Procedures for Standard Evaluation and Data Management of Advanced Potato Clones

1. Introduction

The International Potato Center (CIP) currently manages potato selection trials in no less than 50 different countries in Africa, Asia and Latin America. Each region or sub-region has scientists in charge of monitoring breeding advances and varietal selection. The procedures presented in this basic guide are designed to assist CIP staff in organizing trials and data collection in such a way that data can be shared, centrally stored and uploaded to the Global Trial Data Management system that is currently being proof-tested by CIP's Research Information Unit (RIU).

Among breeders and collaborators we do at least need agreements about: (i) the most important traits to be observed and measured, (ii) standardized procedures and formats to record data, (iii) a user-friendly and practical global system to upload, store and share data.

Module 2

Healthy Tuber Yield Trials

Healthy Tuber Yield Trials (HTYT) can be conducted with 1 up to 30 clones and is recommended for advanced materials that have already shown superior performance in intentional exposure trials for key traits.

Location

***Season 1:** During the first season, yield trials are established in a location representative of the targeted production area. However, the number and quality of the seed used might force this first evaluation to be located in an experimental station.

***Season 2 onward:** Yield trials are established in one or more locations representative of the targeted production areas. Yield trials can be combined with on and off-farm experiments, management trials, participatory selection and/or GxE interaction trials.

Genetic materials

Clones or varieties from CIP and/or national breeding programs can be evaluated. At least two of the most commonly-used varieties should be used as controls. High-quality seed of the same origin should be used as control varieties and clones. The tuber yield trial in the first season requires at least 40 seed tubers per entry (10 plants per row), to be planted in three replications in one location. During the following seasons, the plot size and number of locations should be increased depending on seed availability.

Experimental Design

The Healthy Tuber Yield Trials uses a **Randomized Complete Block Design (RCBD**), where replications of clones are planted in blocks and within each block all genotypes are randomized.

In the RCBD design, all treatments (advanced clones/varieties) are grouped into uniform blocks of equal size. The main purpose of blocking is to reduce experimental error by eliminating sources of heterogeneity such as soil fertility or field slopes. With a predictable pattern of field variability, plot shape and block orientation can be carefully chosen so that the experimental conditions within each block are as uniform as possible. When the pattern of field variability is unidirectional, long and narrow blocks should be used. When the pattern of variability is not predictable, blocks should be as square or rectangular plots of double or multiple rows. These are preferable to long, single row plots. Single row plot should not be carried out because of the inter-plot competition (border affects due to neighbor plot within a block).

Ideally, Healthy Tuber Yield Trials must be carried out in at least 3 locations. The clear advantage to conduct tuber yield trials in three locations is that this saves time, because in potato trials temporal variation of test environments can be replaced by spatial variation of test environments (locations). Trials carried out across locations allow for the separation of effects due to genotypes, genotype by environment interaction and plot error. Furthermore, with 3 divergent locations it is possible to determine for each genotype stability parameters, which must be considered as an additional character associated with yield.

The randomization process for a RCBD design is applied to each of the blocks. Randomizing can be done with "data collector". Analysis of Variance (ANOVA) is used to analyze the data collected in a RCBD. The three sources of variability used in the statistical model are the treatment (variety/ potato clone), the blocks (repetition) and the experimental error for each environment.

Others Designs.- Depending on the type of trial and its objectives, additional trial designs (such as a split-plot design and lattice design) can be applied. Brief descriptions and the randomization processes of those designs are provided in the annex 1. Further information can also be obtained from technical manuals dealing with experimental trial designs (Gomez and Gomez, 1984).

Field Management and Information on Environmental Factors

Field management should follow standard agronomic practices and local procedures to protect the crop from pests and diseases. Meteorological data and soil analyses are ideally collected as to identify spatial patterns among experimental sides and agro-ecological zones. Climatic data may be easily accessible only for on-station trials, whereas availability for other experiments may depend on the proximity of the test site to a meteorological station.

Geographical Information System (GIS) – taking waypoint

For plant breeders, the strength of spatial data management systems is its capacity to provide information on test location that can be used in supporting the analysis of genotype x environment interactions. Ideally a so-called waypoint is taken with a GIS device to record longitude, latitude and altitude for each trials site.

Evaluation parameters

Once the Healthy Tuber Yield Trial(s) have been established, the following agronomic data should be collected during:

Period of vegetative development

a) Number of Tubers Planted (NTP): this is recorded directly at planting.

b) Number of Emerged Plants/plot (NEP): this is 45 days after planting.

c) Plant Growth Habit (PGH)[1]: this is collected 45 days after planting for bred potatoes using three values. (Gomez, 2004).

Scale	State	Description
1	Erect	Erect: the stems are almost vertical and the angle of insertion between the leaves rachis and the main stem is sharp, around 30 °.
2	Semi-erect	The stems have more or less a vertical growth, but some secondary stems open up a bit and the insertion angle between the leaves rachis and the main stem is more open, around 45 °.
3	Decumbent	The stems are more open, some secondary stems are open to the point of reaching the ground. From there the stems tend to recover some vertical growth. The angle of insertion of the leaf rachis with the main stem is very open, from 60 to 90 °. Such plants cover the ground very well and have most of the leaf area exposed to sunlight.

[1] It is not necessary to collect this variable for multiple plots or years. Once the plant habit is established no re-recording is required,

d) Plant Uniformity (Plant_Unif): This data is collected 45 days after planting and should be evaluated using a scale from 1 to 9. (Salas, 2007)

Scale	State	Description
1	Very heterogeneous	Height, vigor, growth stage very heterogeneous.
3	Heterogeneous	75% of the plants show height, vigor and growth stage heterogeneity.
5	Intermediate	50% of the plants show height, vigor and growth stage heterogeneity.
7	Uniform	75% of the plants show height, vigor and growth stage homogeneity.
9	Very uniform	100% of the plants show height, vigor, growth stage homogeneity.

e) Plant Vigor (Plant_Vigor): this data is collected 45 days after planting and should be evaluated using a scale from 1 to 9. (Salas, 2007).

Scale	State	Description
1	Very weak	All the plants are small (< 20 cm), few leaves, weak plants, very thin stems and/or light green color.
3	Weak	75% of the plants are small (< 20 cm) or all the plants are between 20 and 30 cm, the plants have few leaves, thin stems and/or light green color.
5	Medium	Intermediate or normal.
7	Vigorous	75% of the plants are over 50 cm, robust with foliage of dark green color, thick stems and leaves very well developed.
9	Very vigorous	All the plants are over 70 cm and ground coverage is complete. The plants are robust, with thick stems and abundant foliage of dark green color

f) Flowering Degree (Flower)[2]: this data is collected 60 days after planting and recorded using a scale from 1 to 7 (Biodiversity & CIP, 2009; Gomez, 2004)

Scale	State	Description
0	No bud	No inflorescence although inflorescence are rudimentary and consequently of buttons.
1	Aborted bud	Presence of small or rudimentary inflorescences that can show an abortion or abscission point at the joint of the pedicel.
3	Low	Flowering is scarce with the presence of 2 to 3 flowers (buds, flower buds, flowers, fruits and flower abscissions) per inflorescence.
5	Moderate	Flowering is moderate with 8 to 12 flowers (buds, flower buds, flowers, fruits and flower abscissions) per inflorescence.
7	Profuse	Profuse flowering with 20 or more flowers (buds, flower buds, flowers, fruits and flower abscissions) per inflorescence.

g) Senescence stage (SE): this evaluation is conducted 90 days after planting and should be evaluated using a scale from 1 to 9. (Amoros & Gastelo, 2011. Personal communication)

Scale	State	Description
1	Very late	All the plants still show green foliage and flowers
3	Late	Most of the plants are still green, flowering is over and berries might be formed.
5	Medium	The plants are still be green or on the onset of senescence, there may be a slight yellowing. The angle of insertion of the leaves on the stems may have become more obtuse than in the younger plants of the same clone. The formation of berries can be advanced and abundant in fertile clones.
7	Early	The plants have senescent foliage, yellowing is more advanced but the stems may still be upright. If berries are present, their color will turn from green to pale green or yellow green.
9	Very early	The plants are completely senescent, yellowing is complete and uniform, and the stems are decumbent.

[2] It is not necessary to collect this variable for multiple plots or years. Once the flowering degree is established no re-recording is required.

Certainly observations on disease and pest damage can also be recorded. Because the purpose of the trial is to evaluate yield under standard or optimum crop management, integrated crop management practices should be used to control pests and diseases.

Period of harvest

The foliage should be burned or cut 10 to 15 days prior to harvesting. It is recommended that evaluations are conducted in the following sequence:

a) Number of Plants Harvested (NPH):

b) Number of Stolons (Num_Stolon)[3]: Overall assessment of the number and length of the stolons based on inspection of the stolons using a 1 to 9 scale.
(Amoros & Gastelo, 2011. Personal communication)

Scale	State	Description
1	Very few	Plants show no stolon or very few (0 to 4).
3	Few	Plants with 5 to 10 stolons.
5	Medium	Plants with 11 to 15 stolons.
7	High	Plants with 16 to 25 stolons.
9	Very high	Plants with more than 25 stolons.

c) Lenght of the Stolons (Leng_Stolon)[4]

Scale	State	Description
1	Very short	Stolons are less than 5 cm long.
3	Short	Stolons are between 6-10 cm long.
5	Medium	Stolons are between 11- 20 cm long.
7	Long	Stolons are between 21- 50 cm long.
9	Very long	Stolons are more than 50 cm long.

[3] It is not necessary to collect this variable for multiple plots or years. Once the variable is established no re-recording is required.

[4] It is not necessary to collect this variable for multiple plots or years. Once the variable is established no re-recording is required.

d) Tuber Appearance (Tuber_Apper). (Amoros & Gastelo, 2011. Personal communication)

scale	State	Description
1	Very poor	Very low yield, totally misshapes and non uniform size
3	Poor	Low yield, some misshapes but non uniform size
5	Regular	Medium yield, good shape but non uniform size
7	Good	Good yield, good shape and uniform size
9	Very good	High yield, good shape and very uniform size

e) Tuber Uniformity (Tub_Unif): Overall assessment of tuber uniformity is based on the inspection of the harvested tubers using a 1 to 9 scale.
(Amoros & Gastelo, 2011. Personal communication)

Scale	State	Description
1	Very heterogeneous	All tuber sizes are present (from very small to large)
3	Heterogeneous	All tuber sizes are present but there is a predominant size
5	Intermediate	There are only 2 or 3 tuber sizes with a predominant size
7	Uniform	Only two sizes are present with a predominant tuber size
9	Very uniform	Only one tuber size

f) Tuber size (Tub_size): this data is collected using a 1 to 9 scale.
(Amoros & Gastelo, 2011. Personal communication)

Scale	State	Description
1	Very small	Most tubers are very small (<2cm).
3	Small	Tubers are small, between 2 and 4cm.
5	Medium	Tubers are between 4 and 6cm
7	Large	Tubers are large, between 6 and 9 cm.
9	Very large	Tubers are over 9 cm.

g) Number Marketable Tubers Category I/plot (NMTCI): Count the number of marketable tubers for category I with weighing between 200-300g or tubers of 60 mm.

h) Number Marketable Tubers Category II/Plot (NMTCII): Count the number of marketable tubers category II with weighing between 80-200g or tubers between 30 -60 mm.

These categories I and II are arbitrary and can be change according to the country or region where are being evaluated. Each evaluator is free to use locally relevant criteria; however, each category should be defined in order to facilitate comparison of data between countries.

i) Number of Non-Marketable Tubers/Plot (NNoMTP): Count the number of Non marketable tubers with weighing less of 80 g or less of 30 mm.

j) Marketable Tuber Weight Category I/Plot (MTWCI): Weigh marketable tuber category I /plot. The unit of measure is Kilograms.

k) Marketable Tuber Weight Category II/plot (MTWCII): Weigh the marketable tuber category II /plot. The unit of measure is Kilograms.

m) Non-Marketable Tuber Weight/Plot (NoMTWP): Weigh the non-marketable tuber / plot. The unit of measure is Kilograms.

Data should be checked for any errors made during collection or transcription and checked as soon as possible afterwards so that corrections can be made where necessary.

Calculated of variables.- Several variables can be derived from the raw data the Healthy Tuber Yiels Trials. We consider: Total Tuber Yield, Marketable Tuber Yield and Average Tuber Weight as a must.

Variable	Abbreviations	Unit	Formula
Percentage of Plants Emerged	**PPE**	Percentage	$PPE = \frac{NPE * 100}{NTP}$
Percentage of Plants Harvested	**PPH**	Percentage	$PPH = \frac{NPH * 100}{NTP}$
Number Marketable Tubers/Plot	**NMTP**	Count	$TTWPL = \frac{TTWP}{NPH}$
Total Number of Tubers/Plot	**TNTP**	Count	$TNTP = NMTP + NNoMTP$
Total Number of Tubers/Plant	**TNTPL**	Count	$TNT\,PL = \frac{TNTP}{NPH}$
Number Marketable Tubers/Plant	**NMTPL**	Count	$NMTPL = \frac{NMTP}{NPH}$
Total Tuber Weight/Plot	**TTWP**	kg	$TTWP = MTWCI + MTWCII + NoMTWP$
Total Tuber Weight/Plant	**TTWPL**	kg/pl	$TTWPL = \frac{TTWP}{NPH}$
Total Tuber Yield Adjusted	**TTYA**	t/ha	$TTYA = \frac{TTWPL * PLD}{1000}$
Total Tuber Yield No Adjusted	**TTYNA**	t/ha	$TTYNA = \left(\frac{TTWP}{PLS}\right) * 10$

Variable	Abbreviations	Unit	Formula
Marketable Tuber Weight/Plot	**MTWP**	kg	$MTWP = MTWCI + MTWCII$
Marketable Tuber Weight/Plant	**MTWPL**	kg	$MTWPL = \frac{MTWP}{NPH}$
Marketable Tuber Yield Adjusted	**MTYA**	tons/ha	$MTYA = \frac{MTWPL * PLD}{1000}$
Marketable Tuber Yield No Adjusted	**MTYNA**	tons/ha	$MTYNA = \left(\frac{MTWP}{PLS}\right) * 10$
Average Tuber Weight	**ATW**	g	$ATW = \left(\frac{TTWP}{TNTP}\right) * 1000$

Where: PLS= Size of plot and **PLD**=Planting Density.

Other evaluations.- A random sample of 10 tubers per clone should be cut transversally and checked for:

a) External defects: such as cracking, secondary growth and warts, and

b) Internal problems: such as hollow heart, black spots, heat necrosis, and rot. Internal defects should be reported at harvest time. This is critical for estimating processing quality.

c) Percentage of defected tubers: For each entry, the number of affected tubers is recorded on the tuber yield datasheet.

Data analysis.- By using some initial simple statistics entries to be excluded from the analysis can be identified.
Variables which have to be analyzed can be:

(i) Quantitative continuous/discrete variables.- Numeric variables following approximately a normal distribution (e.g. Total Tuber Yield, Dry Matter, etc..) are analyzed using parametric statistics.

(ii) Quantitative ordinal (pseudo- quantitative) variable.- Numeric variables which show in their distribution strong deviation from a normal distribution (e.g. The percent of plant infection (which is used in evaluating clonal resistance to a disease). This variable, which represents the evaluator's estimation of the damage, is more a rank than a measurement.

(iii) Qualitative ordinal variables.- Data cannot be measured, they are ranked or attached to a rating scale. (e.g. Scores with a scale of 1 to 9 for plant uniformity or scores with a scale 1 to 3 for plant growth habit). Ordinal variables are analyzed and compared using non-parametric methods of analysis.

Simple statistics such as mean, standard error, frequency distribution and boxplots should be used to explore the data. Yield data are analyzed using variance analysis (ANOVA) and means are compared using statistical comparison tests such as LSD, Tukey, Waller-Duncan, and Bonferroni. Orthogonal contrasts and Dunnett tests can be used to compare the advanced clones with the control(s). The analysis of residuals is recommended to test the validity of the model and to analyze the behavior of the variance (homogeneous or not). All analysis can be performed using R or other statistical packages that facilitates analysis and reports of the results.

Example and Data interpretation

Validation of the experiment.- An experimental trial for tuber yield evaluation is considered to have been carried out under appropriate conditions if the experiment's coefficient of variation does not exceed 30%.

Selection criteria.- Performance of each advanced clone is compared with the performance of the control(s). It is important to consider the commercial yield of the entry rather than the total yield. In most situations, the ability of a clone to develop numerous small tubers will be viewed as a negative characteristic.

Annex 1

A brief description of commonly used incomplete block designs

Split-Plot Design

Characteristics

The split-plot design is a special kind of incomplete block design. The underlying principle of the split-plot design is that whole plots, subject to one or more treatments (factor A), are divided into subplots to which one or more additional treatments are applied (factor B). Thus, each whole plot may be considered as a block for subplot treatments (factor B), but only as an incomplete block as far as the full set of treatments is concerned (factor A + B). The design may be used when an additional factor (such as planting density or fertilizer use) is to be incorporated into an experiment to increase its scope.

Randomization

Randomization is a two-stage process. First, factor A treatments are randomized over the whole plot; then factor B treatments are randomized within the subplots.

A3	B2	2
		1
		3
		4
		5
	B1	3
		5
		4
		1
		2
	B3	4
		1
		5
		3
		2

A1	B3	4
		3
		5
		2
		1
	B2	1
		5
		4
		3
		2
	B1	3
		5
		1
		4
		2

A2	B2	3
		2
		1
		5
		4
	B3	3
		2
		4
		5
		1
	B1	5
		1
		4
		2
		3

Partially Balanced Lattice Design

Characteristics

The partially balanced lattice design is recommended when the number of treatments is very large or when the experimental units are very heterogeneous. Lattice designs are incomplete block designs. Each block does not contain all treatments, so the precision of comparison between treatments differs depending if the treatments belong to the same block or not. The lattice design (also called double lattice or square lattice), is a partially balanced design in which the number of treatments is a perfect square (9, 16, 25, 36, 49, 64, 81, 121 etc.) and the number of treatments within each block is equal to the square-root of the total number of treatments. This design needs two or multiples of two replications. The experimental units within each incomplete block should be as homogeneous as possible.

Randomizing

Treatments are arranged in the form of a square (step 1). Treatments are grouped by row, and then by columns. The row grouping is generally known as X grouping. The group of treatments in one row will form a block.

Step 1: Arrangement of treatments into a square

1	2	3	4	5
6	7	8	9	10
11	12	13	14	15
16	17	18	19	20
21	22	23	24	25

All the rows (blocks) will make one repetition (step 2). The column grouping is generally known as Y grouping. The group of treatments in one column will constitute another block. This Y grouping will form the other repetition (step 3). The X grouping and Y grouping ensure that treatments occurring together in the same block once do not appear together in the same block again.

Step 2: repetition 1 (grouping by row or X-grouping)

Block 1	1	2	3	4	5
Block 2	6	7	8	9	10
Block 3	11	12	13	14	15
Block 4	16	17	18	19	20
Block 5	21	22	23	24	25

Step 3: repetition 2 (grouping by column or Y-grouping)

Block 6	1	6	11	16	21
Block 7	2	7	12	17	22
Block 8	3	8	13	18	23
Block 9	4	9	14	19	24

For each repetition, the randomization is a three-stage process: the blocks are randomized, each treatment is randomized within each block (step 4), and ultimately a treatment is randomly assigned to each plot.

Step 4: randomization

1- repetition

2- block

3- treatment

Block 8	3	18	23	13	8
Block 10	10	5	25	20	15
Block 9	14	4	9	24	19
Block 6	16	11	21	1	6
Block 7	22	17	7	2	12

Block 5	22	23	25	21	24
Block 1	4	2	1	3	5
Block 3	12	15	14	11	13
Block 2	6	8	10	9	7

Balanced Incomplete Block Design (BIBD)

Characteristics

Each block in a balanced incomplete block design does not contain all treatments; the precision of comparisons between treatments differs depending if the treatments belong to the same block or not. The design is called “balanced” when the experiment is planned in such a way that every pair of treatments occurs the same number of times. Balanced incomplete block design can be used when assessing potato clone processing and cooking performance used a panel of evaluators in which each evaluator tastes/characterizes a limited number of clones. Each evaluator represents a block and evaluates a different selection of potato clones/varieties.

Design

Organizing an experiment with a balanced incomplete block design requires use of the following parameters:

- t = number of treatments (ex. 15 clones)
- b = number of blocks (ex. 15 tasters)
- k=number of experimental units within each block having k<t (ex. 10: each taster can evaluate 10 clones)
- r = number of times each treatment appears having r < b
- λ= number of blocks in which the ith treatment and the jth treatment appear together (λ is the same for all pairs of treatments)

The parameters have to be chosen carefully so that $bk = tr$ and $\lambda (t-1) = r (k-1)$. All parameters should be whole numbers. Cochran and Cox (1957) provide tables assigning suitable values to parameters.

Randomizing

Since the design is linked to many constraints, it is appropriate to use design generated by software, or pre-established tables (Cochran and Cox, 1957).

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