects due to genotype, due to genotype by environment interactions, and due the plot error for each trait. Furthermore, with 3 locations it is possible to determine for each genotype, stability parameters, which must be considered as an additional character associated with yield.

To summarize, since 2008 CIP has aimed to conduct PTs as follows: (i) at least two row plots with (ii) at least 30 plants per plot, (iii) two plot replications per genotype and (iv) in at least three locations and environments, respectively (you can generate environments in the same location by treatments i.e. irrigation or fertilization). The set of attributes and traits, respectively, to be recorded in PTs is fixed. For details of data to record for PTs, see section 3. Data forms for the PTs are provided in the Appendix. However, the recording of additional traits is optional if the breeder thinks one or more traits must be recorded for an appropriate selection in his target environment.

Note: The main question in selection is how many genotypes should be selected? If almost none of the clones in a trial meet the lowest acceptable value in each trait there is not much choice. However, after good OTs most genotypes should meet the lowest acceptable value across all character. Variety selection is a multi-stage process, and for fixed entries (all genotypes of clonally propagated crops are fixed entries) this multi-stage selection problem has been well solved by selection theory. The results of selection theory show for very different selection scenarios [different ratios of variance components for genotypes, GxE and plot error, and different numbers of test capacities (total number of possible field plots to be allocated to genotypes, locations and replications)] that at each selection stage 5 to 15% of the total number of genotypes should be selected. Moreover, they show that more than 3 to 4 selection stages do not result in a significant increase of genetic gains. Hence a 3-step selection (one in OTs, one in PTs, and one in ATs) is sufficient to identify the most appropriate clones for variety registration in a breeding population. However, if the breeding population is still at an unsatisfactory level, there might be no or only very few clones which can be recommend for variety registration. In such a case the breeder must allocate more resources to population improvement (to increase the variety-generating ability of the breeding population) by (i) conducting more crosses (controlled crosses), (ii) using more parents, and (iii) shorter recurrent selection cycles (1 year to recombine parents and 1 year to select parents, which are more

close to his breeding targets).

The **advanced trial (AT)** is the next selection stage of variety selection. It is usually planted as a RCB design, but in larger plots than in the PT. Our ATs in the breeding program of CIP have 5 row plots (15 plants per row) with 75-plant per plot, and two replications per location. The ATs are carried out at 4 or more locations. The coefficient of variation (CV%) for the storage root yield error term in a single AT (five rows, 15 plants per row) at CIP typically ranges between 25% and 32%, which is still large compared to ATs for grain crops. This shows that there is still room for improvement in trial designs for sweetpotato, and better experimental designs might be developed for sweetpotato in the future. The result of the selection process in ATs should be 5 to 8 clones with good performance over all traits and which have, in at least one trait, a clear advantage compared to all sweetpotato varieties available in the region. As mentioned above formal plant breeding has been criticized for not being successful at developing better varieties for resource poor farmers. For this reason, at least at one location, the AT has to be carried our with farmer participation (details see below). This eliminates the possibility of putting forward genotypes for official variety release that are not accepted by farmers. The selected clones (5 to 8) are re-evaluated in the next growing season in a similar design at the same locations and additionally in more than 10 on-farm trials (OFT), which should be linked with the process of official variety release (for details of OFTs, see below).

To summarize: Since 2008 CIP proposes that ATs be carried out: (i) in three and more row plots with (ii) at least 75 plants per plot, (iii) 2 plot replications per genotype, and (iv) at least four locations. Farmer participatory variety selection is required in at least one location, and at least 10 OFTs in the final selection stage should be linked to official variety release. Instructions for data recording for ATs are given in section 3. Data forms for the ATs are provided in the Appendix. Note, the same forms are used for ATs as for PTs, but provide space for detailing details of plot layout. Space is also provided for the collection of additional attributes.

Note: Extended PTs and ATs to evaluate vine survival and piecemeal harvest, are of special importance in sweetpotato. Therefore we give here a brief discussion of these traits and a recommended method to evaluate these traits (this procedure might change in the future).

Sweetpotato has the highest food production per unit area per unit time. However, in drought-prone environments, a critical character of sweetpotato is vine survival from harvest to the next planting season. Additionally, an important trait of sweetpotato is the ability for use in piecemeal harvest, especially when it is used in home gardens. Neither trait has been addressed in PTs and ATs to

date, because typically the complete plot was harvested and no plants remained to determine vine survival and piecemeal harvest quality. We propose a design which allows observing these traits, by using extended plots in PTs and ATs. These trials are carried out as described above, but rows are longer (about 5 planting positions per row). The first and larger part of the plot is used to record characters as usual. The second and smaller part of the plot is used later (2 to 3 months after the first harvest) to determine vine survival and piecemeal harvest traits. We recommend that each partner should carry out extended PTs and ATs at 2 locations with two plot replications. It should be noted that in drought prone regions vine survival and sprouting potential of small roots determines the acceptance of a variety, since varieties that fail in these attributes will have no planting material available when the rain comes. Moreover, the ability to use a variety for piecemeal harvest is one of the most important characteristics in Sub-Saharan Africa; varieties that develop undesirable fiber or taste at later growing stages (5 months and more) are nearly always rejected by farmers. CIP hopes to come to an agreement with partners that these characters be determined in extended PTs and ATs in the future, and we are working to develop standard methods for data collection from these trials.

Farmer Participatory Variety Selection (FPVS)

This is an important part of the evaluation of AT clone. It should be carried out at a minimum of one AT location per country. Farmers are invited to give their evaluations and comments on clones in ATs. The evaluation is carried out on the basis of frequencies for the assessment of a limited number of traits (6 traits and one overall assessment) for each variety. The assessment is recorded using color cards to score each entry in the trial. (red = not acceptable; yellow = more or less acceptable; and green = clearly acceptable) and the overall performance of each variety. To assess the genotypes each farmer obtains 21 color cards for each genotype to be evaluated (one card of each color for each attribute to be evaluated). As each attribute is discussed, the farmer values each genotype by placing one color card into a bag (based on the degree of acceptance for that attribute). There should be separate bags for women's and men's votes, or cards may be marked with M or F to indicated gender. Results are tallied using the data collection forms provided in the Appendix.

On-Farmer Trials (OFTs)

ATs at the 2nd stage, which are carried out at several locations are used as mother trials for OFTs. Each farmer obtains 4 varieties from the AT and has to assess these varieties relative to his currently used variety. As with FPVS, the evaluation is carried out on the basis of frequencies for a limited number of traits (6 traits and one overall assessment for each variety). The farmer ranks the new

genotypes relative to the performance of his currently preferred variety for each trait as well as for the overall performance across traits. We recommend conducting this on the basis of color cards as for FPVS: Green = improved or better than the local check, Yellow = equal or nearly equal to the local check, and Red = inferior compared to the local check. These data should be collected from farmers during visits to farms.

2.3. Data analysis, selection of clones and reporting of results

To facilitate analysis and decision-marking, the raw data collected in trials using standardized methods described in section 3 should be transformed into reference units. For instance, the number of harvested plants divided by the number of cuttings planted would be survival, yield measured in kg/plot must be converted to t/ha, etc. After this processing step sort the data to be analyzed by location, genotype and replication. The ANOVA (Analysis of Variance), and mean comparisons (e.g., LSD or Tukey test) become useful tools for clonal selection. In section 4 we give recommendations for breeders to analyze data from their trials, select clones and report results. On the other hand we all must follow the description of data collection in selection 3 (Appendix 1, 2 and 3) in order to report the results of our sweetpotato variety selection programs to our donors. The raw data recorded in the forms and sheets in appendix 1, 2 and 3 must be made available to CIP from 2008 on. CIP regional breeders will work with national partners on a continuing basis to achieve uniformity, quality, consistency and relevance of data from breeding trials.

3. DESCRIPTION OF DATA COLLECTION FORMS AND INSTRUCTIONS FOR THEIR USE

[Note: While under some circumstances, there are different meanings of the words "variable, trait and attribute," for our purposes we use these words synonymously.] In experiments, we distinguish between classification variables, and response variables which have to be analyzed. Classification variables help us to identify plots and how experimental factors and factor levels, are applied to plots (e.g., year, location, genotype, genotype name, replication). The classification variables can be comparable to our home address by which we can identify who is living where (country, town, street, name). Classification variables allow us to identify a plot, its location, which genotype was planted in it, and how the plot was treated. Moreover, classification variables are needed to provide statistical program packages with information about how the data were organized (e.g. data records are sorted by locations, within locations by genotypes and within genotypes by replications) and they are used to inform statistical procedures about how to analyze the data records.

Variables which have to be analyzed can be distinguished: (i) parametric variables (real numbers following an approximately normal distribution (e.g., storage root yield, upper biomass yield, storage root dry matter, etc.), (ii) non parametric variables (real numbers which show strong deviations from a normal distribution (e.g., disease damage), (iii) rank variables (e.g., scores with a scale of 1 to 9 for vine strength or scores with a scale of 1 to 5 for storage root fiber content, or scores from 1 to 3 corresponding to the color cards used during farmer assessment of varieties). It should be noted that the anaylsis of variance (ANOVA) procedure is relatively robust to deviations from the normal distribution, so that even symmetrically distributed rank scores from 1 to 9 can be analyzed by an ANOVA. However, the ANOVA is very sensitive to deviations due to variance in homogeneity of the error [this is the case when a genotype obtains a common score value across replications (e.g., 1 for vine strength = no vine survival), while other genotypes obtain different scores (e.g. 6 to 9 for vine strength = vine survival)]. No variation among replications results in variance in homogeneity, and the requirements of an ANOVA are not fulfilled! Rank variables with scores from 1 to 3 should never be analyzed by an ANOVA; however, the frequency means provide useful information. Rank variables with scores from 1 to 3 have to be analyzed by rank statistics and for these, significance tests and multiple comparison procedures (procedures to compare each clone with a check or among all other clones in the trials) are available.

Note on rating scales: In general, the approach that we have taken with rating scales is to use a 1 to 9 scale, setting 1 as good and 9 as bad (in the case of hedonic scales), or setting 1 as absence of a problem (in the case of diseases and pests). For a few traits including vine vigor and cooked root storage quality

traits, this logic doesn't hold perfectly. Thus, for vine vigor, we have set 1 to lowest and 9 to highest. in some places, established breeding programs may have already developed different rating scales (e.g. 1 to 3, or 1 to 5 or 1 to 9). It is our hope that all of our partners in this collaborative sweetpotato breeding effort will be willing to adopt and use the scales given below, so that all may benefit from the power of the information provided from comparative analysis of our combined results.

Below we provide detailed forms and instructions for use. We have also now developed a computer program, CloneSelector, which contains the same forms and which significantly automates the tasks of making field books, collecting and analyzing data. The CloneSelector program and instruction manual can be found at http://sweetpotatoknowledge.org.

Forms 1a and 1b: Sweetpotato Observational Trial (General Information)

This form requests **essential** information for the observation trial such as location, plot size, and trial management practices. It also provides space for the results of soil analyses and meteorological data which adds value to the information on performance of genotypes in the trials by helping to identify patterns among experimental sites and agro-ecological zones, respectively.

The general OT information to be recorded:

1. Country (see form 3a for codes)
2. Name of Contact Scientist
3. Institution
4. Address
5. Phone numbers
6. Location of Trial
7. Latitude, Longitude and Altitude
8. Type of Trial (single plant or row observations and season)
9. Names of the check varieties
10. Planting and Harvest Dates (including crop duration)
11. Plot Description
12. Plot Size
13. Crop rotation
14. Soil Description (see form for description of categories)
15. Meteorological data during the trial
16. Traits evaluated in the OT
17. Numbers of families (polycross or controlled cross) in trial, and number of genotypes
18. Comments on the observation trail

Form2: Sweetpotato Observational Trial (Data Sheet)

The observations in the OT to be recorded are restricted to selected clones. This will allow us to identify the most successful crosses which will then be repeated on a larger scale (i.e. 500 to 1000 seeds per cross combination). The data to be recorded are:

1. Clone Number formed by a number for the father, a number for the mother and the number of the clones within the family. In the case of clones from polycrosses, leave the columns for the father empty or set them to zero.
2. Pedigree name if the father and mother of the clone have already names for example Jonathan x SPK004
3. Indicate check varieties and take data on checks for comparisons
4. Indicate the traits recorded in the OT and in the case of scores identify the meaning of the scores. Examples given in the data sheet are root and vine yield per plot, which would be used to calculate harvest index, and flesh color
5. Record the observation of traits for each selected clone

Forms 3a and 3b: Sweetpotato Preliminary (PT) and Advanced Yield Trial (AT) (General Information)

This form requests **essential** information for preliminary and advanced yield trials such as location, plot size, and trial management practices. It also provides space for the results of soil analyses and meteorological data which are needed to add value to the information recorded. This additional information will help to identify patterns among experimental sites and agro-ecological zones, respectively.

The general PT and AT information to be recorded:

1. Country (form for codes)
2. Name of Contact Scientist
3. Institution
4. Address
5. Phone numbers
6. Location of the trial including district, site name, agro-ecological zone
7. Latitude, Longitude and Altitude
8. Type of Trial
    a. 1- Preliminary (PT), 2- Advanced (AT), 3- On-farm (OFT)
    b. 1- CRB design, 2- other designs (specify)
    c. 1- Standard trial, 2- Quality specific trial
    d. Season: 1- wet, long rains; 2- wet, short rains; 3- dry

9. Dates
    a. Planting
    b. Verification of Establishment (3-4 weeks after planting)
    c. Date of gap filling, if done
    d. 1st Virus Symptom Evaluation (6-8 weeks after planting)
    e. 2nd Virus Symptom Evaluation (1 month before harvest)
    f. Harvest
    g. Crop duration in days from planting to harvest
10. Plot Description
    a. Plot type: 1- Rows/ridges, 2- Mounds, 3- Rows/flat
    b. Number of rows/mounds per plot (includes the border rows)
    c. Number of border rows or rows of mounds per plot
    d. Number of plants intended for final harvest (excludes border rows & end plants)
    e. Cuttings per plot actually used to achieve target plant density per plot
    f. Target plant spacing WITHIN rows (m)
    g. Space BETWEEN rows (m)
11. Determine NET Plot Size (meters squared excluding border rows & plants)
12. Crop Rotation
    a. Crop(s) from previous season
    b. Crop(s) from two seasons ago
13. Soil Description (see form for description)
    a. Soil type
    b. Soil texture
    c. Soil pH
    d. Percent Organic Matter
14. Meteorological data during the trial
    a. Specify Month
    b. Code for Month
    c. Rainfall (mm) for each month
    d. Temperature (In Centigrade) mean for each month
    e. Temperature (In Centigrade) mean minimum for each month
    f. Temperature (In Centigrade) maximum for each month
15. Specify and describe the number of check varieties used. Please use MORE than one check (6 recommended).
    a. Check 1
    b. Check 2
    c. Check 3
    d. Check 4
    e. Check 5
    f. Check 6
16. Other comments on events that occurred during the trial

Form4a. Sweetpotato genotypes in trial.

This is the form for maintaining a detailed record of the names of clones entered in the trial and seems to be quite superfluous, though it may be useful for assigning a simple code number to each genotype.

Forms 4b, c, and d are for pre-harvest, harvest and post-harvest data from PT and ATs and must be filled in completely. Variables 1 through 10 in are classification variables and are repeated in each form. Form 4d is for postharvest data, which does not need to be collected from all entries. If response data are missing for a particular trait, the cells with missing data receive the data record "*****" **for missing value**.

1. C = Country code
2. L = Location or SITE
3. T = Trial type
4. Y = Year
5. S = Season
6. PN = Plot Number
7. R = Replication
8. G = genotype number (3 digits for the year of the cross the clone is tracing back to, + 3 digits for the father number, + 3 digits for the mother number, and 4 digits for the clone number in the family. The form from the breeders meeting looks different.
9. SC = simple genotype code for 1 to N (N total number of clones in trial, assure that the same genotype has the same number across locations).
10. CL = Clone; 1 = new clone, 2 = check clone

4b) Pre-harvest data sheet

11. NOPS = number of plants (cuttings) planted per plot.
12. NOPE = number of plants (cuttings) established per plot (to be determined 3 weeks after planting).
13. VIR1 = virus symptoms 1st evaluation (to be determined 4 to 6 weeks after planting); recorded in scores from 1 to 9, where 1 indicates no virus symptoms, 2 = unclear virus symptoms, 3 = Clear virus symptoms < 5% of plants per plot, 4 = Clear virus symptoms at 6 to 15% of plants per plot, 5 = Clear virus symptoms at 16 to 33% of plants per plot, 6 = Clear virus symptoms at 34 to 66% of plants per plot (more than 1/3, less than 2/3), 7 = Clear virus symptoms at 67 to 99 % of plants per plot (2/3 to almost all), 8 = Clear virus symptoms at all plants per plot (not stunted), 9 = Severe virus symptoms in all plants per plot (stunted).
14. VIR2 = virus symptoms 2nd evaluation (to be determined 1 month before harvest; recorded in scores from 1 to 9 as described for VIR1).
15. ALT1 = Alternaria symptoms 1st evaluation (to be determined 4 to 6 weeks after planting); recorded in scores from 1 to 9, where 1 indicates no symptoms, 2 = Unclear symptoms, 3 = Clear symptoms at <5% per

plot, 4 = Clear symptoms at 6 to 15% of plants per plot, 5 = Clear symptoms at 16 to 33% of plants per plot (less than 1/3), 6 = Clear symptoms at 34 to 66% of plants per plot (more than 1/3, less than 2/3), 7 = Clear symptoms at 67 to 99 % of plants per plot (2/3 to almost all), 8 = Clear symptoms at all plants (not fully defoliated), 9 = Severe symptoms at all plants per plot (fully defoliated).

16. ALT2 = Alternaria symptoms, 2nd evaluation (to be determined 1 month before harvest; recorded in scores from 1 to 9 as described for ALT1).
17. VV = vine vigor 1st evaluation (to be determined 1 month before harvest; recorded in scores from 1 to 9, where 1 = nearly no vines, 2= weak vines, thin stems, very long internode distances, 3 = weak to medium strong vines, medium thick stems, and long internode distances, 4 = medium strong vines, medium thick stems, and medium internode distances, 5 = medium strong vines, thick vines, and long internode distances, 6 = medium strong vines, thick stems, and medium internode distances, 7 = Strong vines, thick stems, short internode distances, and medium-long vines, 8 = Strong vines, thick stems, short internode distances, and long vines, 9 = very strong vine strength, thick stems, short internode distances, and very long vines).

4c) Sweetpotato Harvest

18. NOPH = Number of plants harvested.
19. NOPR = Number of plants with storage roots.
20. NOCR = Number of commercial storage roots per NET plot
21. NONC = Number of non-commercial storage roots per NET plot
22. CRW = weight of commercial storage roots per NET plot in kg
23. NCRW = weight of non- commercial storage roots per NET plot in kg
24. VW = weight of vines per NET plot in kg
25. SCOL = storage root skin color where 1 = white, 2 = cream, 3 = yellow, 4 = orange, 5 = brownish orange, 6 = pink, 7 = red, 8 = purple red and 9 = dark purple.
26. FCOL = storage root flesh color to be determined on 4 storage roots per plot using CIP color chart, noting the page number from the color chart on the data sheet. If you don't have a color chart, use a 1 to 9 scale where 1 = white, 2 = cream, 3 = dark cream, 4 = pale yellow, 5 = dark yellow, 6 = pale orange, 7 = intermediate orange, 8 = dark orange, and 9 = strongly pigmented with anthocyanins (purple). [Note: some may find it more convenient to determine skin and flesh color in the laboratory using samples taken for dry matter determination.]
27. RS = Overall assessment of storage root size based on inspection of the harvested roots. Use a 1 to 9 scale, where 1 = excellent, 3 = good, 5 = fair, 7 = poor and 9 = terrible, with numbers in between representing intermediate ratings.
28. RF = Overall assessment of storage root form based on inspection of the harvested roots. Use a 1 to 9 scale, where 1 = excellent, 3 = good, 5

= fair, 7 = poor and 9 = terrible, with numbers in between representing intermediate ratings.

29. DAMR = Note storage root defects if prominent, including cracks, veins, constrictions and grooves, or a predominance of pencil roots.
30. WED1 = Overall assessment of weevil damage based on inspection of the harvested roots. Use a 1 to 9 scale, where 1 = no damage, 3 = minor, 5 = moderate, 7 = heavy and 9 = severe damage, with numbers in between representing intermediate ratings.

4d) Sweetpotato Quality.

Note in PTs only the top fraction of clones (15 to 25% of all PT clones) needs to be determined, whereas in ATs all clones have to be determined

31. DMF = fresh weight of storage root samples (roughly 200g recommended sample size)
32. DMD = dry weight of storage root samples
33. DMM = dry matter assessment method (1- Sun-dried, 2- Laboratory oven dried, 3- freeze dried, 4-specific gravity)
34. COOF = Fibers in cooked storage root samples assessed by inspection and tasting. Use a 1 to 9 scale where 1 = non-fibrous, 3 = slightly fibrous, 5 = moderately fibrous, 7 = fibrous and 9 = very fibrous, with numbers in between representing intermediate ratings.
35. COOSU= storage root sweetness in cooked samples, determined by taste test. Use a 1 to 9 scale where 1 = non-sweet, 3 = slightly sweet, 5 = moderately sweet, 7 = sweet and 9 = very sweet, with numbers in between representing intermediate ratings.
36. COOST= storage root texture in cooked samples, determined by taste test. Use a 1 to 9 scale where 1 = very moist, 3 = moist, 5 = moderately dry, 7 = dry and 9 = very dry, with numbers in between representing intermediate ratings.
37. COOT = Overall taste of cooked samples assessed using a 1 to 9 scale where 1 = excellent, 3 = good, 5 = fair, 7 = poor and 9 = horrible, , with numbers in between representing intermediate ratings.
38. COOAP= Appearance of cooked samples assessed using a 1 to 9 scale where 1 = very appealing, 3 = appealing, 5 = somewhat appealing, 7 = unappealing, 9 = very unappealing, with numbers in between representing intermediate ratings.

FORM 5a. Sweetpotato farmer participator field evaluation

To be carried out at one AT location. Farmers are asked to give their opinion about each genotype in the AT in one plot replication by "providing their cards (green = good, yellow = medium, red = unacceptable – men are given cards

marked with M, women are given cards marked with F) on the following **seven attributes and traits, respectively"**:

1. The ability to produce enough planting material (foliage production);
2. The ability to tolerate diseases, especially SPVD;
3. The ability to tolerate pest damage (mainly weevils);
4. The yielding ability (i.e. number and size of mature roots);
5. The attractiveness of the root skin colour. Probe more to know which colour(s) are most preferred and why they are preferred;
6. The attractiveness of the root flesh colour? Probe more to know which colour(s) are most preferred and why?
7. Overall opinion on the acceptability of the variety?

Data are recorded on plot basis and card frequencies [number of red (R), yellow (Y), and green (G) cards] on the form using count data – give the number of red, yellow and red cards provided by the farmer group by gender for each trait.

4. SUGGESTIONS FOR DATA ANALYSIS AND CLONAL SELECTION

Several variables can be derived from the raw data of agronomic trials and can be effective in evaluating the performance of clones. Here we consider only the total storage root yield per hectare and storage root dry matter content as a must:

Yield of total Roots Per Hectare **RYTha = (CRW + NCRW)/NET plot area in m2) x 10.**

Storage root dry matter content **DM = (DMF / DMD) x 100.**

Further variables derived from raw data should be calculated within the analysis of statistical program packages. Suggestions for further variables to calculate are:

Survival or Establishment **(SHI)= (NPH / Number of cuttings per NET plot area) x 100.**

Harvest Index (HI) = 100 x CRW + NCRW/(VW + CRW + NCRW), i.e. root weight/total root + foliage weight)

4.1 Statistic Program Packages

CIP provides statistical support for data recorded with the Forms 4b and 4c. The support is restricted to the program packages PLABSTAT (a free to down load program from the internet), SAS and R.

PLABSTAT is a statistical program for plant breeders written by a plant breeder. It provides in the output important parameters such as the variance components, the least significant difference (LSD), heritability, stability parameters (ecovalence, slope of the regression line and deviations from the regression line), as well as covariances and genotypic correlations.

SAS is a widely available statistical program package, but SAS requires a deeper statistical knowledge to be used: Variance components must be calculated by hand from MQ values if your data have missing values (exception is proc mixed). Moreover, you can use many different multiple comparison procedures (LSD, Tukey, Scheffe, and Dunnet). Heritability, stability parameters, and analysis of covariances and genotypic correlations can only be calculated by user-written programs in SAS-IML. However, SAS-IML allows you write your own programs for AMMI analysis, index selection procedures for several characters, etc., and are readily available for sharing among breeders.

R is a free programming language and software environment for statistical computing.

As with SAS, it requires a high statistical knowledge and programming abilities to be used. Linear models can be fitted with the base distribution of R. To fit linear and nonlinear mixed effects models you will need to download and install library nlme. The base distribution, contributed packages and documentation about R can be found at http://www.r-project.org.

4.2 Data Analysis Example

Data set

The example data set was taken from Mega Clone trials. It was reduced to 5 clones (SantoAmaro, Jonathan, Resisto, Xushu18, Tanzania) and 3 locations (Chiclayo, La_Molina, San_Ramon). Our Mega Clone trials generally have only 2 replications. The data set comprises

1) **the classification variables L, Y, S, Check, Geno** (here the CIP number), **G** and **R** (see forms in Appendix 1) with the additional variable **Name** (because clone are already varieties with a name).
2) the observation variables **VY** (vine yield) and **FYLDha** (VY per hectare) from the Vine Observation Data (forms 4B & 4C),
3) the observation variables **TRW =** CRW+NCRW, **NOPH**, **SHI** = NOPH x Number of cuttings per NET plot area) x 100, **TYLDha** (TRW per hectare) from the Root Observation Data (form 4C),
4) the observation variables **DMM**, **DM** = DMD **/** DMF x 100, **BCM** and **BC** from the Quality Observation Data (form 4D),
5) no observation variables were taken from Vine Survival and Piecemeal Harvest Quality.

Note that some of the abbreviations used in the data analysis may differ slightly from those on the forms in Appendix 1.

Y	L	S	CHECK	GENO	NAME	G	R	VY	TRW	NOPH	SHI	FYLDha	TYLDha	DMM	DM	BCM	BC
2006	Chiclayo	summer	NO	400011	SantoAmaro	1	1	13.7	66.0	11	36.67	10.15	48.89	2	33.33	4	-39.30
2006	Chiclayo	summer	NO	400011	SantoAmaro	1	2	17.1	34.0	18	60.00	12.67	25.19	2	37.25	4	-60.43
2006	Chiclayo	summer	YES	420014	Jonathan	2	1	12.5	46.3	13	43.33	9.26	34.30	2	28.88	4	146.07
2006	Chiclayo	summer	YES	420014	Jonathan	2	2	12.5	27.8	12	40.00	9.26	20.59	2	32.60	4	*
2006	Chiclayo	summer	NO	440001	Resisto	3	1	9.3	18.6	11	35.00	6.89	13.78	2	26.59	4	442.26
2006	Chiclayo	summer	NO	440001	Resisto	3	2	8.9	29.5	16	51.67	6.59	21.85	2	26.98	4	204.52
2006	Chiclayo	summer	NO	440025	Xushu18	4	1	14.7	27.0	16	53.33	10.89	20.00	2	30.28	4	-60.14
2006	Chiclayo	summer	NO	440025	Xushu18	4	2	10.7	52.0	18	60.00	7.93	38.52	2	29.08	4	-92.69
2006	Chiclayo	summer	NO	440166	Tanzania	5	1	16.2	7.5	12	38.33	12.00	5.56	2	33.40	4	-93.45
2006	Chiclayo	summer	NO	440166	Tanzania	5	2	20.1	39.0	16	53.33	14.89	28.89	2	37.80	4	-97.61
2006	La Molina	summer	NO	400011	SantoAmaro	1	1	37.0	12.0	19	63.33	54.81	17.78	2	31.69	4	-26.88
2006	La Molina	summer	NO	400011	SantoAmaro	1	2	53.5	6.0	24	80.00	79.26	8.89	2	31.04	4	-18.87
2006	La Molina	summer	YES	420014	Jonathan	2	1	29.0	8.5	16	53.33	42.96	12.59	2	25.79	4	572.40
2006	La Molina	summer	YES	420014	Jonathan	2	2	30.0	3.5	20	66.67	44.44	5.19	2	26.77	4	174.80
2006	La Molina	summer	NO	440001	Resisto	3	1	22.0	19.0	15	50.00	32.59	28.15	2	23.61	4	629.40
2006	La Molina	summer	NO	440001	Resisto	3	2	41.0	22.5	24	80.00	60.74	33.33	2	23.61	4	653.90
2006	La Molina	summer	NO	440025	Xushu18	4	1	32.0	25.5	25	83.33	47.41	37.78	2	30.63	4	-14.03
2006	La Molina	summer	NO	440025	Xushu18	4	2	37.0	30.2	27	90.00	54.81	44.74	2	29.70	4	-13.87
2006	La Molina	summer	NO	440166	Tanzania	5	1	56.0	7.0	24	80.00	82.96	10.37	2	32.47	4	-12.51
2006	La Molina	summer	NO	440166	Tanzania	5	2	90.0	14.0	26	86.67	133.33	20.74	2	32.74	4	-7.59
2006	San Ramon	summer	NO	400011	SantoAmaro	1	1	18.2	18.1	20	66.67	26.96	26.81	2	30.65	4	-21.46
2006	San Ramon	summer	NO	400011	SantoAmaro	1	2	4.2	5.8	22	73.33	6.22	8.59	2	*	4	*
2006	San Ramon	summer	YES	420014	Jonathan	2	1	3.9	8.5	19	63.33	5.78	12.59	2	35.25	4	119.70
2006	San Ramon	summer	YES	420014	Jonathan	2	2	6.2	7.9	27	90.00	9.19	11.70	2	28.83	4	147.60
2006	San Ramon	summer	NO	440001	Resisto	3	1	6.4	10.1	25	83.33	9.48	14.96	2	33.00	4	498.10
2006	San Ramon	summer	NO	440001	Resisto	3	2	13.4	21.4	28	93.33	19.85	31.70	2	28.94	4	483.30
2006	San Ramon	summer	NO	440025	Xushu18	4	1	5.1	16.0	27	90.00	7.56	23.70	2	33.04	4	-8.11
2006	San Ramon	summer	NO	440025	Xushu18	4	2	1.3	2.0	15	50.00	1.93	2.96	2	31.21	4	-12.53
2006	San Ramon	summer	NO	440166	Tanzania	5	1	11.4	6.1	17	56.67	16.89	9.04	2	33.93	4	-12.87
2006	San Ramon	summer	NO	440166	Tanzania	5	2	11.0	4.1	18	60.00	16.30	6.07	2	34.73	4	-14.84

Model

The statistical model is

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \gamma_{k(j)} + \varepsilon_{ijl}$$

where:

- Y_{ijk} is the response variable (TYLDha, DM or BC) with genotype *i*, at location *j*, repetition *k*.
- α_i is the fixed effect of genotype *i*.
- β_j is the random effect of location *j*. We assume that β_j has a normal distribution with mean 0 and variance σ_b^2.
- $(\alpha\beta)_{ij}$ is the random interaction effect between genotype *i* and location *j*.
- $\gamma_{k(j)}$ is the random effect of repetition whiting location *j*.
- ε_{ijk} is the random error term. We assume that ε_{ijk} has a normal distribution with mean 0 and variance σ_e^2.

Not always nice to read, but useful for publications. It is important is to know the difference between fixed and random effects. If we compare means of genotypes in advanced multi-location trials the effect of genotypes is always fixed and all other effects are random. If we want information about variance component and heritabilities all effects are random.

4.3 Computations for our example using PLABSTAT

PLABSTAT Input

Important: In the way we use PLABSTAT here the data must be sorted according to the order of the factors in the 'factor' statement. In our example location, genotype and replication. PLABSTAT reads the data lines (rows) according to this sorted order and if the data lines or rows are not sorted in this order our results would be a meaningless mess due to mixed up factor levels.

```
'REFERENCE' 5 Megaclones at 3 Locations
'FACTORS' L=3 G=5 R=2
'MODEL' L + G + LG + R:L + RLG
'ANOVA/1111' 8 8 8
'VARIABLE_NAMES' VY TRW NOPH SHI FYLDha TYLDha DMM DM BCM BC
'NAMES_OF_TR/L' Chiclayo La_Molina San_Ramon
'NAMES_OF_TR/G' SantoAmaro Jonathan Resisto Xushu18 Tanzania
'RANDOM' L R
'HERITAB' G
'SUBINT'LG
'MEAN' GL
'TBT_TAB' GL
'RUN'
2006 Chicl sum NO  400011 SantoAmaro 1 1 13.7 66.0 11 36.67  10.15 48.89 2 33.33 4 -39.30
2006 Chicl sum NO  400011 SantoAmaro 1 2 17.1 34.0 18 60.00  12.67 25.19 2 37.25 4 -60.43
2006 Chicl sum YES 420014 Jonathan   2 1 12.5 46.3 13 43.33   9.26 34.30 2 28.88 4 146.07
2006 Chicl sum YES 420014 Jonathan   2 2 12.5 27.8 12 40.00   9.26 20.59 2 32.60 4      *
2006 Chicl sum NO  440001 Resisto    3 1  9.3 18.6 11 35.00   6.89 13.78 2 26.59 4 442.26
2006 Chicl sum NO  440001 Resisto    3 2  8.9 29.5 16 51.67   6.59 21.85 2 26.98 4 204.52
2006 Chicl sum NO  440025 Xushu18    4 1 14.7 27.0 16 53.33  10.89 20.00 2 30.28 4 -60.14
2006 Chicl sum NO  440025 Xushu18    4 2 10.7 52.0 18 60.00   7.93 38.52 2 29.08 4 -92.69
2006 Chicl sum NO  440166 Tanzania   5 1 16.2  7.5 12 38.33  12.00  5.56 2 33.40 4 -93.45
2006 Chicl sum NO  440166 Tanzania   5 2 20.1 39.0 16 53.33  14.89 28.89 2 37.80 4 -97.61
2006 La_Mo sum NO  400011 SantoAmaro 1 1 37.0 12.0 19 63.33  54.81 17.78 2 31.69 4 -26.88
2006 La_Mo sum NO  400011 SantoAmaro 1 2 53.5  6.0 24 80.00  79.26  8.89 2 31.04 4 -18.87
2006 La_Mo sum YES 420014 Jonathan   2 1 29.0  8.5 16 53.33  42.96 12.59 2 25.79 4 572.40
2006 La_Mo sum YES 420014 Jonathan   2 2 30.0  3.5 20 66.67  44.44  5.19 2 26.77 4 174.80
2006 La_Mo sum NO  440001 Resisto    3 1 22.0 19.0 15 50.00  32.59 28.15 2 23.61 4 629.40
2006 La_Mo sum NO  440001 Resisto    3 2 41.0 22.5 24 80.00  60.74 33.33 2 23.61 4 653.90
2006 La_Mo sum NO  440025 Xushu18    4 1 32.0 25.5 25 83.33  47.41 37.78 2 30.63 4 -14.03
2006 La_Mo sum NO  440025 Xushu18    4 2 37.0 30.2 27 90.00  54.81 44.74 2 29.70 4 -13.87
2006 La_Mo sum NO  440166 Tanzania   5 1 56.0  7.0 24 80.00  82.96 10.37 2 32.47 4 -12.51
2006 La_Mo sum NO  440166 Tanzania   5 2 90.0 14.0 26 86.67 133.33 20.74 2 32.74 4  -7.59
2006 San_R sum NO  400011 SantoAmaro 1 1 18.2 18.1 20 66.67  26.96 26.81 2 30.65 4 -21.46
2006 San_R sum NO  400011 SantoAmaro 1 2  4.2  5.8 22 73.33   6.22  8.59 2     * 4      *
2006 San_R sum YES 420014 Jonathan   2 1  3.9  8.5 19 63.33   5.78 12.59 2 35.25 4 119.70
2006 San_R sum YES 420014 Jonathan   2 2  6.2  7.9 27 90.00   9.19 11.70 2 28.83 4 147.60
2006 San_R sum NO  440001 Resisto    3 1  6.4 10.1 25 83.33   9.48 14.96 2 33.00 4 498.10
2006 San_R sum NO  440001 Resisto    3 2 13.4 21.4 28 93.33  19.85 31.70 2 28.94 4 483.30
2006 San_R sum NO  440025 Xushu18    4 1  5.1 16.0 27 90.00   7.56 23.70 2 33.04 4  -8.11
2006 San_R sum NO  440025 Xushu18    4 2  1.3  2.0 15 50.00   1.93 12.96 2 31.21 4 -12.53
2006 San_R sum NO  440166 Tanzania   5 1 11.4  6.1 17 56.67  16.89  9.04 2 33.93 4 -12.87
2006 San_R sum NO  440166 Tanzania   5 2 11.0  4.1 18 60.00  16.30  6.07 2 34.73 4 -14.84
'EOD'
'STOP'
```

- After the 'REFERENCE' statement, the 1st statement line gives you the option for a reference, comment or name of the data set you are going to analyze.

- In the 2nd statement line you have to specify the factors in your experiment. Here we have 3 factors (Location, genotype and replication) named L G R. The 1st factor – location - has three factor levels (L=3). The 2nd factor - genotype - has five factor levels (G=5). The 3rd factor has two factor levels. Again note that the way we use PLABSTAT here the data have to be sorted by location, genotype and replication prior to the analysis.

- In the 3rd statement line, specify the model (after the 'MODEL' statement). In our case a complete randomized block experiment (CRBE) carried out at a series of locations [see also randomized complete block design with 1 factor in a series over places in the PLABSTAT manual (English version)]. In the APPENDIX C of the PLABSTAT manual a VERY useful collection of 'MODEL' statement lines for experimental designs is given.

- With **the 'ANOVA' statement** you read data and conduct an analysis of variance for balanced data (missing values up to 15% will be estimated to obtain a balanced data set). The qualifier after the forward slash "/" is used for controlling input and output. The qualifier Q consists of 4 digits namely MISS, EXTR, PRIN, and NEWF (e.g. 1111: MISS = 0 zeros are not interpreted as missing values or MISS = 1 zeros are interpreted as missing values (default); EXTR = 0 no test on extreme values or outliers or EXTR = 1 test of residuals on extreme values (default); EXTR = 2 test of residuals and effects on extreme values (for PRIN, and NEWF please see PLABSTAT manual). The qualifier is followed by 3 numbers. The 1st is used for the number of variables (columns) to be ignored for the analysis of variance – these are the variables (columns) that are used as classification variables for our data set. In our example (see below) the first 8 columns (see 1st row: 2006 Chicl sum NO 400011 SantoAmaro 1 1) are used as classification variables for our data set. The 2nd number is used for the number of variables (columns) to be read for the analysis of variance. The 3rd number is used for the number of variables to be analyzed in the analysis of variance.

- With the statement 'VARIABLE_NAMES' you can assign names to the 8 variables to be read and the 8 variable to be analyzed by the ANOVA in our example.

- With the statement ' NAMES_OF_TR/L' you can assign names to the 3 levels of the factor L.

With the statement ' NAMES_OF_TR/G' you can assign names to the 5 levels of the factor G .

- With **the 'RANDOM' statement** you can define the random factors (and random effects) in your design. All factors not listed are assumed to be fixed. This statement results in changes of the error term used for testing the different effects in the analysis of variance. For example if G and L are fixed, the main effects of G and L, as well as the interaction term (GxL), are tested against the error term in the F-test. However, if G is fixed and L is random, the main effect of G must be tested against the interaction term (GxL), whereas the main effects of L and the interaction term (GxL) must be tested against the error term in the F-test.

> Note 1: In all cases the factor replication (R) is a random factor.
>
> Note 2: In 99.9% of all cases in plant breeding the factor location (L) is a random factor!!!
>
> Note 3: The factor genotype (G) is a fixed factor when you want to compare mean differences among genotypes for example by the least significant difference (LSD) test. However, factor genotype (G) is a random factor when you want to estimate and compare variance components as well as heritabilities.

- The statement 'HERITAB' requests the calculation of heritabilities on the basis of the variance component estimations in the ANOVA – this is usually done for the factor genotype (G).

- The **statement 'SUBINT'** requests the calculation of a stability analysis and stability parameters for the interaction term [in our case the genotype by environment interaction (LG)]. The stability parameters (slope of regression lines, deviations from regression lines, ecovalence, etc.) are calculated for both factors (in our case genotypes and locations).

> Note 4: In plant breeding the stability of environments is often of interest, because breeders want to select in environment in which they can distinguish well between genotypes.

- The statement 'MEAN' requests a table with means across the factor levels and by factor levels.

- The statement 'TBT_TAB' allows you to write the calculations of the 'MEAN' statement in a file separate from the rest of the output file for further analysis (e.g. AMMI analysis or index selection procedure).

- With the statement 'RUN' PLABSTAT starts to read your data set.

- With the statement 'EOD' PLABSTAT stops reading your data set.

- With the statement 'STOP' the PLABSTAT program stops (exit and no further analysis).

Note 5: You can run several analyses (several blocks from statement 'REFERENCE' until statement 'EOD'). In this way you can analyze your data for all factors random and for 1 factor (genotype) fixed with all other factors random.

PLABSTAT Output

From the PLABSTAT output of the example "Mega Clone Trial" and the 8 variables to analyze we selected here 3 variables namely TYLDh, DM, and BC.

```
      TYLDh    DM      BC
MIN    5.19   23.61  -97.61
MAX   48.89   37.80  653.90
```

This output allows you to identify in a 1st step outliers in our data set. Values which are clearly out of the biological range [e.g. a TYLDh of 448.89 (a total root yield of 448 tones per hectare)] must be set to "*" in your input data set, which is the symbol for a missing value in PLABSTAT – such values were not observed in our example. Note: negative values for ß-carotene are possible if these were estimated by NIRS.

```
----------  Character  1  TYLDh   ----------
Please check for outliers (test after ANSCOMBE and TUKEY)

Source   DF       SS           MS         Var.cp      s(V.cp)      F      DF-NM   DF-DN   s.e.   LSD5
L         2    503.6645     251.8323     23.6707      17.8329   16.65*     2.00    3.00   1.23   5.53
G         4    997.3840     249.3460     13.9130      26.9912    1.50      4.00    8.00   5.26  17.15
GL        8   1326.9457     165.8682     27.4119      42.6146    1.49      8.00   12.00   7.45  22.96
R:L       3     45.3755      15.1252    -19.1839       8.6094    0.14      3.00   12.00   4.71  14.52
RGL      12   1332.5335     111.0445    111.0445      41.9709
Total    29   4205.9032
HERITAB  33.48 (-497.81  86.82)
```

These are the results for the analysis of variance which includes in PLABSTAT the variance components (Var.cp), which are very important parameters for plant breeding.

The asterisk after the F value for L indicates that this effect is significant and the least significant differences at the 5% level (LSD5) are given in the last column, with the value for L (5.53) being the only one of interest since the G and GL effects are not significant.

--------- Subdivision of two-way table G * L ---------

COMPOUND ANOVA

Source of variation	DF	SS	MS	Varcomp	Fvalue
G *L	8	1326.9457	165.8682	27.4119	1.49
Non-additiv (TUKEY)	1	1.5572	1.5572	-6.2595	0.01
Het.Regr.G	3	264.4549	88.1516	-28.4489	0.34
Het.Regr.L	1	284.3989	284.3989	2.5554	1.10
Deviat. from regr.	3	776.5347	258.8449	73.9002	2.33

Regr.coeff. of interaction effects on product of both main effects C = -0.00964

Estimates for the factor G

Level	Mean	Corr.	Regr.	MSdev	MSentry	MSinteract.	MSdevXHY
1 Santo	22.7	0.6729	1.692	174.19	159.17	99.15	218.09
2 Jonat	16.2	0.6794	1.341	105.69	98.16	55.78	161.67
3 Resis	24.0	-0.2993	-0.387	76.59	42.06	86.73	92.67
4 Xushu	29.6	0.5906	1.350	171.30	131.54	88.74	15.54
5 Tanza	13.4	0.9745	1.004	2.69	26.72	1.34	24.94

Estimates for the factor L

Level	Mean	Corr.	Regr.	MSdev	MSentry	MSinteract.	MSdevXHY
1 Chicl	25.8	0.3814	0.494	79.27	69.57	70.11	46.70
2 La_Mo	22.0	0.8274	1.741	77.37	183.95	80.83	130.76
3 San_R	15.8	0.8114	0.766	16.86	37.01	14.93	41.95

These are the results for the stability analysis of variance. Note: Neither the heterogeneity due to the regression on genotypes (Het.Regr.G) nor the heterogeneity due to the regression on locations are significant (Het.Regr.L). Important stability parameters for genotypes and locations are the slope of the regression line (Regr.) – should be close to 1, the deviations from the regression line (MSdev) – should be close to zero, and the ecovalence (MSinteract.) – should be low.

The yield differences between genotypes are remarkable [i.e. between Tanzania (13.4 t/ha) and Xushu (29.6 t /ha), but they are not significant due to the very large LSD5% of 17.15]. Please note that "no significant differences" does not mean there are no differences – the large differences in the example were simply not possible to verify at the 5% significance level because of the relative large error. The ratio of variance components in this experiment for genotype : genotype by location interaction : error was 13.91 : 27.41 : 111.04 (1 : 1.97 : 7.98). This is a very extreme ratio. Usually the ratio of variance components are not so extreme in sweetpotato (see Grüneberg et al., 2005). This is also reflected by the low heritability for storage root yield of 33.48. This is to low for advanced yield trials. Here it is recommended to carefully check the data for suspicious values (indeed, Xushu18 had a yield of 23.70 t/ha in replication 1 in San_R and a yield of 2.96 t/ha in replication 2 in San_R. This is very suspect against the background that the clone Xushu18 has a yield of 29.6 t/ha across locations. In such cases it might be worthwhile to check the original data and/or to use a "better" estimation of 12.96 t /ha, or by setting this value to a missing value "*" and re-analyzing the data.

```
----------  Character  2  DM      ----------
Missing data    1
    4  Iterations
       38    27.772

Please check for outliers (test after ANSCOMBE and TUKEY)

Source   DF       SS           MS         Var.cp      s(V.cp)      F      DF-NM   DF-DN   s.e.   LSD5
L         2     55.0616      27.5308      1.6423       2.0696    2.48      2.00    3.00   1.05   4.74
G         4    165.0367      41.2592      5.0079       4.0572    3.68+     4.00    8.00   1.37   4.46
GL        8     89.6934      11.2117      4.3376       2.5559    4.42*     8.00   11.00   1.13   3.51
R:L       3     33.3222      11.1074      1.7142       1.4190    4.38*     3.00   11.00   0.71   2.22
RGL      11     27.9010       2.5365      2.5365       0.9949
Total    28    371.0149
HERITAB  72.83 (-144.20  94.62)
Note:  Tests approximative, since treatment variances are overestimated in case of missing data
***  NO CORRECTION OF DEGREES OF FREEDOM FOR MISSING VALUES IN SUBINT
```

The results of the analysis of variance of DM are next. There are striking differences among the dry matter content of genotypes (F value of 3.68+), but no significant difference (all differences among genotypes are smaller than the LSD5 (4.46). The genotype by environment interaction is significant (F value of 4.42*), but we are not interested in comparing the means of genotypes by location, since we considered the factor location to be random. The heritability for storage root dry matter content is high (72.83), which is typical for this quality trait.

We do not examine the results of the stability analysis of DM, since stability analysis of plant quality parameters like dry matter, starch, sugars, carotenoids and minerals is usually not very useful.

```
----------  Character  3  BC      ----------
Missing data    2
    4  Iterations
        5    72.175       38   -19.783

Please check for outliers (test after ANSCOMBE and TUKEY)
Suspect%  117    in RGL     1   2   2    obs. =  572.4
Suspect% -117    in RGL     2   2   2    obs. =  174.8

Source   DF        SS            MS         Var.cp     s(V.cp)      F      DF-NM   DF-DN   s.e.    LSD5
L         2  114840.1179    57420.0589   4854.7080   4098.8090   6.47+     2.00    3.00  29.79  134.06
G         4  1275770.5442   318942.6361  51326.5752  30701.1858  29.04**   4.00    8.00  42.78  139.53
GL        8    87865.4777    10983.1847   1239.9849   3007.3596   1.29     8.00   10.00  65.20  205.46
R:L       3    26618.9355     8872.9785     73.9527   1319.7375   1.04     3.00   10.00  41.24  129.95
RGL      10    85032.1487     8503.2149   8503.2149   3471.4229
Total    27  1590127.2240
HERITAB  96.56 (  69.05  99.32)
Note:  Tests approximative, since treatment variances are overestimated in case of missing data
***  NO CORRECTION OF DEGREES OF FREEDOM FOR MISSING VALUES IN SUBINT
```

Results of the analysis of ß-carotene come next. There are significant differences among the ß-carotene contents of genotypes (F value of 29.04**), so differences among genotypes will be found which are larger than the LSD5 (139.53). The genotype by environment interaction is not significant (F value of 1.29). The heritability for ß-carotene content is remarkable high (96.56) with a 95% confidence lower limit of 69.05 and upper limit of 99.32. Note: There is a suspect value of 572.4 in rep 2 at La Molina. The analysis of BC can be probably be improved by setting the highly unlikely value of 572.4 to 174.8 or designating it as missing (*).

4.4 Computations for our example using SAS

SAS Input Example

Now we illustrate how to fit the model with SAS. The stability analysis is not possible here but CIP will make SAS IML programs available for region analysis and AMMI.

In the first section the data is loaded.
- The 1st statement line gives a name for the data, in this case "All".
- The 2nd statement line gives the names of the variables in the data. Non-numeric variables must be followed by $ sign.
- The **cards** statement indicates that the data lines follow immediately. Missing values are indicated with dots in SAS.
- The **run** statement after the data lines reads the data.

In the second section the **Proc means** is used to calculate means, minima and maxima.
- In the 1st line, the **data=All** statement tells **Proc means** that the data set with name **All** must be used.
- In the 2nd statement **Var** tells **Proc means** which observation variables (here TYLDha DM BC) the **Proc means** has to use to calculate the mean, standard deviation, minimum and maximum values.
- The statement **run** ends each proc section – here the section proc means.

In the third section the **Proc glm** is used to fit a general linear model (not to be confused with with the Generalized Linear Model) in order to get the analysis of variance results.
- In the 1st line, the **data=All** statement tells **Proc glm** that the data with name **All** must be used.
- The **class** statement indicates the classification variables (factors) that are going to be included in the analysis.
- The **model** statement indicates the response variable (to the left side of =) and the complete model specification, that is, the fixed and random factors as well as their interactions (to the right side of =). Since **Proc glm** assumes fixed effects, due to this statement we will get an analysis of variance where all the effects are considered as fixed. Note that the F test for factor G would not be valid since its mean square must be compared with the interaction mean square and not with the error mean square.
- The **random** statement indicates the factors and interactions which are random. The **/test** statement ask for the F tests for these effects. Here SAS take into account which effects are fixed and which are random to calculate appropriate F ratios.
- Alternatively we can ask for specific tests with the statement **test** in a new line. For instance, the statement **test H=G E=L*G** will considered **G** as the main effect to evaluate and **L*G** as the error term for the F ratio.
- The **lsmeans** statement computes least-squares means (LS-means). In this case we are asking LS-means for the levels of factor **G**. After the / sign some options are defined. **cl** requests confidence limits for the individual LS-means or for differences between pairs. **pdiff** requests p-values for differences of the LS-means, and for these differences several adjustments are available. Here, **adjust = T** signifies no adjustment for multiple comparisons, so a Student-t distribution based confidence interval is computed. **E=L*G** specifies the effect of the model to use as error term.

- Finally the **run** statement tells SAS to run the **Proc glm** computations.

```
data All;
input Y L $ S $ GENO NAME $ G R TYLDha DM BC;
cards;
2006  Chiclayo   summer  400011  SantoAmaro  1  1  48.89  33.33  -39.30
2006  Chiclayo   summer  400011  SantoAmaro  1  2  25.19  37.25  -60.43
2006  Chiclayo   summer  420014  Jonathan    2  1  34.30  28.88  146.07
2006  Chiclayo   summer  420014  Jonathan    2  2  20.59  32.60  .
2006  Chiclayo   summer  440001  Resisto     3  1  13.78  26.59  442.26
2006  Chiclayo   summer  440001  Resisto     3  2  21.85  26.98  204.52
2006  Chiclayo   summer  440025  Xushu18     4  1  20.00  30.28  -60.14
2006  Chiclayo   summer  440025  Xushu18     4  2  38.52  29.08  -92.69
2006  Chiclayo   summer  440166  Tanzania    5  1  5.56   33.40  -93.45
2006  Chiclayo   summer  440166  Tanzania    5  2  28.89  37.80  -97.61
2006  La_Molina  summer  400011  SantoAmaro  1  1  17.78  31.69  -26.88
2006  La_Molina  summer  400011  SantoAmaro  1  2  8.89   31.04  -18.87
2006  La_Molina  summer  420014  Jonathan    2  1  12.59  25.79  572.40
2006  La_Molina  summer  420014  Jonathan    2  2  5.19   26.77  174.80
2006  La_Molina  summer  440001  Resisto     3  1  28.15  23.61  629.40
2006  La_Molina  summer  440001  Resisto     3  2  33.33  23.61  653.90
2006  La_Molina  summer  440025  Xushu18     4  1  37.78  30.63  -14.03
2006  La_Molina  summer  440025  Xushu18     4  2  44.74  29.70  -13.87
2006  La_Molina  summer  440166  Tanzania    5  1  10.37  32.47  -12.51
2006  La_Molina  summer  440166  Tanzania    5  2  20.74  32.74  -7.59
2006  San_Ramon  summer  400011  SantoAmaro  1  1  26.81  30.65  -21.46
2006  San_Ramon  summer  400011  SantoAmaro  1  2  8.59   .      .
2006  San_Ramon  summer  420014  Jonathan    2  1  12.59  35.25  119.70
2006  San_Ramon  summer  420014  Jonathan    2  2  11.70  28.83  147.60
2006  San_Ramon  summer  440001  Resisto     3  1  14.96  33.00  498.10
2006  San_Ramon  summer  440001  Resisto     3  2  31.70  28.94  483.30
2006  San_Ramon  summer  440025  Xushu18     4  1  23.70  33.04  -8.11
2006  San_Ramon  summer  440025  Xushu18     4  2  12.96   31.21  -12.53
2006  San_Ramon  summer  440166  Tanzania    5  1  9.04   33.93  -12.87
2006  San_Ramon  summer  440166  Tanzania    5  2  6.07   34.73  -14.84
run;

proc means data=all;
var TYLDha DM BC;
/* proc means compute the mean, the standard deviation, the minimum and
maximum for each variable in the data. Note this is a comment is starts with
(/*) and ends with */
run;

Proc glm data=All;
class L G R;
model TYLDha DM BC = L G L*G R(L);
random L L*G R(L) /test;
/* test H=G E=L*G */
lsmeans G / cl pdiff adjust=T E=L*G;
/* Two further important multiple comparison procedures
1) the Tukey test, which compares all possible differences among the factor
levels of G - in our example 5*(5-1)/2 = 10 differences. Note with more and
more differences the power of a test goes down */
/*lsmeans G / pdiff=all cl adjust=tukey E=L*G;*/
/*
2) the Dunnett test, which allows us to compare against a control. Here we test
against the factor level 2 of the factor G - this is the variety Jonathan - and
we test if the noncontrol levels are greater than the control */
/*lsmeans G / pdiff=controlu('2') cl adjust=dunnett E=L*G; */
run;
```

SAS Output

Here we have the expected mean squares for each source of variation and the ANOVA results computed with **Proc glm** for the TYLDha variable:

The GLM Procedure

Source	Type III Expected Mean Square
L	Var(Error) + 5 Var(R(L)) + 2 Var(L*G) + 10 Var(L)
G	Var(Error) + 2 Var(L*G) + Q(G)
L*G	Var(Error) + 2 Var(L*G)
R(L)	Var(Error) + 5 Var(R(L))

Please note that mean square estimates are not variance component estimates! However, variance component estimates can be calculated from mean square estimates with the above equations!

The GLM Procedure
Tests of Hypotheses for Mixed Model Analysis of Variance

Dependent Variable: TYLDha

Source	DF	Type III SS	Mean Square	F Value	Pr > F
L	2	503.664540	251.832270	3.60	0.3335
Error	1.077	75.337912	69.948928		
Error: MS(L*G) + MS(R(L)) - MS(Error)					

Source	DF	Type III SS	Mean Square	F Value	Pr > F
G	4	997.383900	249.345975	1.50	0.2885
Error: MS(L*G)	8	1326.945760	165.868220		

Source	DF	Type III SS	Mean Square	F Value	Pr > F
L*G	8	1326.945760	165.868220	1.49	0.2560
R(L)	3	45.375490	15.125163	0.14	0.9365
Error: MS(Error)	12	1332.533460	111.044455		

In contrast to PLABSTAT you do not get a note of significant effects on the 5% or 1% level by "*" or "**". Instead you get the probability of the F value (Pr > F) directly. In our case the probability is 0.2885 to get a result as the observed one in the case that the statement "no effect due to genotypes" is true.

For each response variable in the model statement of the procedure glm an output table is printed. In our example the expected mean squares for each source of variation and the ANOVA results computed for the DM variable are given below:

The GLM Procedure

Source	Type III Expected Mean Square
L	Var(Error) + 4.6429 Var(R(L)) + 1.8571 Var(L*G) + 9.2857 Var(L)
G	Var(Error) + 1.875 Var(L*G) + Q(G)
L*G	Var(Error) + 1.9 Var(L*G)
R(L)	Var(Error) + 4.6667 Var(R(L))

The GLM Procedure
Tests of Hypotheses for Mixed Model Analysis of Variance

Dependent Variable: DM

Source	DF	Type III SS	Mean Square	F Value	Pr > F
L	2	52.851783	26.425892	1.70	0.2608
Error	5.9894	93.312982	15.579698		

Error: 0.9774*MS(L*G) + 0.9949*MS(R(L)) - 0.9723*MS(Error)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
G	4	162.173806	40.543451	4.78	0.0286
Error	8.0632	68.392971	8.482073		

Error: 0.9868*MS(L*G) + 0.0132*MS(Error)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
L*G	8	68.490787	8.561348	3.38	0.0328
R(L)	3	29.182193	9.727398	3.84	0.0421
Error: MS(Error)	11	27.901058	2.536460		

There are significant differences among genotypes for DM on the 5% level – see Pr > F of 0.0286 for the source of variation G, which is smaller than 0.05.

In the following the expected mean squares for each source of variation and the ANOVA results given for the BC variable:

The GLM Procedure

Source	Type III Expected Mean Square
L	Var(Error) + 4.3077 Var(R(L)) + 1.7231 Var(L*G) + 8.6154 Var(L)
G	Var(Error) + 1.7529 Var(L*G) + Q(G)
L*G	Var(Error) + 1.8008 Var(L*G)
R(L)	Var(Error) + 4.3333 Var(R(L))

The GLM Procedure
Tests of Hypotheses for Mixed Model Analysis of Variance

Dependent Variable: BC

Source	DF	Type III SS	Mean Square	F Value	Pr > F
L	2	102657	51329	5.02	0.1303
Error	2.5552	26134	10228		

Error: 0.9569*MS(L*G) + 0.9941*MS(R(L)) - 0.9509*MS(Error)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
G	4	1222768	305692	28.29	<.0001
Error	8.3421	90133	10805		

Error: 0.9734*MS(L*G) + 0.0266*MS(Error)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
L*G	8	86939	10867	1.28	0.3512
R(L)	3	23887	7962.430024	0.94	0.4589
Error: MS(Error)	10	85032	8503.214093		

There are significant differences among genotypes for BC on the 1% level – see Pr > F of 0.0001 for the source of variation G, which is smaller than 0.01.

Considering the output of multiple comparison procedures first we have the LS-means values for the TYDLha variable. LS-means are better estimates than the mean values for the observed variables in cases where there are missing values. The results of the multiple comparison procedure based on a Student-t test are not given for TYLDha because the G effect was not significant in the F-test of the analysis of variance. However, not significant does not mean that there are no differences. Not significant means in a statistical test that the observed differences between factor levels are not significant compared to the error (in our case L*G). In cases where a significant L*G effect and a non-significant G effect is observed, care has to be taken. Compare variances and variance components due to G and L * G, and if the variance component due to interaction is larger than the variance component due to the main effect

genotype, it becomes interesting to look at the performance of genotypes across locations. Usually main effects are larger than interaction effects in biology. In our case the interaction effect L * G is significant and the main effect G is not significant, so your genotypes react very differently across locations. There might be patterns in response of genotypes to locations (genotypes with different adaptation to locations).

Least Squares Means
Standard Errors and Probabilities Calculated Using the Type III MS for L*G as an Error Term

G	TYLDha LSMEAN	LSMEAN Number
1	22.6916667	1
2	16.1600000	2
3	23.9616667	3
4	29.6166667	4
5	13.4450000	5

Second we present here the LS-means for the variable DM together with the p-values for differences between pairs of LS-means based on a Student-t test:

Least Squares Means
Standard Errors and Probabilities Calculated Using the Type III MS for L*G as an Error Term

G	DM LSMEAN	LSMEAN Number
1	31.9554167	1
2	29.6866667	2
3	27.1216667	3
4	30.6566667	4
5	34.1783333	5

Least Squares Means for effect G
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: DM

i/j	1	2	3	4	5
1	0.2566	0.0315	0.5041	0.2655	
2	0.2566	0.1674	0.5816	0.0289	
3	0.0315	0.1674	0.0697	0.0031	
4	0.5041	0.5816	0.0697	0.0706	
5	0.2655	0.0289	0.0031	0.0706	

In contrast to PLABSTAT the least significant difference is not given. SAS uses here another method to present the results of the Student-t test: The p-values of each comparison. The Student-t test indicates for our example significant differences between DM lsmean 1 and 3, lsmean 2 and 5, and lsmean 3 and 5 (see p values < 0.05).

Another way to present the results of the Student-t test follows below: The confidence limits of the lsmeans values. In our example the DM lsmean of genotype 5 is estimated with 34.178333% DM and there is a 95% confidence that the computed interval 31.178333% and 36.932914% contains the "true mean value" of genotype 5. Genotypes with non overlapping confidence limits are significantly different for the observed variable.

G	DM LSMEAN	95% Confidence Limits	
1	31.955417	28.676810	35.234024
2	29.686667	26.932086	32.441248
3	27.121667	24.367086	29.876248
4	30.656667	27.902086	33.411248
5	34.178333	31.423752	36.932914

Finally, for the BC variable we have the following lsmean, LSD and confidence limit estimates:

Least Squares Means
Standard Errors and Probabilities Calculated Using the Type III MS for L*G as an Error Term

G	BC LSMEAN	LSMEAN Number
1	-31.120417	1
2	205.457500	2
3	485.246667	3
4	-33.561667	4
5	-39.811667	5

Least Squares Means for effect G
Pr > |t| for H0: LSMean(i)=LSMean(j)

Dependent Variable: BC

i/j	1	2	3	4	5
1	0.0108	<.0001	0.9715	0.8987	
2	0.0108	0.0029	0.0069	0.0060	
3	<.0001	0.0029	<.0001	<.0001	
4	0.9715	0.0069	<.0001	0.9198	
5	0.8987	0.0060	<.0001	0.9198	

G	BC LSMEAN	95% Confidence Limits	
1	-31.120417	-147.930741	85.689908
2	205.457500	88.647175	322.267825
3	485.246667	387.106364	583.386969
4	-33.561667	-131.701969	64.578636
5	-39.811667	-137.951969	58.328636

Here we get significant differences between BC lsmean 1 and 2, lsmean 1 and 3, lsmean 2 and 3, lsmean 2 and 4, lsmean 2 and 5, lsmean 3 and 4, and lsmean 3 and 5 (see p values < 0.05).

4.5 Multiple Comparison Procedures in Plant Breeding

In our output examples we presented the Student-t test. In our input example we gave also the Tukey test and the Dunnett test in a statement set into a comment (see /* */. Which test shall be used? The Student-t test is informative but it can control the 5% error level only up to a comparison of all differences among three lsmeans (or a factor with 3 factor levels) if you have the previous information of significant differences of the F-test. What happens when you have more lsmeans? There can be situations where the F-test is not significant and you find significant differences by Student-t test, which is not nice. The results of F-test and multiple comparison procedures must be consistent. For this reason the Tukey test was developed which allows comparisons among all lsmeans and controls the 5% error level so that F-test results and Tukey test results are consistent (the Tukey will never give you a significant difference if the F-test is not significant). However, with more and more comparisons the precision/power of tests declines. There are cases in which the F-test is significant but Tukey finds no significant difference, especially in situations of many comparisions. With fewer comparisons you have a higher precision (for example, with 5 genotypes you have 5 * (5-1) / 2 = 10 comparisons). In plant breeding, usually sufficient information is obtained by comparing

with a check, and often only the information significantly larger (or smaller) than the check genotype is of interest. For this type of comparisons the Dunnett test was developed. The Dunnett controls the 5% level in the case of a test against one check (in such cases the Dunnett has a higher precision/power compared to the Tukey test). There are three test possibilities: 1) Smaller or larger than the check, 2) smaller than the check, 3) larger than the check. The latter two have a higher precision/power compared to the first test strategy, and they control for all comparisons against the check at the 5% level (there will not be significant differences among genotypes when the F-test shows no significant differences).

How important are these multiple comparison procedures in plant breeding. They are important when you want to release varieties at the final stages of a breeding program. You want to obtain information with 5% error (not more) about which among your best candidates is better than the most widely grown variety (check variety) in at least one variable (trait), and you want to present this information to national authorities. with 5% error (this can be considered as a quality label for a new genotype) that your new genotype is better in at least one relevant variable (trait) compared to existing varieties. The test situation is: **There are very few top genotypes and these have to be compared with a standard or check. In this situation, exact multiple comparison procedures are useful in plant breeding and they provide a quality label to the released material.** However, the most import issue is that none of the top clones is close to or below the lowest acceptable value (according to the needs of farmers) in any trait. In early breeding stages (observation trials or preliminary trials) forget exact multiple comparison procedures and the 5% level. The F-test and the least significant difference in PLABSTAT is more than enough. Realize that you must operate with thousands of new genotypes to increase the chances of ”good” genotypes among your material. If you want to compare with exact multiple procedures (e.g. 5000 genotypes => 12,497,500 comparisons with the Tukey test; => 4999 with the Dunnett test) the precision/power of these comparisons is extremely low, or you find many striking and interesting differences but nearly no significant differences at the 5% level. Note: Multiple comparison procedures in statistics are designed to control the alpha error with 5%. This is the situation where you make the statement that something is better than another, and are confident that in only 5 out of 100 cases you will be wrong. When you work with many genotypes the beta error becomes more and more important. This is the case where you make the statement “not different” and you make an error with the statement. The situation in early breeding stages is that – provided you have made good crosses – it is nearly certain that there will be some genotypes which are better than the best widely grown genotypes among thousands of new genotypes which the results of your statistical analysis indicate are of equal or lower value than the best widely grown genotypes. Multiple comparison procedures like LSD, Tukey and Dunnett do not control the beta-error. To control the beta-error selection procedures must be used in which candidates are discarded step by step to enrich the frequency of genotypes with good performance overall variables (traits) in the selected fraction. However, beta-error controlled multiple comparison procedures are still a research field in mathematical statistics. In case where a breeder has made good crosses, there are more than few good genotypes in the population, and it is no problem to discard some good genotypes as long as the frequency of good genotypes in the selected fraction is clearly increased. A parameter to measure ”good” genotypes in the selected fraction is the response to selection, which can be estimated by statistical

procedures of quantitative genetic and selection theory, respectively. At the end of this chapter we will give suggestions to select (or discard genotypes) in advanced breeding material. Note in the future also intend to provide suggestions on strategies for selection in observation trials.

4.6 Computations for our example using R

R Input Example

There are different ways to analyze a linear model in R. For linear mixed effects models command **lme** must be used. However, **lme** is intended for nested random effects so it will not be able to manage the L*G interaction that we have in our example. Assuming that all the effects are fixed, we can use **lm** or **aov** commands. Here we use the **lm** command to get the analysis of variance. Although the degrees of freedom, sums of squares and mean squares are correct, the F ratios are not correct since we are assuming that all the effects are fixed. Below we show code for this analysis.

- In the 1st line we load the data stored in the file example.dat. The data is loaded and stored in the object **all** in R.
- In the 2nd line we fit the linear model. **L*factor(G)** means the effect of L plus the effect of G plus the interaction effect L*G. We write factor(G) and factor(R) because G and R are qualitative factors, otherwise R will interpreted them as quantitative (since they are coded with numbers in the data). **Factor(R)%in%L** means that blocks (R) are nested in locations. The results are stored in the object **model_TYLDha** in R.
- In the third line the command **anova(model_TYLDha)** extracts and prints the analysis of variance results stored in the **model_TYLDha** object.
- The same steps are repeated for the **DM** and **BC** variables. In these variables we have some missing values. Missing values must be identified with **NA** in the data. Because of differences in the estimation of the missing values, we get slightly different results for the sums of squares with PLABSTAT, SAS and R.

```
all <- read.table("D:/example.dat",header=T)
model_TYLDha <- lm(TYLDha~L*factor(G) + factor(R)%in%L, data=all)
anova(model_TYLDha)
model_DM <- lm(DM~L*factor(G) + factor(R)%in%L, data=all)
anova(model_DM)
model_BC <- lm(BC~L*factor(G) + factor(R)%in%L, data=all)
anova(model_BC)
```

R Output Example

```
Analysis of Variance Table

Response: TYLDha
            Df  Sum Sq Mean Sq F value Pr(>F)
L            2  503.66  251.83  2.2679 0.1461
```

```
factor(G)    4  997.38  249.35  2.2455 0.1249
L:factor(G)  8 1326.95  165.87  1.4937 0.2560
L:factor(R)  3   45.38   15.13  0.1362 0.9365
Residuals   12 1332.53  111.04

Analysis of Variance Table

Response: DM
            Df  Sum Sq Mean Sq F value    Pr(>F)
L            2  63.524  31.762 12.5222  0.001462 **
factor(G)    4 182.837  45.709 18.0208 8.544e-05 ***
L:factor(G)  8  58.585   7.323  2.8872  0.053227 .
L:factor(R)  3  29.182   9.727  3.8350  0.042089 *
Residuals   11  27.901   2.536
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Analysis of Variance Table

Response: BC
            Df  Sum Sq Mean Sq F value   Pr(>F)
L            2  114310   57155  6.7216  0.01412 *
factor(G)    4 1259027  314757 37.0162 5.76e-06 ***
L:factor(G)  8   85880   10735  1.2625  0.35787
L:factor(R)  3   23887    7962  0.9364  0.45887
Residuals   10   85032    8503
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Remember that the F ratios are incorrect, since **lm** assumes that all the factors are fixed. You must compute the correct F ratios and their probability values in R by yourself. Indeed, you can also compute any kind of results using the capabilities of **R** as a programming language.

4.7 Suggestions for selection in advanced breeding trials

In advanced breeding trials a relative low number of genotypes have to be compared (20 to 60 genotypes) depending on the size of the breeding program. Usually for about 100 to 300 genotypes entering preliminary and advanced trials only 2 to 10 genotypes (not more) are tested for variety release. A common rule is to select from 5 to 20% at every breeding stage.

Advance breeding trials: The comparison of 20 to 60 genotypes still results in many multiple comparisons. We recommend to first determine the lowest acceptable value (according to the needs of farmers) for each variable (trait), except yield. Discard all genotypes which do not meet the lowest acceptable values for each trait for further comparisons. Depending on the quality of your selection in previous breeding stages you should not have too many genotypes which meet or exceed the lowest acceptable value

for all traits. Then, use the least significant difference (LSD) for yield, and compare the best among the remaining genotypes with all other remaining genotypes. Discard all genotypes which have differences in comparison to the best genotype that are larger than the LSD. However, this is a comparison only within your breeding material and does not provide information about the performance of new genotypes in relation to other breeding material and programs, respectively. Such information is only possible by check clones comprising successful varieties from other sub-regions and regions e.g. Mega-clones. **A set of recommendable mega-clones to be used as check clones are described by (Eyzaguirre et al. 2009). These are Blesbok, CEMSA 74-228, Xushu 18, Brondal, Jonathan and Tanzania, and are available from CIP for distribution across regions (health status 2).** It should be noted that experienced breeders often work with a larger set of check clones to obtain information and to characterize advanced breeding clones. Often they simply express performance of new advanced breeding clones relative to these checks. For example: 110% in yield to CEMSA 74-228, 95% dry matter to Tanzania, 120% β-carotene to Jonathan, and 100% SPVD symptoms to Tanzania – such a genotype would surely be a VERY interesting clone for Elite breeding trials within our breeding program as well as for other breeding programs. It should be noted that for effective comparisons, adapted check mega-clones should be identified in a particular region.

Elite breeding trials (these would correspond to second stage advanced trials): In these trials only very few genotypes are tested. These trials should be designed so that results can be used as information in variety release by national authorities (this depends on the country). However, elite material from other breeding programs can and should be used provided that clones can be imported due to quarantine regulations. The check clones to be used should include the above mentioned check clones, although national authorities request only a local checks (usually the mainly grown varieties in our country). Here multiple comparison procedures that control the 5% error clearly makes sense. With few new Elite-clones (2 to 4) and a larger number check clones (6 to 8) test each elite clones against the check clones (Dunnett) to identify in which traits your new genotype is significantly different compared to checks.

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