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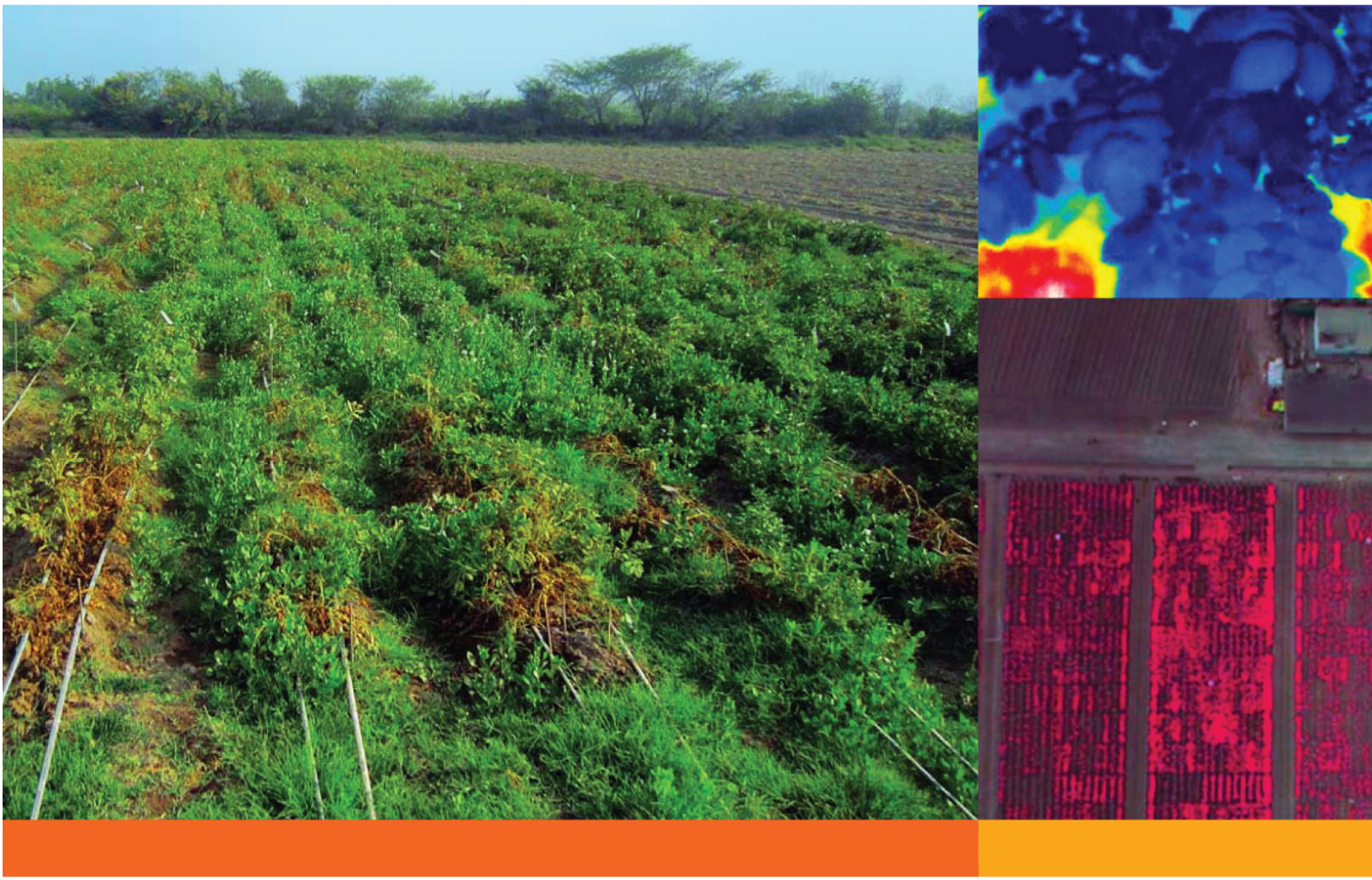

Procedures for standard evaluation and data management of advanced potato clones

Practical guide to assessing potato clones for drought tolerance under field conditions

SEPTEMBER
2020

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CGIAR RESEARCH PROGRAM ON Roots, Tubers and Bananas

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Practical guide to assessing potato clones for drought tolerance under field conditions

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Contents

Summary

This protocol aims to aid in the design of field trials to characterize the performance of potatoes under drought stress, to understand the relationship of agro-morphological, physiological, and tuber traits with tolerance and susceptibility; and to identify the underlying genetics. Precise and accurate phenotyping of component traits are a prerequisite for proper genetic dissection, therefore, most of the methods recommended here are quantitative, to reduce subjectivity and increase genetic studies effectiveness. These are general guidelines that should be taken as recommendations to conduct drought trials, particularly in field, but could also be used in greenhouse or controlled-environment chambers. It should be noted that this protocol has not been intended for large germplasm screenings or *in vitro* screenings; therefore, the number of clones, the experimental design, the plant physiological stage at drought initiation, and types of evaluations depend solely on researcher's specific objectives. Specific formats for data collection with a minimum size of metadata, and defined trait dictionaries have been added in the International Potato Center's Global Data Management System, where they can be analyzed using CIP's user friendly analytical platform (HiDAP), which include a wide range of statistical functions; from basic descriptive statistics to multivariate analysis and selection of top performing clones for further studies and use in breeding programs.

Background theory, terms and definitions

Drought tolerance is a plant capability to live, grow, and reproduce adequately with a limited supply of water/under water shortage conditions during its growing cycle. Drought tolerance is a complex quantitative trait controlled by several small-effect genes or quantitative trait loci (QTL). A plant's response to drought and the impact on yield depends on physiological stage i.e., tuber initiation, tuber bulking, and maturity, as well as drought initiation (Khan *et al*., 2015; Monneveux *et al*., 2014; Schafleitner et al., 2007), intensity, and frequency. For example, due to different drought tolerant genes, intermittent drought stress can occur at tuber initiation stage for one clone and at tuber bulking stage for another; which implies one month compared to each other time according to their physiological stages. Additionally, different germplasm sources might use different gene networks and pathways to tolerate drought stress, depending on the environmental scenario.

Therefore, a critical point for preparing a useful experiment for drought tolerance evaluation in potato is to clearly define the specific physiological stage of artificial drought initiation (a), drought stress intensity, and frequency. The most likely drought scenario in the targeted potato production area should be considered to reflect the most relevant information and to identify the best material for the particular situation. In some germplasm, flowering time coincides with tuber initiation and can be a good physiological marker to set a reference point for starting drought stress and to account days for trait evaluations. This reference point will also facilitate the comparison of results across different regional and global experiments as well as allowing a better interpretation of results. However, some genotypes might not flower in all locations and 5% flowering might not correspond to tuber initiation. Additionally, evaluating a diverse set of potato germplasm, with different maturity stages and lifespan may lead to false interpretations; this factor should be accounted before experimental setup. Due to different germplasm maturity, the physiology of different genotypes will be different at a specific day; therefore, grouping genotypes based on their maturity time is optimal for drought trials analysis. Bulking-based maturity tests with sequential harvests at 70, 90, and 110 days after planting could be done to estimate potato yield and to assign germplasm maturity groups.

Tuber uniformity in terms of size and vigor has significant impact on plants establishment and experiment uniformity. Therefore, evaluating emergence days after tuber planting can be used as factors while analyzing data to interpret effects over dor mancy rupture and non-uniformity of tubers. If multi-environment and multiyear trials are conducted, initiation of drought at a similar physiological stage as well as following the same evaluation protocol and calendar (± 3 days) is advisable.

A. Pre-experimental preparations

A.1. Experiment conceptualization and first practical steps

a) **Conceiving the experiment**: According to the objectives, get a basic idea the experiment and the number of clones needed in order to start the experiment, keeping in mind elevation and latitude of the experimental site as well as the access to basic required facilities/equipment/tools. One important point to be considered is to classify the material to be evaluated based on maturity. A plant's response to stress will be different to drought at different maturity levels or physiological stages. Therefore, applying drought stress to plants that have different maturities at one fixed time will result in different plant responses with different explanations, making the comparison of their drought responses more difficult.

b) **Facilities/equipment/tools:** It is recommended to have access to the following basic facilities, equipment and tools for conducting a potato drought experiment: 1) Tuber seed multiplication unit (TMU); 2) Laboratory for soil analysis; 3) Weather stations; 4) Soil moisture sensors; 5) Control over water input e.g., drip irrigation etc.; 6) Fertilization and pest management; and 7) Weighing balances and an oven to dry samples. The use of any other equipment will depend on which trait will be evaluated during the experiment.

c) **Tuber multiplication/Tuber seed request**: Tubers should be requested from TMU with enough anticipation to ensure the required number of tubers for the experiment initiation. Uniform, and well-sprouted seeds are a pre-requisite for a good experiment. In order to obtain good quality seeds, keep in mind whether the clones that you intend to use are under *in vitro* or field conditions. Another aspect to be considered is that different genotypes might require different times to break dormancy and to have uniform and well-sprouted tubers (Fig. 1). The Number of tubers/clone to be requested from TMU depends on experimental design and number of treatments (see section A.2 - "experimental design and treatment" for more details). In general, the number of tubers for each clone could be estimated as:

$$\frac{tubers}{clone}\ needed = (5-10)\ plants * clone * replications * (2-3) treatments$$

It is recommended to use plots of 10 plants per clone instead of 5, since some plants can be affected by diseases or not germinate. Tubers from each clone as well as checks should be placed in barcode-labeled bags for dormancy break and later for transporting to field site.

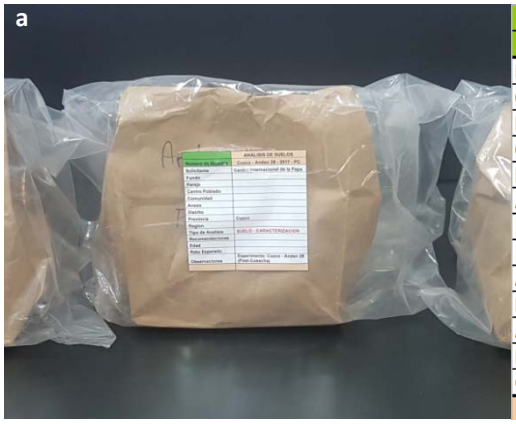
Fig.1 Tuber seed preparation. a) Tuber seeds without sprouts treated with green apples for sprout development. b) Optimal tuber seeds ready for planting.

d) **Field selection**: The experimental site should be selected from previous experience of different locations, discussion with colleagues, and suitability for drought tolerance evaluation. This suitability must be based on the area typical weather; soil compositions; the effect of long and short days over the genotypes, as well as diseases; accessibility; infrastructure, such as the distance from the road, transportation disposal, irrigation system, rain-out shelter, access to temporary workers or students, and collaborations in the area. Geographical Information System (GIS) information of environments homologous to the target environment can be used to make a strong and informed decision for selection of the appropriate site.

e) **Site and Infrastructure Survey:** Once experimental sites have been selected, field inspections of the current state of the site is required. This site should include: good field condition, an available irrigation system, rain shelter, transportation and field accessibility, facilities available for workers, a warehouse for temporal storage of harvest products, infrastructure for evaluations, and power supply for equipment, such as ovens, balances, equipment batteries, scanners, computers, etc.

f) **Soil sampling:** For each selected site, the soil type must be defined by chemical evaluation before installing the experiment. Selected field soils should be sampled and evaluated taking in consideration 3 important points: 1) Representative places in the field must be defined and labeled for posterior collection of 1-2 subsamples per ½ ha, using zig-zag collection pattern; 2) Soil samples must be collected with a clean and rust free auger at two different tillage depths: 15-30 cm and to 30- 50 cm; 3) Each collected soil sample containing approximately 500 g to 1 kg must be stored in double layered paper bag which should be placed in a clearly labeled polyethylene bag afterwards (Fig.2). All soil samples must be shipped to the soil analysis laboratory as soon as possible.

a



b

SOIL ANALYSIS	
Sample Number	Hyo - VHT - 2017 - Sample 1
Requested by	International Potato Center (CIP)
Country	Peru
Region / Estate / Province / Prefecture	Junin
City	Huancayo
District	El Tambo
Area / Field / Annex	La Victoria
Latitude	12° 3'56.87"S
Longitude	75°12'32.33"O
Analysis type	Soil - CARACTERIZATION + Micronutrients (Fe & Zn)
Recommendations	
Age	
Expected result	
Observations	Experiment: VHT

Fig.2 Soil sample preparation. a) Soil sample properly packaged within 3 containers, consisting of 2 paper bags and a polyethylene bag as an external protector. b) Soil sample label information model.

A.2. Experimental design and treatments

a) **Experimental design:** Experimental designs should be decided based on the objective of the experiment and the type of experimental population. It is recommended to separate genotypes according to earliness, since early and late potato plants have different physiological behaviors and responses; this characteristic might mislead drought tolerance while analyzing both types together. An augmented block design (ABD) is practical for QTL and association mapping experiments, in which un-replicated

clones are planted with checks repeated in each block. Check could be chosen from a wide range of types: advanced breeding lines, commercial varieties, or crosses parents that are suitable for the targeted region and compatible with the experimental population; however, it is important to take in consideration a check previous knowledge related to its tolerance or susceptibility before making a decision.

If there is a gradient in the field, block size should not be very large and variation within blocks should be minimized by locating each block in the direction of the least variation. Whereas, if variation between blocks exists, repetitions of the same checks in each block should be adjusted. Having at least 10 degrees of freedom (df) is recommended to handle error in the ANOVA analysis. To find the number of checks required for good estimation of experimental error use the formula:

$$Error\ df = (r-1)(c-1) = 10$$

Where:

"c"= number of different checks per block

"r"= number of blocks = number of replicates of a check Minimum blocks would be $r > \left[\frac{(10)}{(c-1)}\right]$

For example, with 4 checks, [(10)/(4-1)]+1=(10/3)+1=3.33+1=4.33 ~ **5**, which means, 5 blocks are needed.

The purpose of an augmented design is to facilitate the evaluation of a large number of clones. Number of plants per clone could be reduced to 5 instead of 10 for practical purposes; however, it is safer to start the experiment with 10 plants per clone, due to cases in which some plants do not germinate, die out, or become infected with diseases. The same should be done with checks. Two replications per augmented block design are recommended, if there are enough tuber seeds and a great logistic capacity to perform evaluations. In cases in which the number of clones is fewer, a strip plot design must be used to properly randomized the experiment using the treatments.

Table 1 An augmented block design with 100 clones, and 4 checks divided into 4 blocks. "T": clones; "C": checks.

Block 1: (T88, T86, T67, C4, T2, T81, T70, T89, T50, C2, C1, T84, T15, T24, C3, T5, T100, T42, T6, T33, C4, T68, T41, C3, T63, C2, T12, T80, T77, T64, T22, T28, T65, C1, T58)
Block 2: (C1, T62, T44, T45, T95, T36, T52, C4, T35, T83, T4, T57, T17, T18, C2, T31, T92, C3, T96, C1, T7, T93, C3, T61, T79, T94, T51, T91, T11, T20, C4, T16, T43, C2, T25)
Block 3: (C2, T38, T10, T71, T40, C3, T9, T48, T54, T23, T53, T56, T69, T26, C3, T8, T87, T34, T73, C4, T60, T30, T3, T14, T27, C1, T39, T13, T72, C2, T37, C1, T29, T32, C4)
Block 4: (C2, C3, T46, T19, C4, T82, C3, C1, T21, T59, T47, T75, T98, C1, T76, T97, C4, T1, T55, T49, T66, T90, T74, T85, T99, C2, T78)

b) **Treatments:** For drought experiments, 3 treatments must be taken in consideration: I) Normal Irrigation, II) Recovery, and III) Terminal drought (Fig. 3). All treatments should be watered equally until 40 to 45 days after planting (DAP) in which drought treatments start. In some cases where the abiotic conditions suggest another management, the experimental site should be visited frequently, and plants should be carefully observed for the presence of flower buds. Drought should be initiated when 5% of plants have flower buds (with +/- 2-3 days variation). According to the literature, the start of flowering coincides with tuber initiation, therefore, using this physiological marker (5% flowering) should help capture plant tolerance to drought at the same developmental phase, allowing comparison between trials. The exact irrigation intervals (normally 5-7 days in clay sand loamy soil) in all treatments, before and after drought initiation, should be determined during the trial through visual evaluation of wilting of the plants and measurement of soil water content. It is necessary to diligently adapt the intervals of irrigation to the level of stress observed on the plants. Ideally, the soil water potential in the control should not decline below -0.03 MPa.

The field must be properly separated so that it is possible to give different amounts of irrigation to the different treatment blocks. Water inflow from one block into the other must be avoided.

b.1.) Normal irrigation (NI): In this treatment, water is applied as normally required by the potato crop and determined from previous trials for normal growth at the site and by looking at soil water potential, environmental and seasonal conditions. Field capacity must be determined and maintained with an optimal moisture level (-1/3 or -0.33 bar = -0.033 MPa). It is important to evaluate irrigation amount and frequency of this treatment at the site of the experiment. Frequency, duration and intensity of irrigation depends on soil type, weather conditions and crop stage, therefore amount and frequency of water needed might vary between sites.

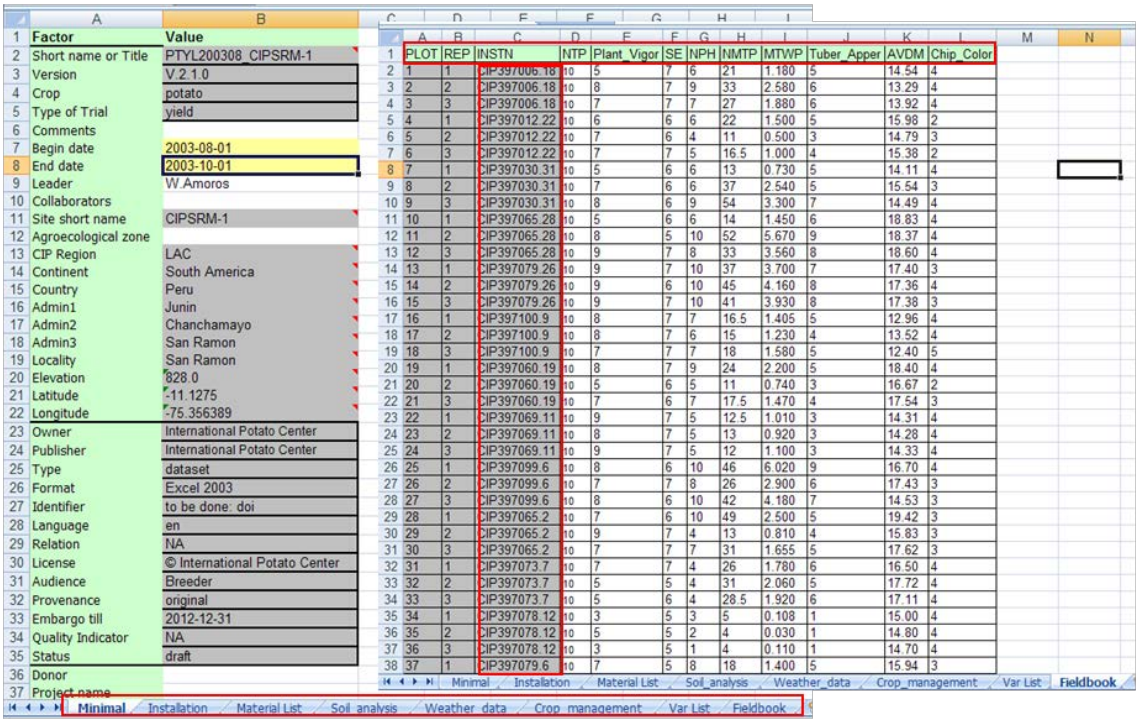
b.2) Water Restriction (WR): Irrigate this treatment normally as described above for normal irrigation by maintaining moisture level at field capacity (-1/3 or -0.33 bar = -0.033 MPa) until the initiation of drought treatments; where this treatment is watered less frequently than NI and its moisture level is maintained at -4 bars= -0.4 MPa (below field capacity but not above wilting point = -15 bars or -1.5 MPa).

b.3) Terminal drought (TD): Same as normal irrigation and deficit irrigation, irrigate this treatment normally to maintain moisture level at field capacity (-1/3 or -0.33 bar = -0.033 MPa) until the initiation of drought treatments. This treatment irrigation is completely suspended until plant harvest.

Fig.3 Water treatments performed in drought experiment. a) Normal Irrigation, b) Water restriction; c) Terminal Drought

A.3. Preparation of field layout and field book

a) **Field book:** For preparing the experimental layout for augmented design, randomize the clones and checks in blocks using R-code or HIDAP (https://research.cip.cgiar.org/gtdms/hidap/). After randomization, prepare a layout in Microsoft Excel or in HIDAP. First write the randomized out-put results from R-code in an excel column. Then for each clone and check, insert five (or 10) rows (corresponding to number of plants/clone) to add the same clone/check at least five times to indicate planting of at least five plants/clone or check next to each other in a plot manner. For RCBD and alpha designs randomize the clones in replications and blocks to prepare the layout in the similar way as explained above. Also prepare a field book in Excel that should have multiple tab sheets with minimal information, installation, material list, soil analysis, weather data, crop management, weather data and field layout information. Field book tab sheet has multiple columns with experimental layout (prepared above), blocks, location, time, and the evaluations to be made (Fig. 4). Also generate and print the barcodes to be used at different stages of the experiment (e.g., multiplication, transfer to the field, planting in the field, at evaluation for leaf trait evaluations, at harvest for tubers, foliar, and roots) and tuber seed transfers (Fig. 5a).



Factor	Value
Short name or Title	PTYL200308_CIPSRM-1
Version	V.2.1.0
Crop	potato
Type of Trial	yield
Comments	
Begin date	2003-08-01
End date	2003-10-01
Leader	W.Amoros
Collaborators	
Site short name	CIPSRM-1
Agroecological zone	
CIP Region	LAC
Continent	South America
Country	Peru
Admin1	Junin
Admin2	Chanchamayo
Admin3	San Ramon
Locality	San Ramon
Elevation	828.0
Latitude	-11.1275
Longitude	-75.356389
Owner	International Potato Center
Publisher	International Potato Center
Type	dataset
Format	Excel 2003
Identifier	to be done: doi
Language	en
Relation	NA
License	© International Potato Center
Audience	Breeder
Provenance	original
Embargo till	2012-12-31
Quality Indicator	NA
Status	draft
Donor	
Project name	

PLOT	REP	INSTN	NTP	Plant_Vigor	SE	NPH	NMTP	MTWP	Tuber_Apper	AVDM	Chip_Color
1	1	CIP397006.18	10	5	7	6	21	1.180	5	14.54	4
2	2	CIP397006.18	10	8	7	9	33	2.580	6	13.29	4
3	3	CIP397006.18	10	7	7	7	27	1.880	6	13.92	4
4	1	CIP397012.22	10	6	6	6	22	1.500	5	15.98	2
5	2	CIP397012.22	10	7	6	4	11	0.500	3	14.79	3
6	3	CIP397012.22	10	7	7	5	16.5	1.000	4	15.38	2
7	1	CIP397030.31	10	5	6	6	13	0.730	5	14.11	4
8	2	CIP397030.31	10	7	6	6	37	2.540	5	15.54	3
9	3	CIP397030.31	10	8	6	9	54	3.300	7	14.49	4
10	1	CIP397065.28	10	5	6	6	14	1.450	6	18.83	4
11	2	CIP397065.28	10	8	5	10	52	5.670	9	18.37	4
12	3	CIP397065.28	10	9	7	8	33	3.560	8	18.60	4
13	1	CIP397079.26	10	9	7	10	37	3.700	7	17.40	3
14	2	CIP397079.26	10	9	6	10	45	4.160	8	17.36	4
15	3	CIP397079.26	10	9	7	10	41	3.930	8	17.38	3
16	1	CIP397100.9	10	8	7	7	16.5	1.405	5	12.96	4
17	2	CIP397100.9	10	8	7	6	15	1.230	4	13.52	4
18	3	CIP397100.9	10	7	7	7	18	1.580	5	12.40	5
19	1	CIP397060.19	10	8	7	9	24	2.200	5	18.40	4
20	2	CIP397060.19	10	5	6	5	11	0.740	3	16.67	2
21	3	CIP397060.19	10	7	6	7	17.5	1.470	4	17.54	3
22	1	CIP397069.11	10	9	7	5	12.5	1.010	3	14.31	4
23	2	CIP397069.11	10	8	7	5	13	0.920	3	14.28	4
24	3	CIP397069.11	10	9	7	5	12	1.100	3	14.33	4
25	1	CIP397099.6	10	8	6	10	46	6.020	9	16.70	4
26	2	CIP397099.6	10	7	7	8	26	2.900	6	17.43	3
27	3	CIP397099.6	10	8	6	10	42	4.180	7	14.53	3
28	1	CIP397065.2	10	7	6	10	49	2.500	5	19.42	3
29	2	CIP397065.2	10	9	7	4	13	0.810	4	15.83	3
30	3	CIP397065.2	10	7	7	7	31	1.655	5	17.62	3
31	1	CIP397073.7	10	7	7	4	26	1.780	6	16.50	4
32	2	CIP397073.7	10	5	5	4	31	2.060	5	17.72	4
33	3	CIP397073.7	10	5	6	4	28.5	1.920	6	17.11	4
34	1	CIP397078.12	10	3	5	3	5	0.108	1	15.00	4
35	2	CIP397078.12	10	5	5	2	4	0.030	1	14.80	4
36	3	CIP397078.12	10	3	5	1	4	0.110	1	14.70	4
37	1	CIP397079.6	10	7	5	8	18	1.400	5	15.94	3

Minimal | Installation | Material List | Soil_analysis | Weather_data | Crop_management | Var List | Fieldbook

Fig.4 Fielbdook example which shows how the data must be presented. Left: data related to the location, date and researchers in charge. Right: experiment data display in the fieldbook. Bottom: order and names of sheets used to organize the experiment.

b) **Barcoding:** Barcodes should be created using a label design and printing software (e.g., Zebra designer software), where a unique code should be assigned to every plant. A barcode must contain a unique CIP number, the year, the trial name, the site name, the current treatment, the replication and the plant number information. Bi-dimensional barcode labels should be printed on either long or squared labels using ZM400 or RW 220 mobile printer (ZEBRA Technologies, USA) (Fig. 5). In some situations, barcodes are assigned to the clones only, and once plants are established, the best five plants/clone are selected and assigned barcodes in the greenhouse as described above before moving them to the field. The selected plants should be on the middle of the furrow. Avoid selecting plants at the beginning or at the end. If using any Android smart phone, use "Open Data Kit" (ODK, http://opendatakit.org/) to prepare a field book with clones arranged according to experimental layout, blocks, location, time, and the evaluations to be made.

Fig.5 Barcode Labeling examples. a) example of content; b) example of usage.

A.4. Preparation of field and planting

a) **Field Leveling:** In order to comply this fundamental step, the field must be weed, flattened, harrowed, and plough twice to a depth between 25 to 40 cm using a tractor. It is highly recommended to use a tractor; however, any agricultural tool based on available facilities can be used. While harrowing, leveling and furrowing of soil must be supervised, thus any misplaced plot can drag data analysis mistakes. Big and medium sized stones should be removed, and finally grooves should be made with 0.7 to 0.9 meters spacing. Finally, grooves must be irrigated for 4 to 5 hours using a sub-main line to soften the arable soil (Fig. 6).

Fig.6 Phases of field furrowing. a) Tractor has to removed weeds, then b) it has to leveling the field; later c) heavy irrigation (irrigation for 4 to 5 hours) is done and finally d) furrowing is realized.

b) **Installation of irrigation system:** Install drip irrigation to have better control of water in the field. For installation of the drip irrigation system, it is recommended to purchase a water pumping engine with a liquid flowmeter, and a tank of an appropriate size for the field, to be placed on an elevated place in the field (Fig. 7a). After hilling, a second line of drip irrigation is added (Fig. 7b).

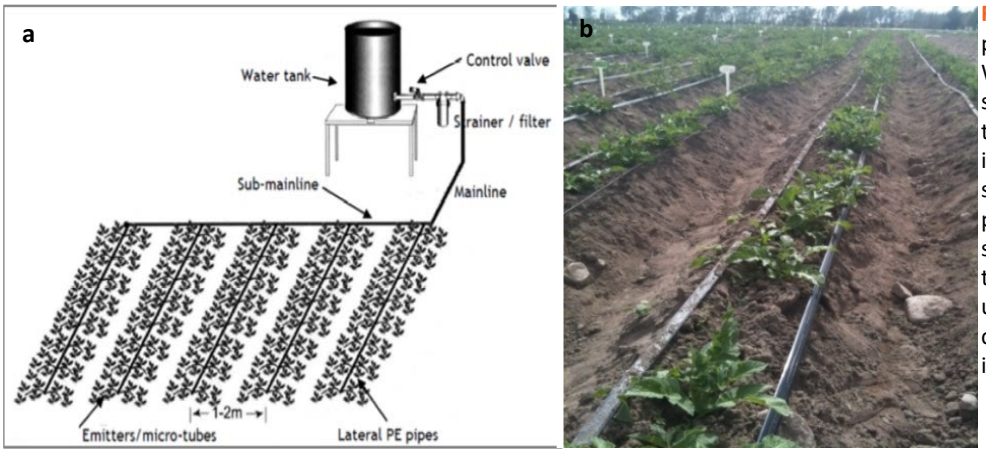


Fig.7 Potato field preparation. a) Water distribution scheme in which the tank and irrigation lines are shown. b) Field picture of ¨A¨ scheme, showing the double hose usage for potato crops, not shown in the scheme.

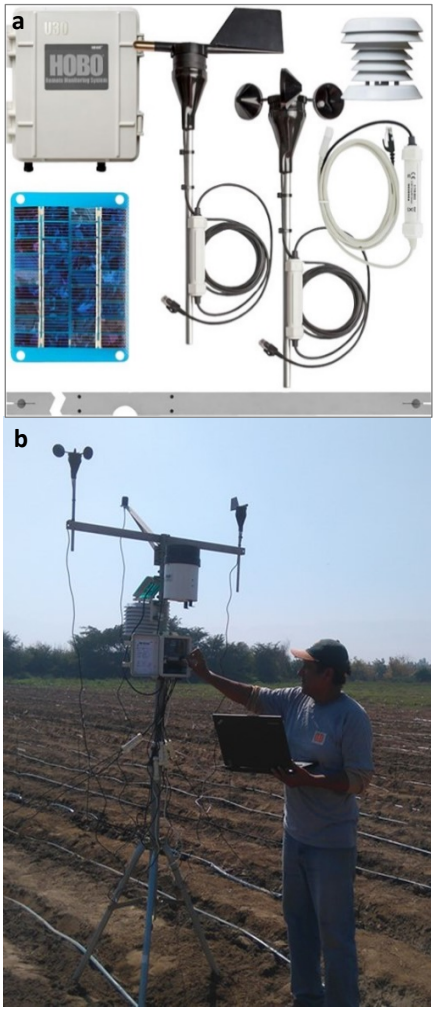


Fig.8 Hobo Onset System examples. a) Picture a complete Weather station model U30 HOBO (Onset Corporation, Bourne, Massachusetts). b) Example of field installed Hobo U30 complete weather station.

c) **Installation of weather station:** A weather station model U30 HOBO (Onset Corporation, Bourne, Massachusetts, USA) (Fig. 8a) should be installed in the field to monitor and record the following parameters:

- Air Relative humidity
- Temperature
- Soil moisture
- Soil temperature
- Water pressure deficit
- Wind velocity
- Wind direction
- Precipitation
- Solar irradiation
- Photosynthetically active radiation (PAR)

Each sensor should be tested before field installation including the Station Logger Hobo U30. The weather station support must be strongly stablished under the soil (about 30 to 60 cm belowground) to avoid falling equipment damages. Hobo U30 must be launched at the field using Onset Hoboware®Pro software (The software must be tested before bringing the portable computer to the field to avoid further configuration problems). It is strongly recommended to set the sensors to log and record every 15 to 30 minutes/day. If PAR sensors are not available, they can be replaced with a formula in which half of the total incoming solar radiation is estimated as PAR.

d) **Mark field according to layout:** Lime must be used to mark the field according to experimental field design for posterior setting of each plot bag with the exact number of tubers. In order to be precise, candlewick is used to create a straight line to help field workers easily follow field layout. (Fig. 9)

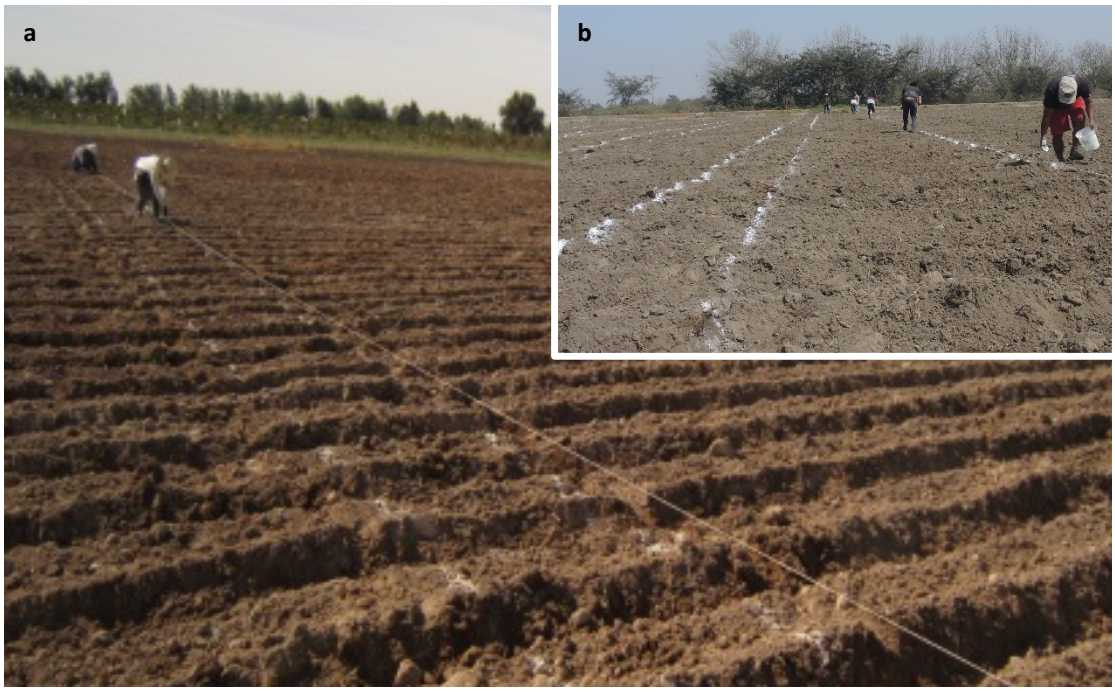


Fig.9 Field preparation before planting. a) and b) show the correct use of candlewick and lime to mark the field according to the experimental design.

e) **Tuber preparation and planting:** Verify the marked layout and make sure the tubers are prepared to be planted manually at 8-10 cm depth according to the field layout. Distance between rows should be 70 to 90 cm and between plants 25 to 30 cm. This is a very crucial step in the experiment where there might several chances, mislabeling, and mistakes; and therefore, the physical presence of the researcher is needed. (Fig. 10)

Fig.10 Tuber preparation before planting. a) Experimental field design verification after material repartition. b) Field tuber seed distribution per plot. c) Designated area for fertilization between tubers. d) Tuber seed covering after application of insecticide nematicide and fungicide

f) **Barcodes installation:** Each tuber seed bag should have its respective plot label inside prior experiment installation. Before or at the same time the tuber preparation is performed, labels must be taken out of their bags and set in their respective plot layout using iron field markers or bamboo sticks (Fig. 11b). Try to locate the markers or sticks in the middle of the plot to avoid losing the identity of each plot.

Fig.11 Installation of barcodes per plot. a) b) Tie labels are put in bamboo sticks in from of each plot.

B. During the experiment

B.1. Monitoring weather parameters

The weather station should be monitored as many times as possible to avoid any failure; as well as each logger, depending on the recorded parameters. It is important to take in consideration not to turn off any data logger since that real-time data can be shown by plotting the data without shutting down any logger, while using HOBO-OnSet®.

B.2. Irrigation and water flow (m^3)

The amount of water used for irrigation must be uniform across the drip lines in field. Use a pressure gauge to make sure that water pressure is uniform. Another way to test water flow uniformity in the field can be done by measuring the amount of water collected in a given time at random location in field. The irrigation must be maintained as required for each treatment (described above in Section A.2 - b).

B.3. Soil humidity, temperature, and water quantity

a) **Soil moisture (MPa):** Soil moisture in the field could be monitored with 2 methods. In the **tensiometer method**, daily monitoring of soil moisture at three different spots in each irrigation treatment should be recorded using a tensiometer (Fig. 12). In this method, soil moisture interacts with the tensiometer through the ceramic tip which is set 40 cm below ground. Soil water tension tries to remove the water from the instrument, which creates a measurable tension inside the column that can be read with either a mechanical gauge or a transducer attached to the instrument. Tensiometers require periodical maintenance to keep them filled with water; specially during winter, they must be removed from the field to avoid equipment freezing damages. In **gravimetric moisture** method, soil samples should be

taken by drilling down to a depth of 50 cm at random sites in the experimental field; afterwards, soil samples should be packed in nylon bags and the bags should be tightly closed until weighing. After weighing, the soil samples should be oven dried (75ºC for 3 days) and weighed again. Soil water content percentage is calculated as:

Soil water (%) = (fresh weight – dry weight) *100 / fresh weight.

Fig.12 Tensiometer (irrometer®) Installation. a) Lateral and b) front view of tensiometers correct positon and location when installed on a field experiment.

b) **Soil moisture (kPa) and temperature (°C) sensors:** The soil temperature can be recorded by installing soil temperature and moisture sensors in the weather station model U30 HOBO (Onset Corporation, Bourne, Massachusetts, USA). The soil temperature sensor should be installed between two potato furrows at 10 cm depth, and it should be at least one sensor in each corner and middle part of the field.

B.4. Fertilization/ Pest and disease management

Fertilization and pest management must be performed in all treatments in the same manner (same dose and frequency).

a) **Fertilization:** Fertilization should be based on recommendations after soil analysis and done while avoiding high nitrogen concentrations in the field which can generate an ideal substrate for weeds; therefore, it is recommended to apply half the nitrogen fertilizer dose at planting and half at hilling. Some examples of fertilizers that can be used are: animal manure, di-ammonium phosphate, potassium sulfate, and ammonium nitrate. Fertilization at each site should be performed before planting and added next to the tubers to avoid damaging the sprouts. The application of the fertilizers should be placed in deep grooves between tubers (at planting, see Fig. 10c). Fertilizers should be previously weighed, by using a Mettler balance 400P, and transported to the experimental site using polyethylene bags. The preparation of the fertilization mixture must be prepared in the field to perform a uniform distribution (Fig. 13).

Fig.13 Fertilization. a) Fertilizers mixture preparation in the field; b) Nitrogen application prior hilling, note the fertilizer is applied close to the plants to allow a complete mixture when covering the plants.

b) **Pest and disease management:** Pests should be monitored in all the field and must be managed according to their prevalence. According to the pest type, different pesticides and fungicides could be applied; the main pests which infect potato crops are: *Agrotis* spp., *Phthorimaea operculella*, *Tuta absoluta*, *Liriomyza* spp., aphids, *Leptinotarsa decemlineata*, *Russelliana solanicola*, and fungal diseases such as *Alternaria solani* and *Phytophthora infestans*. Traps of pheromones combined with water and insecticide can also be placed in the experimental field to control *Phthorimaea operculella* and *Tuta absoluta*. Adding regular detergent helps the water to penetrate insect wings thus preventing them from escaping (Fig 14).

Fig.14 Pesticide application to potato plants as pest management. a) Pest application example. b) Minimum personal protective equipment required for every pesticide applicator (Image from Montana State University Pesticide education program).

B.5. Hilling and Weeding

Weeding should be done through the whole experiments and especially before planting and at hilling. Hilling; or plant coverage; should be done manually or using tractors between 30 to 45 days after planting (DAP) (Fig. 15). This date can vary depending on the plant phenological time of distinct genotypes and climatological conditions, such as photoperiod and altitude, among other factors.

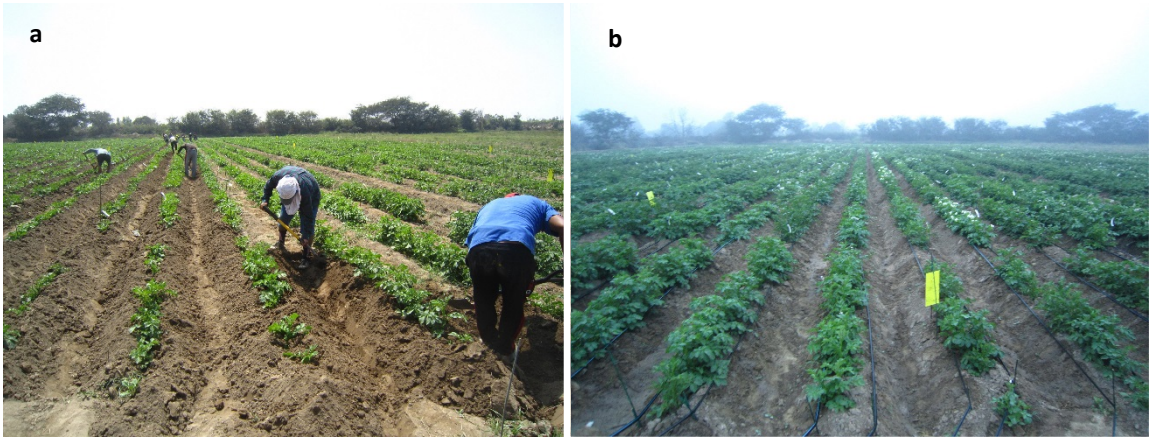


Fig.15 Hiiling. a) Plant coverage to allow tuber formation and bulking afterwards; b) two drip lines should install after hilling

C. Pre-stress evaluations

The following evaluations should be made for every plant per clone before initiation of drought stress at plant maturity at 40-45 DAPS, unless otherwise stated. For evaluations, use pocket pc or smart phones. If you consider using a smart phone, Galaxy Notes II is recommended, in which is possible to install android application ODK (Open Data Kit, http://opendatakit.org/), prepare or upload field book according to field layout as described in the previous section, and scan each plot barcode to enter the corresponding data in the corresponding column. All the evaluations (data collected) performed throughout the experiment using either equipment or a qualitative visual scale must be entered in the electronic field book in smart phone and on the same day should be saved in the computer and uploaded to the database.

C.1. Emergence/Survival/Establishment

Emergence/survival/establishment date and percentage is important to keep track of total number of plants per clone that are alive and are available for data collection, and to account the effect of emergence efficiency on results interpretation and comparison. A clear distinction should be made between emergence and survival. Survival/establishment of each plant should be recorded until all plants have 30-35 days of being planted. At 10 DAP, the field should be observed on regular basis until 30 DAP (Fig. 16). The date of emergence should be recorded for each plant per genotype and must be taken every 3rd day. Additionally, percentage of plants emerged (%) can be calculated using the following formula:

$$Percentage\ of\ Plants\ Emerged\ (\%) = \left(\frac{number\ of\ plants\ emerged}{number\ of\ tubers\ planted}\right) * 100$$

Fig.16 Emergence evaluation between 20-30 days after planting

C.2. Plant Disease Incidence (Disease)

Disease incidence should be monitored visually and accessed as "yes" or "no" in the field book. If the answer is "yes", additional information about the presumed disease must be registered by adding an observation column in the field book. (Fig. 17). This trait must be taken in consideration in order to select which plants will be evaluated in each plot. It is recommended to register this trait one time before hilling; however, this evaluation has to be constantly performed, because is important for crop management.

Fig.17 Potato plant with viral symptoms

C.3. Plant Vigor (PVG)

This data should be recorded once at 45 days after planting and must be evaluated using a scale from 1 to 9 based on plant greenness. (Tab. 2)

Table 2 Plant vigor degree scale developed by CIP Genetics, Genomics and Crop Improvement Science Division (GGCI).

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.

C.4. Plant Uniformity (Plant_Unif)

This trait must be measured using a scale (1, 3, 5, 7, 9) explain below. Evaluation must be done before drought initiation.

Table 3 Plant uniform degree scale developed by CIP Genetics, Genomics and Crop Improvement Science Division (GGCI).

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

D. Drought Initiation

Initiate drought treatments as described above. Proceed with irrigation according to treatment as detailed above in experimental design. Also, keep monitoring irrigation and water flow (m^3), soil humidity, temperature, water quantity, and soil temperature as mentioned in the corresponding sections. Last irrigation is recommended to be from 5 to 7 days after hilling.

E. Post-stress evaluation

These evaluations can be taken from 2 to 6 days after drought initiation, unless otherwise mentioned (Fig. 18). Evaluations such as drought wilting, plant vigor, and disease incidence are taken on a qualitative scale, therefore it is recommended that they should be taken by the same person to avoid different scores.

Fig.18 Evaluations during post experiment. It is recommended that one person take note about measures do by other persons.

E.1. Plant Height (PLAHE, cm)

The plant height (cm) of the main stem should be measured with a ruler or a measuring tape to asses height parallel from the main stem, from the tip of the plant to ground level (Fig. 19 a,b). This evaluation must be done once right after hilling and at least twice after drought initiation. It is recommended to leave a space of 2 weeks between each assessment.

E.2. Stem Diameter (SD, cm)

Use Vernier calipers to measure diameter of the stem just above the first leaves (Fig. 19 c,d). This evaluation should be done one time before, and two times after drought initiation. Note Vernier caliper readings are made in millimeters and this trait must be written in centimeters in the field book. In case a Digital caliper is not available, the correct Vernier caliper measurement is indicated by the number designated by 0 in the Vernier scale (Fig. 19c red arrow), if 0 does not fit any number value in the Main scale (Fig. 19c blue arrow) the next line after 0 is used for fitting, and so on until a match is found; each number after 0 corresponds a +0.1mm to the number value right before the missed matched number value in the main scale.

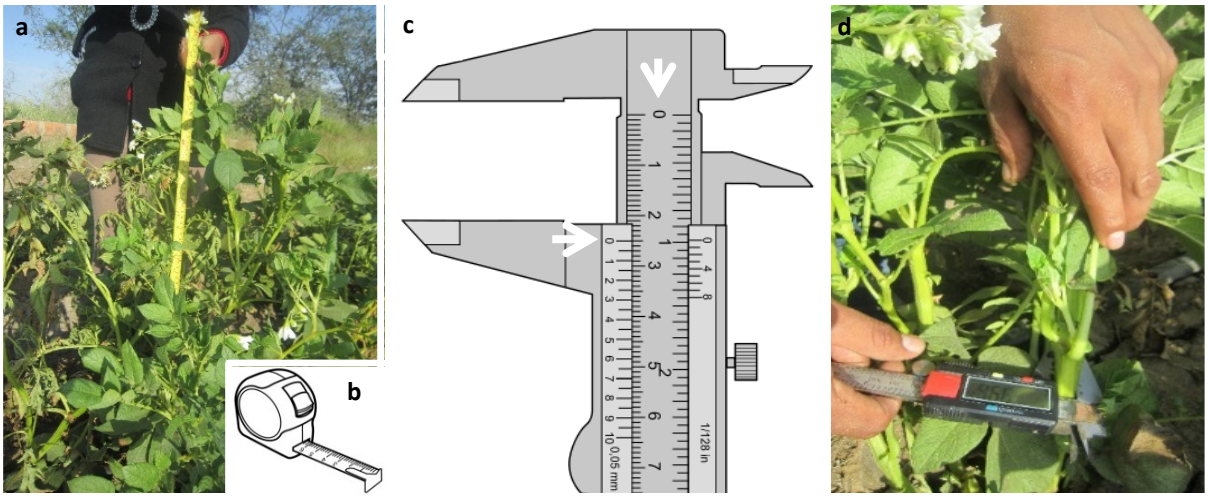


Fig.19 Plant height and stem diameter measurement. A) Proper potato PLAHE measurement; b) Recommended measuring tape type to use in PLAHE evaluation: c) Regular Vernier caliper measuring 2.5cm. c) Proper point of measurement in SD evaluation.

E.3. Stem Number Per Plant (SNPP)

The number of stems can give an idea of tuber uniformity, physical injuries, and independent root/system per plant in a narrow space that effect plants performance and thus interpretation of results. Therefore, accounting for number of plants and using them as co-founding factor in analysis can improve interpretation. Number of stems per plant should be counted once at or before hilling.

E.4. Leaflet Area (LFA, cm^2)

Three representative fully mature leaves should be harvested and kept in polyethylene bags from the selected plant/clone before and after water stress treatment (Fig. 20a). Once between hilling and before drought treatments and twice after drought initiation with a variation range of ± 3-5 days, depending on a plant's performance. The Leaflet area can be measured using 2 methodologies:

a) **Lab scan (LFA_LS):** The Collected leaves should be scanned on an ultra-black or ultra-white background and saved in BMP/JPEG format. Before starting to scan the leaves, the background should be examined first for darkness or whiteness by scanning and analyzing a blank image (without leaf) using the candidate background, the resolution DPI, pixel etc. since these parameters should be noted for the scanner. The software for measuring leaf area must be calibrated using a colored paper of known dimensions before use. The LA software and indications for LA measurement can be found here: http://www.ehabsoft.com/CompuEye/LeafSArea/HLeafSAmain.htm.

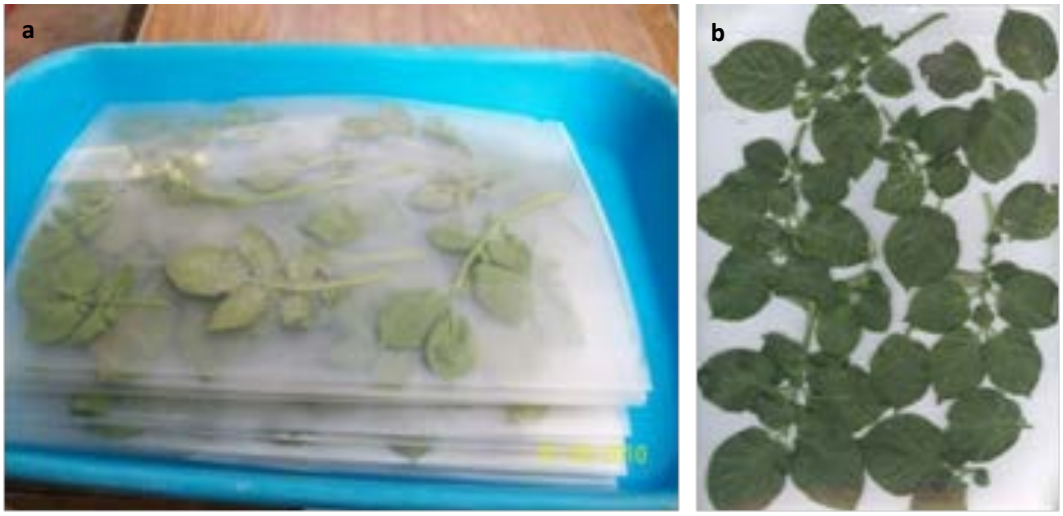


Fig.20 Leaf area measurement using the first method. a) Leaf samples collected correctly; b) Leaf samples places over the scanner and ready to be scanned.

b) **Field scan (LFA_FS):** 3 leaflets; which will be subsequently used in Relative Water Content analysis; are placed in a fabricated picture table made of a white or black clipboard with a plastic 2cm^2 square sticker centered at 2cm of the bottom used as pattern size (Fig. 21), in order to take a perpendicular JPG picture with a cellphone or a digital camera at distance in which only the clipboard background along with the leaflets and the red square are covered. After taking pictures the whole set of pictures, they are processed in the EasyLeafArea program (Easlon & Bloom, 2014): https://github.com/heaslon/Easy-Leaf-Area/archive/master.zip

Fig.21 Example of a fabricated picture table for determination of Leaf Area.

E.5. Relative Water Content (RWC, %)

From the first fully mature leaf, terminal leaflet from each selected plant/clone/replication (at least three per clone) should be harvested once between hilling and before drought treatments and twice after drought initiation (± 5 to 10 days, depending on plant performance). Leaf collection should be done between 9:00 and 11:00 am or 3:00 and 5:00 pm. Leaflet should be packed in a zip-top bag and fresh weight (g) **(Leaflet_FW)** should be taken within 30 minutes of the leaflet harvest. This leaflet should be scanned for determining the **leaflet area (LFA),** on an ultra-black or ultra-white background and saved in BMP/JPEG format. Then, the bag should be filled up with distilled water containing the leaflets and left for 12 hours (Max 24 hours) under a continuous low level of fluorescent cool white light, to reach full capacity of water storage. After the 12h, surface of leaflets should be dried using paper towels and weighed on an analytical balance (Mettler AE-50) to record the turgid weight (g) **(leaflet_TW).** Afterwards, place the leaflet in the bag and put it in oven at 60°C for two days and take the leaflet dry weight **(Leaflet_DW)** using an analytical balance (Mettler AE-50) (Fig.22).

Calculate RWC as:

$$RWC = \left(\frac{Leaflet_{FW} - Leaflet_{DW}}{Leaflet_{TW} - Leaflet_{DW}}\right) * 100$$

Fig.22 Scheme of steps to calculate the relative water content. Arrow indicates a terminal leaflet.

E.6. Specific Leaf Area (SLA, $cm^2\ g^{-1}$)

SLA is used to measure differences of leaf morphology between clones as well as changes in leaf thickness under drought. It could be calculated based on leaflet area (cm2) and leaflet dry weight obtained for relative water content as follows:

$$SLA = (Leaflet\ area/Leaflet\_DW)$$

E.7. Canopy Cover (CC, %)

Canopy cover gives an indirect estimation of the crop's light interception capacity. There are several methods that can be used to measure canopy cover and here we will describe two of them. This evaluation should be done once between hilling and before drought treatments and twice after drought initiation.

a) **Grid Method (CC_Mgrid):** Ground cover should be measured on a qualitative scale (%) with a quadrate (100 cm x 90 cm or 75cm x 70cm) that is divided into squares (10.0 cm x 9.0 cm or 7.5 cm x 7.0 cm). The number of plants will depend on the distance between plants, e.g., use the grid on three plants with a planting distance of 30 cm, but with 25 cm four plants can be evaluated using the same grid. The grid should be held directly over the center of a row at 3 locations of each block and the number of squares at least are half filled with green are counted and then divided by the total number of squares to determine the percent cover (Fig. 23). It is recommended that the same person takes this measurement, as different persons have the tendency to score differently.

Fig.23 Grid method correct application.

b) **Standard camera method (CC_Dcam):** Canopy cover can also be measured using a digital RGB camera and a computer software applied in a strict monitoring protocol, enabling rapid sampling and recording of quantitative canopy cover values (PSE & CIP. 2013). Image segmentation techniques can be used to separate healthy green vegetation from other components of the scene. A fixed hour should be established to take the photographs, considering the time of day with the best lighting. It is recommended to take three canopy cover photos per plant/clone/replication. The necessary equipment for this method is: a) A digital photo camera with Bluetooth or WIFI connection or a smartphone with standard lens (approximate focal length of 50 mm), b) a scale with a known size, c) Siscob v1.0 Software for data processing, d) Aluminum Stand Mount (450 cm high and 200 cm deep) properly leveled to hold the camera perpendicular to the floor (Fig. 24).

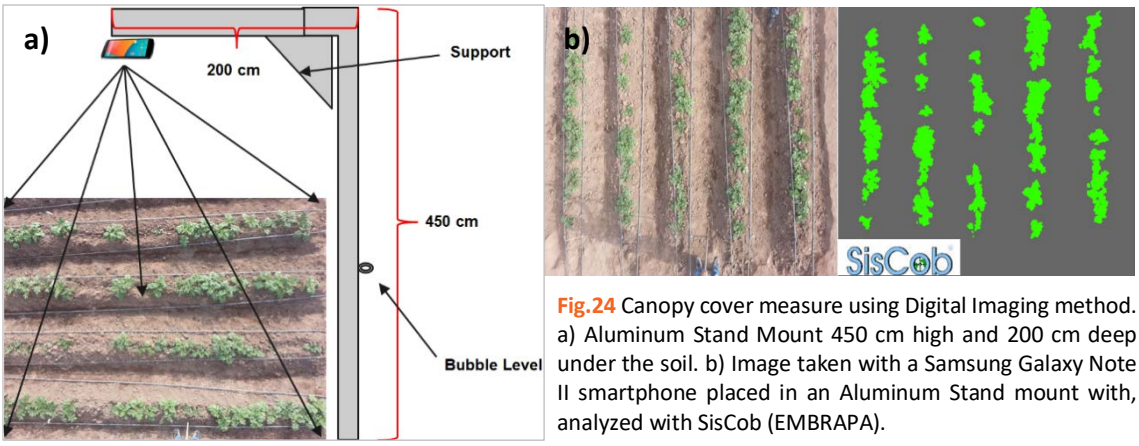


Fig.24 Canopy cover measure using Digital Imaging method. a) Aluminum Stand Mount 450 cm high and 200 cm deep under the soil. b) Image taken with a Samsung Galaxy Note II smartphone placed in an Aluminum Stand mount with, analyzed with SisCob (EMBRAPA).

To start this evaluation the digital camera, or smartphone, must be installed within Its aluminum stand and programed to have maximum image resolution, no zoom, no flash, and to take pictures remotely either with WIFI, Bluetooth or through a physical connector. Once ready, the aluminum stand must be installed perpendicularly to the floor and in the initial fraction of a centric plot, to take picture groups of up to 5 plots depending on the camera resolution. In the specific case of using two android smartphones, we suggest using "RemoteShot" application. After finishing, using the previous camera settings, a scale with known size should be used in a single picture of the field to calibrate all photos for posterior analysis and calculation of Canopy Cover using the software Siscob from EMBRAPA (http://labimagem.cnpdia.embrapa.br/Ferramentas.aspx?ferramenta=3).

c) **Unnamed Aerial Vehicle method (CC_UAV):** Another method to assess the canopy coverage involves a high resolution RGB camera attached to an unnamed air vehicle (UAV), or Drone, to record data among the entire experiment during short periods of time. Oktokopter XL® drone is recommended (Fig. 25). Before each drone flight, the field is georeferenced (including position and altitude) to analyze the field and determine the correct positions to set refencing points according to a flight plan that can allow the UAV to capture the entire field in the less possible amount of flights, taking in consideration altitude and number of pictures. Only when the flying plan has been scheduled and a camera has been attached and programmed, according to the necessities, the drone is launched to fly and take pictures across the field. Images are then converted to 1 single picture using a series of image modifying programs, examples of which are: IMAGE STITCHING V2.0, ENVI 5.0, and QGIS. Processed pictures of the field can be analyzed to obtain the canopy cover percentage using SisCob (EMBRAPA) or other software.

Fig.25 Oktokopter XL® drone with additional equipment designed by CIP, for Remote Sensing. Picture Taken in MWANZA, Tanzania, 2016.

E.8. Plant Wilting (PW, %)

This evaluation is done by looking at the lower part of the plant designating a state base on a 5-degree scale (Tab. 4). This evaluation should be conducted twice after drought stress induction. The starting date for evaluation will depend on the soil and weather variable of the specific trial location. Wilting should be scored three times using descriptions below at intervals of 15 days. The evaluation is recommended between 11:00 am to 3:00 pm. (Fig. 26).

Table 4 Plant wilting degree scale developed by CIP Genetics, Genomics and Crop Improvement Science Division (GGCI)

Scale	State	Description
1	No wilting	All the plants still show green foliage and are turgid.
3	Slight	Most of the plants are still green and turgid; 25% plants within the plot show slight wilting evidence by leaf dropping (wilting) and rolling in the bottom of the canopy.
5	Medium	50% of the plants show leaf rolling in the bottom of the canopy, moderate wilting of leaves throughout the canopy and some loss of petiole turgidity.
7	Severe	75% of the plants show wilt: the petioles severely wilted and dry leaves in the bottom of the canopy.
9	Very severe	100% of the plants show very severe water stress: completely wilted plants and dry leaves.

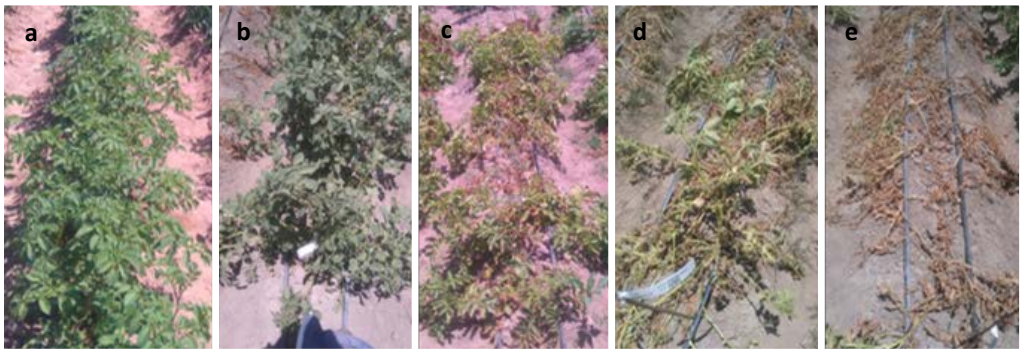


Fig.26 Plant wilting scales. a) no wilting; b) slight wilting; c) medium wilting; d) severe wilting: e) very severe wilting.

E.9. Chlorophyll evaluation (Ch)

Chlorophyll is the pigment which gives most plants their green color, and since its concentration dynamics varies abruptly through the phenological time of a plant, it is highly related to senescence. Evaluating greenness can helps us have an idea of plants chlorophyll content within their cells at a given time whilst facilitating the understanding of their response to abiotic stress which impacts their lifespan and productivity. Chlorophyll content can be measure directly through the measurement of chlorophyll A and B, or indirectly by measuring their stomatal absorbance. This trait must be evaluated once between hilling and before drought treatments and twice after drought initiation.

a) **Chlorophyll Content (ChC, µg/mL leaf):** To determine chlorophyll concentration, leaflets from the third fully developed mature leaf must be sampled for 0.3 to 0.5 grams of leaf material. All samples must be grinded and rinsed in liquid nitrogen as a preparation for posterior extraction with 1.5mL of 80% cold acetone (< -4°C) within a 2mL Eppendorf Tube until separation of phases. After extraction, eppendorf tubes must be centrifuged up to 2 minutes at closely 8000 rpm, to gently sediment plant material. Green organic phase must be separated in a 5mL eppendorf whilst the remaining sediment is re-extracted with another 1.5mL of %80 cold acetone and centrifuged as previously described. After extraction, both solutions are mixed and placed in a spectrophotometric cuvette for absorbance readings at 663 and 646 nm. It is recommended to remain all Eppendorf tubes closed and away from sunlight during extraction.

Chlorophyll a and b are calculated using their respective formulas:

$$\text{Chlorophyll a (µg/ml)} = 12.21\ (A663) - 2.81\ (A646)$$
$$\text{Chlorophyll b (µg/ml)} = 20.13\ (A646) - 5.03\ (A663)$$

Chlorophyll concentrations in µg/ml can be transformed in µg/g as follows:

$$Chlorophyll\ (\mu g/g) = \text{Chlorophyll (µg/ml)}\ \text{x}\frac{\text{Dilution factor (x)}}{Sample\ weight\ (g)}$$

b) Chlorophyll Index (ChI): Chlorophyll index can be assessed mainly with the following recommended equipment: b.1) The SPAD meter from Konica Minolta, b.2) FIELDSCOUT meter from Spectrum Technologies

b.1. SPAD method (ChI_{SPAD}, SPAD Units)

This chlorophyll meter measures units denominated SPAD units (Soil Plant Analyzer Device), these represent a relative chlorophyll concentration in the plant leaf. This chlorophyll index is measured by direct contact with the leaf in an area of 6cm^2 where the leaf absorbance is measured. It is recommended to take readings from three leaflets per plant for each clone and asses an average. Notice that leaves must be taken from the third fully develop mature leave using chlorophyll meter (SPAD 502, Konica Minolta) (Fig. 27).

Fig27 SPAD equipment. Showing the correct position of usage to the left; and the calibration option which must be done before use.

b.2. SCOUT method (ChI_{SCOUT}, index)

This Chlorophyll meter measures units within a chlorophyll index range from 0 to 999. Evaluations with the FIELDSCOUT (FieldScout CM 1000 Chlorophyll Meter, Spectrum Technologies®) are performed at 30 to 45 cm from the targeted canopy. An increase of distance incurs in an augment of sample size which could introduce more effects not attributed to leaves. Equally as SPAD, it is recommended to evaluate leaflets from the third fully develop mature leave. This trait must be evaluated once between hilling and before drought treatments and twice after drought initiation.

E.10. Canopy Reflectance (CR_NDVI)

Normalized Difference Vegetation Index (NDVI) is calculated as a value between 0 to 1 resulting from the relation of visible and near-infrared light reflected by vegetation, and it is used to estimate biomass and changes in leaf water content. Relative vegetation index (reflectance at 800 nm/reflectance at 650 nm) and normalized difference vegetation index (NDVI) ([reflectance at 800 nm−reflectance at 650 nm]/[reflectance at 800 nm + reflectance at 650 nm]) strongly correlates with leaf area index and biomass in potato crops.

a) Greenseeker method (CR_GS): Greenseeker® is one of the most practical hand-held sensors for NDVI measurements to evaluate complete plots on foot (Fig. 28). To take measurements, the Greenseeker® must be placed perpendicular to the canopy being careful of not creating shadow

below the sensor. This evaluation should be done once before drought initiation (right after hilling) and at least 2 times after drought initiation. For better results, it is recommended to evaluate this trait using high resolution NDVI cameras attached to flying drones, since this can reduce the trait variability through time

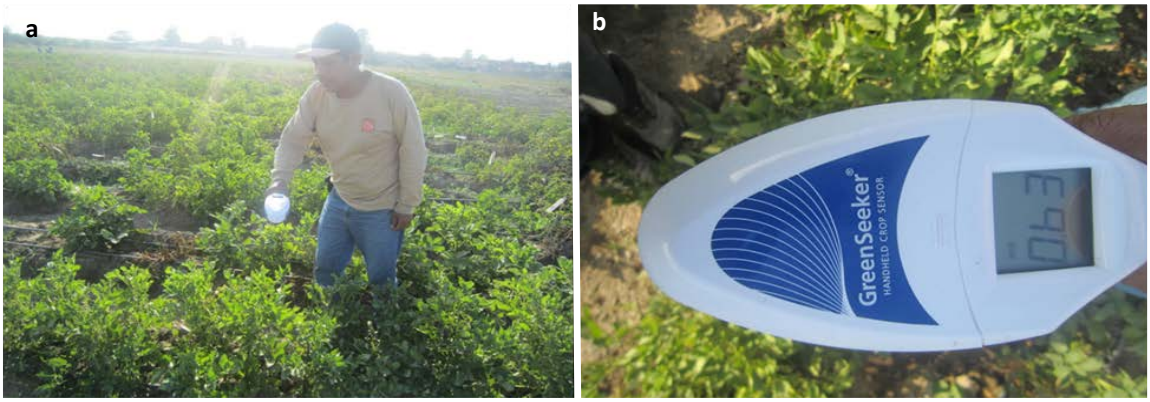


Fig.28 Greenseeker®. a) Showing the correct usage during field evaluation; b) perpendicular view of the equipment.

b) TetraCam method (CR_TC): Tetracam's Agricultural Digital Camera (Tetracam, Inc., Chatsworth,cal) uses a single optimized megapixel sensor which captures visible light wavelengths, longer than 520 nm, and near infrared wavelengths, which reach up to 920 nm. To properly estimate the vegetation index of the entire experiment, images must be taken perpendicularly above 0.50 m of the soil, within each plant. It is strongly recommended to calibrate the sensor by taking images under the same light conditions as the images under study, on each plot. This information can be use later in a Software calibration Tile. Based on the reflectance values, vegetation index can be calculated in PixelWrench2 Software environment, which is the main software of tetracam ADC.

c) Unnamed Aerial Vehicle method (CR_UAV): This assessment uses the same principle of the previously explained method in E.7.c. by using an unnamed air vehicle (UAV) to record data among the entire experiment during short periods of time; however, this method focuses on the evaluation of visible red and near infrared light wavelengths using multispectral cameras with CCD (Charge Coupled Device) or CMOS (Complementary Metal Oxide Semiconductor) image sensors. Drones allow these sensors to cover great amounts of area while achieving precision by reducing measuring time. The captured information could be used to asses NDVI and other series of additional information that could help study different plant responses to environmental conditions. Cameras such as JAI Monochrome CV-A50 IR and Point Grey Firefly MV are recommended (Flores et al. 2009). Since many images are taken during a flight, a single image with high resolution must be assembled using multiple images which can reconstruct the crop area. The construction of this image can be done by many software, which most representative tools are: IMAGE STITCHING V2.0, ENVI 5.0, and QGIS. NDVI computation requires proper alignment of the red and infrared images, which merge display can depict vegetation presence.

E.11. Canopy Temperature (CT, °C)

This evaluation is a requirement to compute the Canopy Temperature Depression. This trait must be evaluated once after hilling, before drought treatments initiation, and at least twice after drought initiation.

a) **Temperature gun method (CT_GM):** This evaluation is assessed using an infrared thermometer gun, at 30 to 60 cm of the targeted area of the canopy. Once triggered the device computes a value in less than a second. It is recommended to evaluate at least 5 points and record an average of the measured points, in each evaluated plant.

b) **Thermal imaging method (CT_TIM):** To perform this evaluation, it is recommended to have an elevated place to stand above the field level to take several overlapping images to cover the whole experiment (Fig. 29a) since this will reduce the background noise and subjectivity of the image at data analysis. The images should be taken between 9:30 hrs to 15:30 hrs on sunny days without wind, in high air temperature and low relative humidity; the emissivity value should be set at 0.97. If using FLIR thermal cameras, the images can be analyzed using FLIR Reporter Professional Software to obtain multiple measurements of temperature that can be averaged.

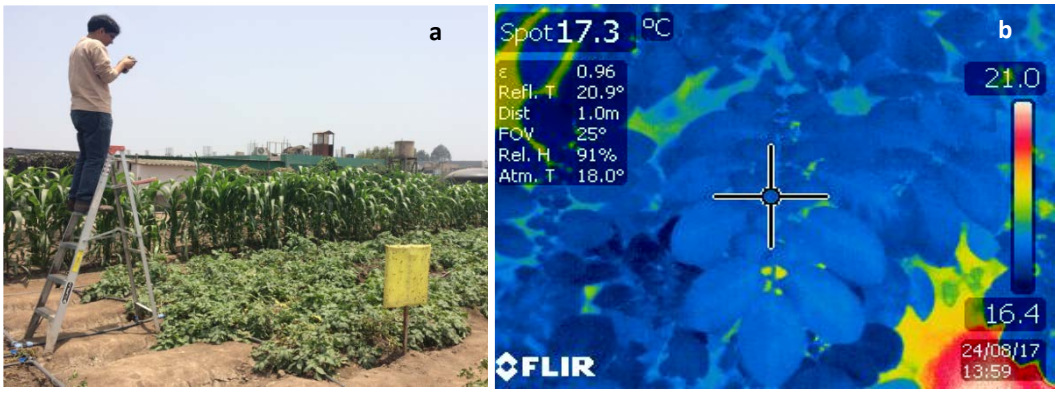


Fig.29 Thermal imaging. a) Appropriate distance for thermal imaging. b) Thermal picture example, note that the information in the superior left corner must be manually updated in the camera every 30 to 60 minutes.

c) **Unnamed Aerial Vehicle method (CT_UAV):**
Thermal images taken from sensors attached to drones can be processed an analyzed equally as previous described techniques (E.7 c and E.10.c).

E.12. Canopy Temperature Depression (CTD)

The change of canopy temperature in relation to environmental air temperature is known as canopy temperature depression (CTD). This trait is an indication of how capable is transpiration to reduce leaf temperature under a demanding environmental load (since a major role of transpiration is leaf cooling); moreover, CTD is an easily measured manifestation of crop metabolic and physiologic response to the environment and has been recognized as an indicator of overall plant water status and used in such practical applications such as evaluation of plant response to environmental stress, irrigation scheduling, cultivar comparison for water use and tolerance to heat and drought. In order to measure the temperature deviation of plant canopies in comparison to ambient temperature, it is necessary to ensure integration of most of the leaves and coverage of different regions of the plot.

CTD= air temperature [Ta] – canopy temperature [Tc])

CTD is positive when the canopy is cooler than the air This trait must be evaluated once between hilling and before drought treatments and twice after drought initiation.

F. Evaluations at Harvest

F.1. Number of Plants Harvested (NPH)

Number of plants that have been harvested should be counted and recorded.

F.2. Numbers of Tubers Per Plant (NTPL)

At harvest, the number of tubers for each plant should be counted in all treatments. Only tubers that have twice the diameter of the stolon where they are inserted at the stem should be considered.

F.3. Fresh Biomass

Total weight and a subsample of up to 250g of the total weight of fresh biomass of each one of the following components must be evaluated by plant within each plot:

1. Leaf fresh weight /plant (LFW, g)[a]
2. Stem fresh weight /plant (SFW, g)[a]
3. Root fresh weight /plant (RFW, g)[b]
4. Stolon fresh weight /plant (STLFW, g)[b]
5. Tuber fresh weight /plant (TFW, g)

Components marked with “a” and “b” can be combined respectively to evaluate above ground (Aerial part fresh weight - APFW) and below ground (Root system fresh weight - RSFW) tissues as groups for crop management purposes (Fig. 30); however, subsamples remain at a 250g limit. Tuber samples, in contrast, can only be assessed isolated from the rest and its separation procedures involve a selection of 6 to 10 tubers, which can vary depending in future Post-Harvest evaluations related to quality.

It is recommended to asses all weights in grams using an analytical balance in the closest time possible to sample collection, and all the weighed samples must be properly labeled and saved in Kraft bags for posterior evaluation of Total Biomass Dry Weight (See Post-Harvest - G.1).

Evaluations can be assessed by plant or by plot depending on the specificity of the experiment objective; however, for practical purposes, it is recommended to use a plot basis.

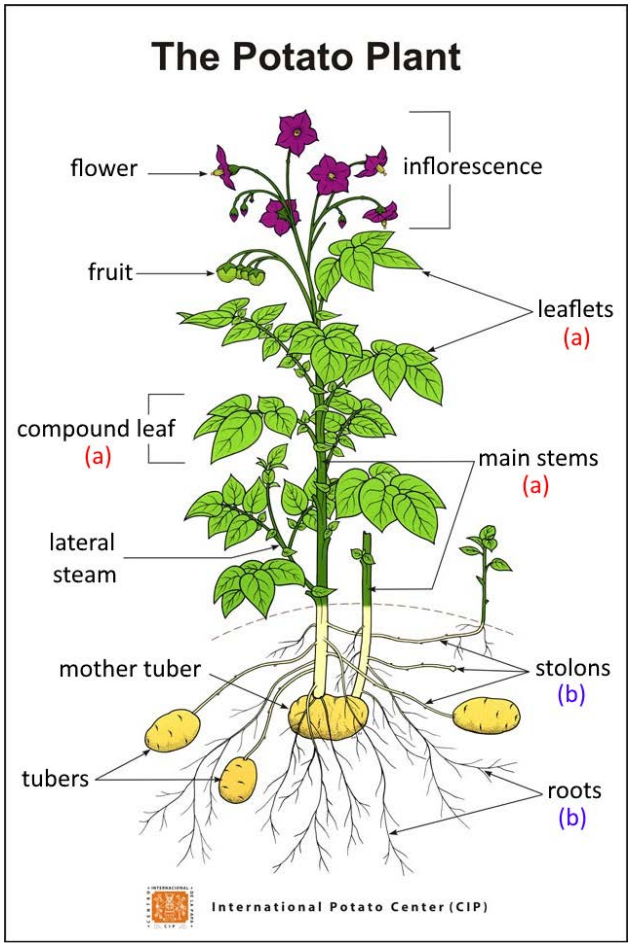


Fig.30 International Potato Center scheme of the potato plant.

F.4. Root Density (RD, scale)

For root density evaluation, extract the root system as intact as possible and in a similar way for all plants. This trait should be measured on a qualitative scale as 1=less roots, loose root system, 2=intermediate, and 3=many roots, dense root system.

F.5. Root Length- longest (RL, cm)

After measuring RD, the length of roots for every plant should be measured using a ruler or measuring tape. Fix the ruler on a hard surface and hold the end of the longest root against the ruler to properly measure the length.

F.6. Length of Stolons (Leng_Stolon)

This evaluation must be done using a 1 to 9 scale (Tab 5). Plants should be extracted from soil using an auger. Digging for all plants should be comparable, which can be assured by maintaining roughly the same depth and distance of auger from each plant, particularly to ensure consistent extraction of root system. Harvested tubers, roots and foliage should be placed in separate Kraft bags labeled with barcodes.

Table 5 Length of stolons degree scale developed by CIP Genetics, Genomics and Crop Improvement Science Division (GGCI)

Scale	State	Description
1	Very short	X ≤ 20 cm long.
3	Short	20 cm < X ≤ 40 cm long.
5	Medium	40 cm < X ≤ 60 cm long.
7	Long	60 cm < X ≤ 80 cm long.
9	Very long	X > 80 cm long.

F.7. Tuber Shape

After harvesting the root system, tubers pictures must be taken before starting the drying process. When taking pictures, it is recommended to set up the camera on a tripod at 50 cm above the tuber. After the whole process, pictures taken should analyzed using software LAMINA (http://lamina.sourceforge.net/).

G. Post-Harvest Evaluations

G.1. Dry Biomass

All components evaluated in the Fresh Biomass Fresh weight step are prepared to have their dry biomass measured. All Kraft bags, properly labeled with barcodes, are introduced in an oven and kept for 2 days at 80°C or 4 days at 65°C (except for tubers). After complete achievement of a maximum dryness point, determined by an average low change (<1g) in weight of a random set of sampled materials through 6-hour intervals, samples are weighed for dry weight.

1. Leaf dry weight /plant (LDW, g)[a]
2. Stem dry weight /plant (SDW, g)[a]
3. Root dry weight /plant (RDW, g)[b]
4. Stolon dry weight /plant (STLDW, g)[b]
5. Tuber dry weight /plant (TDW, g)

Tuber dry weight assessment is divided in 2 subsamples consisting of approximately 200 grams per subsample or its equivalent: 2 to 3 tubers (according to tuber size). The total sample, consisting approximately of 5 tubers, is diced and arranged in their respective 2 subsamples of 200g which are introduced in a properly labeled Kraft bags to be dried for at 100°C for 72 hours. After achievement of maximum dryness point, subsamples are weighed on a balance with a precision of 0.01g or higher. Components marked with “a” and “b”, if combined in APFW and RDFW according to the Total Tuber Fresh Weight step, must be introduced to the oven as they come from the field (Do not separate them).

It is recommended to use the same Kraft bag size for alike samples to allow a fast weighing process, by deducing an average bag weight after sampling up to 20 random samples.

G.2. Dry matter content

All components The dry matter content is the ratio of dry mass to fresh mass of an organ. This evaluation can be performed in point 1 to 5 of (leaves, stem, roots, stolons) or the plant (total biomass)

$$TraitDMC = \frac{TraitDW}{TraitFW} * 100$$

Where:

TraitDMC: is the dry matter content of leaves, stems, roots, stolons or total biomass.

TraitDW: is the average dry weight of the 5 evaluated plants of leaves, stems, roots, stolons or total biomass.

aTraitFW: is the average fresh weight of the 5 evaluated plants of leaves, stems, roots, stolons or total biomass.

H. Calculations and indices

From previously collected data, different ratios and indices can be calculated. Total tuber weight per plot (TTWP) will be used to calculate several indices useful to differentiate tolerant clones. To calculate the following indices, it is important to know these abbreviations:

Y_p = Yield under non-stress
Y_s = Yield under stress
$TTWP_p$ = TTWP of a given genotype in a non-stress environment;
$TTWP_s$ = TTWP of a given genotype in a stress environment;
$\overline{GMTTWP_p}$ = great mean of TTWP in non-stress environment; and
$\overline{GMTTWP_s}$ = great mean of TTWP in stress environment.

H.0. Total Biomass (TB)

Total Biomass is computed using values obtained in evaluations described in sections F.3. and G.1, and they give an idea of the total plant mass in grass. This mass is assessed in either **a)** Fresh Weight or **b)** Dry Weight:

a) Total Biomass Fresh Weight (TBFW, g): Total Biomass allocated to fresh tissues is calculated as the sum of the weights of the total components 1 to 5 in section F.3. It should be noted not to confuse samples with total weights of components.

$$TBFW = LFW + SFW + RFW + STLFW + TFW$$

If Aerial part fresh weight (APFW) and Root system fresh weight (RSFW) have been evaluated, then the formula follows the same principle:

$$TBFW = APFW + RSFW + TFW$$

b) Total Biomass Dry Weight (TBDW, g): Total Biomass of fresh samples dried as described in section G.1, are summed to compute the total mass attributed to dehydrated fractions.

$$TBDW = LDW + SDW + RDW + STLDW + TDW$$

If Aerial part fresh weight (APFW) and Root system fresh weight (RSFW) have been evaluated, then the formula follows the same principle:

$$TBDW = APDW + RSDW + TDW$$

H.1. Harvest Index (HI, %)

Harvest index should be calculated as Tuber fresh (dry) weight/ total biomass on fresh (dry) weight basis per 100. However, as leaves might be in different stages of senescence and under different abiotic circumstances, fresh weight might vary between all plants and clones. Therefore, it is recommended to work with dry weight.

H.2. Drought Susceptibility Index (DSI)

Fischer and Maurer (1978) suggested the drought susceptibility index (DSI) for measurement of yield stability that apprehended the changes in both potential and actual yields in variable environments. In spring wheat cultivars, Guttieri *et al.* (2001) suggested that a DSI with a value above 1 indicates a possible susceptibility to drought stress.

$$DSI = \frac{1 - \left(\frac{TTWP_s}{TTWP_p}\right)}{Stress\ Intensity\ (SI)} = \frac{1 - \left(\frac{TTWP_s}{TTWP_p}\right)}{1 - \left(\frac{\overline{GMTTWP_s}}{\overline{GMTTWP_p}}\right)}$$

The smaller the value of DSI, the greater is the stress tolerance. Selection based on DSI favors genotypes with low yield potential and high yield under stress conditions. In a good drought trial, DSI is around 0.7, which indicates that the plants were exposed to severe drought. If DSI is lower, it might be impossible to identify differences in drought tolerance between genotypes. Instead, a strict correlation between yield potential (control yield) and drought yield will be found, making trials under stress conditions unnecessary.

H.3. Drought Tolerance Index (DTI)

Drought tolerance index (DTI) was defined as a useful tool for determining high yield and stress tolerance potential of genotypes (Fernandez, 1992). Rosielle and Hamblin (1981) demonstrated that when there is a good stress tolerance index value, hybrid yield under normal irrigation and drought condition is close to each other; which can be interpreted as a plant resistance to drought.

$$DTI = \left(\frac{TTWP_p}{\overline{GMTTWP_p}}\right)\left(\frac{TTWP_s}{\overline{GMTTWP_s}}\right)\left(\frac{\overline{GMTTWP_s}}{\overline{GMTTWP_p}}\right) = \frac{(TTWP_p)(TTWP_s)}{\left(\overline{GMTTWP_p}\right)^2}$$

The higher the value of DTI for a genotype, the higher its stress tolerance and yield potential.

H.4. Tolerance (TOL)

A larger value of TOL represents relatively more susceptibility to stress, thus a smaller value of TOL is favored. Selection based on TOL favors genotypes with low yield potential under non-stress conditions and high yield under stress conditions. Under most yield trials, the correlations between TOL and Y_p would be negative and the correlation between TOL and Y_s would be positive. (Rosielle and Hamblin, 1981).

$$TOL = TTWP_p - YTTWP_s$$

H.5. Mean Productivity (MP)

This index favors higher yield potential and lower stress tolerance. Rosielle and Hamblin (1981) showed that under most yield trials, the correlations between MP and Y_p, and MP and Y_s would be positive. Thus, selections based on MP generally increase the average performance in both stress and non-stress environments.

$$MP = \frac{(TTWP_p + YTTWP_s)}{2}$$

H.6. Geometric Mean Productivity (GMP)

MP is based on the arithmetic means and therefore it has an upward bias due to a relatively larger difference between Y_p and Y_s, whereas the geometric mean is less sensitive to large extreme values. (Fernandez,1992)

$$\text{GMP} = \sqrt{(TTWP_p)(TTWP_s)}$$

H.7. Slope (SLP)

It represents a concurrent changing trend in time of a trait. This calculated trait is obtained for each trait which has several time point evaluations (in this protocol, we recommend at least three evaluations per trait). The following formula is required to get the slope value

$$TRAIT\_SLP = \frac{\sum(DAP\_EV - DAP\_AV)(TRAIT\_EV - TRAIT\_AV)}{\sum(DAP\_EV - DAP\_AV)^2}$$

Where:
DAP_EV: number of DAP during an evaluation
DAP_AV: average DAP when evaluations were realized
TRAIT_EV: value obtained for a trait in an evaluation
TRAIT _AV: average value of evaluations of a specific trait

H.8. Rate (R)

This trait gives the average increase or reduction tendency of traits evaluated in different time points. It will be obtained using the following formula:

$$Trait\ Rate = \frac{\left\{\left[\left(\frac{EV_2 * 100}{EV_1}\right)\right] + \left[\left(\frac{EV_3 * 100}{EV_2}\right)\right] + \cdots + \left[\left(\frac{EV_n * 100}{EV_{(n-1)}}\right)\right]\right\}}{(n-1)}$$

I. Data processing

All the Data content in the experiment field book must be examined and cleaned to obtain consistent data prior Statistical analysis. For this purpose, R statistical program is used.

I.1. Data cleaning

Data must be explored for any out layers. Extreme small and large values for one or more traits are searched in order to find any error such as misplaced digits, incorrect units, or systematic errors. As a first step, each missing value must be defined as non-available (written as NA in each registered field) and not as a result of a mistake. After having all the missing values defined, each trait must be submitted to a previous knowledge of standard ranges of values, which can be achieved by either using Microsoft excels filter option to re-order and search for high and low values using professional criteria; using R ¨St4gi¨ package on the data matrix (which uses standard ranges from Sweet Potato crops) or by using Boxplots to visually identify each trait. It is highly recommended to use more than one option while cleaning data in order to avoid missing any possible error. Finding the origin of any mistake is crucial to identify the type of mistake and to apply the best possible correction.

I.2. Data analysis

After generating a consistent data matrix, the information must be arranged by plot prior analysis. The data analysis consists of 3 steps, and for this purpose it is highly recommended to use R statistical program. The first step is to observe the descriptive statistics of the data by generating a matrix which indicates each trait minimum and maximum values, its average, and the respective standard deviations.

The second step is designated to avoid collinearity; for this purpose, the data matrix is re-arranged to evaluate Fisher correlations between all traits. It is expected that same traits taken in different times might have a high correlation between them. Therefore, it is recommended to use slope values of every trait to avoid problematic correlations between the same traits.

In third and last step, the identification of differences between tolerant and non-tolerant clones is started by designating the most informative traits given by the Gini index in a Regression Tree model (Random Forest algorithm - R) in order to use them in a Discrimination analysis. This analysis will show if the selected group of traits allow to visualize if a clone under stress conditions behaves in the same way under non-stress conditions.

I.2.1. Pre-analysis: Before using the codes presented in this brief methodology description, the following R packages must be loaded:

- Readxl
- st4gi
- scales
- ggplot2
- gridExtra
- tidyr
- factoextra
- MASS
- plyr
- reshape2
- polycor
- Formula
- Tcltk
- Tkrplot
- Sp
- SpatialEpi
- Biotools

In order to load the packages mentioned above, use the following codes to install missing packages not added by default in R:
install.packages(c('Matrix', 'lme4',"devtools", 'readxl', 'scales', 'ggplot2', 'lattice', 'gridExtra', 'tidyr', 'factoextra', 'MASS', 'klaR', 'plyr', 'reshape2', 'polycor', 'survival', 'Formula', 'Hmisc', 'caret', 'tcltk', 'rpanel', 'tkrplot', 'sp', 'SpatialEpi', 'biotools', 'randomForest', 'RColorBrewer'))

I.2.2. Descriptive statistics: The following code will create a data matrix in which every trait will be displayed in the first column followed by the minimum, maximum, average and standard deviation values in sub-sequent columns with their respective order.

```
# Code to Load the field book: the names in red must be changed for the address and name of the field book which will be used.

setwd('C:/Users /Dropbox/data_analysis/drought/Hidap')

temp1 <- data.frame(read_excel("PTDrought022217_ICA.xlsx", 1, na = "NA"))


# Code to Re-order the complete data matrix to 3 columns: Treatment, Trait, and Values. All evaluations, and values of each evaluation will appear right next to the original Treatment column.

temp2 <- temp1[,!names(temp1) %in% c("ORD", "BLOCK", "PLOT", 'FACT',"CIPNUMBER")] #type every column title which is not a trait or a treatment

melted <- melt(temp2, id.vars=c("TREAT"))

melted <- na.omit(melted)


# Code to calculate values for every trait by each treatment, and to generate a data matrix with the results.

temp2 <- ddply(melted, c("TREAT", "variable"), summarise,

      min = min(value),

      max = max(value),

      mean = mean(value),

      sd = sd(value))
```

I.2.3. Correlations analysis: To obtain a better idea of how the data behaves within evaluated traits, Spearmen correlation analysis is done to see if there is any unexpected behavior or to eliminate collinearity. The following codes will create a data matrix of every trait correlation by a selected treatment.

```
# Code to load the field book – the names in red must be changed for the address and name of the field book which will be used.

setwd('C:/Users /Dropbox/data_analysis/drought/Hidap')
```

```
temp1 <- data.frame(read_excel("PTDrought022217_ICA.xlsx", 1, na = "NA"))


# Code to order the data frame and to enumerate rows according to “ORD” column, leaving only the treatment column and all which correspond to traits.

temp2 <- temp1[,!names(temp1) %in% c("BLOCK", "PLOT", 'FACT',"CIPNUMBER")]

temp2 <- temp2[,-1]

rownames(temp2) <- temp1[,1] #note “ORD” column must be first in the field book


# Code to select one treatment, to erase the treatment column, and to omit missing values.

temp2 <- temp2[temp2$TREAT == "TD",] #letters in red must be changed for the desired treatment, as shown in the field book.

temp2 <- temp2[,-1]

temp2 <- na.omit(temp2)


# Code to rearrange the data matrix, to calculate each trait correlation for the chosen treatment, and to save the results in a data matrix called “correlations1”.

flattenCorrMatrix <- function(cormat, pmat) {

 ut <- upper.tri(cormat)

 data.frame(

  row = rownames(cormat)[row(cormat)[ut]],

  column = rownames(cormat)[col(cormat)[ut]],

  cor  =(cormat)[ut],

  p = pmat[ut]

 )

}

res<-rcorr(as.matrix(temp2))

correlations1 <- flattenCorrMatrix(res$r, res$P)
```

J. Equipment, materials, and facilities needed

Materials & Equipment	Materials & Equipment	Facilities Access
Weather station	SPAD - 502	Transportation
Soil moisture and temperature sensors	Vernier Caliper	Hygienic facilities
Drip irrigation	Scissors	First Aid Kit
Pressure Gauge	Bar-coded tags and Barcode printer	Power supply
Weighing scale	Zip-top bags	Water supply
Analytical balance (+ / - 0.001)	Bags	Cold room for temporary storage of tubers and biomass
Scanner	Paper towels	Oven to dry tubers and biomass
Digital camera	Distilled water	Equipment storage
Smartphones or hand held pocket PC	Pencils & markers	

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