Standard Operating Procedures (SOPs) for Conducting Field Experiments in Potato

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Standard Operating Procedures (SOPs) for Conducting Field Experiments in Potato

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Contents

Preface

Potato is a unique crop very responsive to environment, but its phenological stages are not clearly visible especially in the subtropics. The system of AICRP started in the early 70s when the need for a system to test varieties at different locations and develop Best Management Practices before their release was felt. High yielding varieties and agro techniques developed at CPRI are tested and adapted through a network of AICRP centres. The mandate of AICRPs is not only the testing of varieties and agrotechniques but also identification of location specific problems and their solutions. When experiments are conducted at many locations and many personnel are involved, it is necessary that common methodology is followed so that the results can be compared across locations. Proper care has also to be taken in recording observations so that we can understand the crop phenology *vis a vis* the environment. Thus, there is a need to delineate a uniform set of essential observations that have to be recorded in all the experiments which would help express the phenology of the crop as affected by the environment or treatments. Moreover, such a systematic list of observations would help to adopt a systematic analysis protocol. Further, the personnel conducting the trials at different centres are of widely different background, hence, well described protocols for conduct of the trials as well as recording observations would reduce the human errors in recording of observations. Hence, it is felt that this bulletin on standard operating procedures will give clearcut guidelines for the personnel involved for conduct of field experiments and recording the observations.

Authors

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Introduction

Potato is grown in a wide range of environments in India ranging from the autumn season in the northern Indogangetic plains where it is grown as an irrigated to the northern, southern & eastern hills where it is grown as rainfed crop. In the plains, the crop experiences short photoperiod and temperatures which are high at planting and cools down, to even less than optimum at some locations, during peak growth stage. On the contrary in the hills the crop experiences long photoperiod with temperature not as diverse as in the plains. Inspite of these environmental constraints potato acreage and production in India registered tremendous growth which has enabled India to emerge as the second largest potato producer in the world. This has been possible due to the development of high yielding varieties adapted to the different environments and appropriate agrotechniques to realize the potential. Therefore, this manual of standard protocols to be followed for experimentation under different disciplines under AICRP has been felt imperative.

AICRP System in India

The high yielding varieties and agro techniques developed at CPRI are tested and adapted through a network of AICRP centres. The system of AICRP started in the early 70s when the need for a system to test varieties at different locations and develop Best Management Practices before their release was felt. It is not only testing of varieties but also identification of location specific problems and develop solutions so as to increase potato production in the country by exploiting G x E interaction. The crop growth and development are a function of the environment and genotype interaction. Multi Environment Trials (METs) are conducted to assess the performance of the hybrids or agro techniques being evaluated in different environments, their yield stability, resistance to pests and diseases as well as develop cost efficient agronomic practices for raising a healthy crop.

Under the AICRP (Potato), field trials are conducted at 25 centres spread out across the country and in different agroclimatic zones. The climatic profile of these centres present a picture of huge diversity. The figures of climatic normals show that the mean maximum temperature of the growing period of 90 days ranges from 21.1 $^{\circ}$C to 32.6 $^{\circ}$C at different AICRP locations, where as the minimum temperature ranges between 8.0 $^{\circ}$C and 20.9 $^{\circ}$C. Classification of the climate data according to its impact on potato shows that at Pantnagar, Srinagar and Jalandhar the mean temperature of the growing season is quite low and hence the growth and development of the crop would be slow at these centres. Kanpur, Shillong, Shimla, Patna, Hisar, Dholi, Chhindwara, Kota and Modipuram centres are expected to have optimum temperatures of 18-20 °C and hence are ideal for evaluating genotypes for their yield potential under optimal temperature conditions. Mild temperature stress i.e. mean temperature of 20-22 °C are expected at Gwalior, Kalyani, Jorhat and Raipur while high stress i.e. mean temperature of >22 °C is expected at Bhubanewswar, Pune, Deesa and Dharwad. Hence, these centres are ideal locations for evaluating genotypic performance against heat stress. The differences in mean temperature are also reflected in the accumulated thermal time which is expected to affect the phenology of the crop. In addition to temperature other climatic variables also present a picture of huge diversity. For testing of hybrids for resistance to pests and diseases they have to be tested at respective hot spots for instance screening of hybrids against PALCV at Hisar, for Sclerotium wilt at Dharwad, for heat stress at Deesa, PCN at Ooty etc. Thus AICRPs offer a unique platform to thoroughly test the hybrids in different environments.

Potato is a unique crop very responsive to environment, but its phenological stages are not clearly visible especially in the subtropics. Some of the phenological stages of the crop are depicted in figure. Proper care has to be taken in recording observations so that we
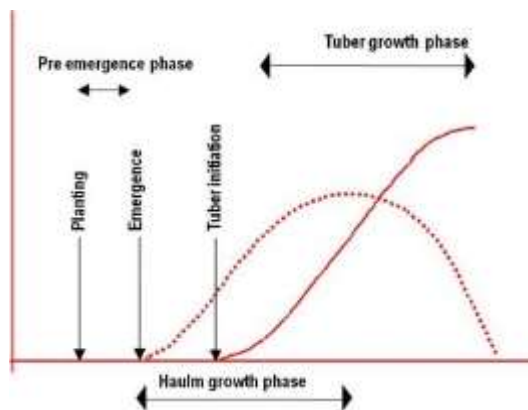
can understand its phenology *vis a vis* the environment. Thus there is a need to delineate a uniform set of essential observations that have to be recorded in all the experiments which would help express the phenology of the crop as affected by the environment or treatments. Moreover, such a systematic list of observations would help to adopt a systematic analysis protocol. Further, the personnel conducting the trials at different centres are of widely different background, hence, well described protocols for conduct of the trials as well as recording observations would reduce the human errors in recording of observations. Hence, it is felt that this bulletin on standard operating procedures will give clearcut guidelines for the personnel involved.

Experimental Layout

Layout of an experiment is the basic and foremost important operation in any testing program and it should be done with care and systematically. It involves field selection and preparation, layout, planting and harvesting etc. The land should be well prepared for potato. Medium textured soils are ideal but it should be representative of the target ecology. The steps in the layout of the field experiment are as below:

1. Establish a base-line using the Pythagoras theorem, so that the four corners of the trial have right angles, mark the plots and assign different treatments to different plots randomly. Randomization assures the unbiased estimates of treatment means and experimental error.

2. Replicate the treatments in different blocks. The number of replications is to be decided based on number of treatments, size of the experiment etc. A block is a unit containing the complete set of treatments as per statistical design of the experiment.

3. Fertilizer as per requirement should be mixed and placed in rows marked as per spacing for the region/variety.

4. Ridges of 15-20 cm high should be made on the rows demarcated.

5. Select seed tubers of uniform size (30-40g) for planting. The seed tubers should be well chitted.

6. Seeds tubers to be dibbled at a depth of about 10 cm so that it is at ½ the ridge depth.

7. Plant full replication or blocks if complete planting of the whole experiment in one day is not possible.

8. Provide at least one border row on either side and at least two border plants at the ends of the row.

9. Irrigation where ever practiced should be upto ½ the height of the ridge only and water should reach the top through capillarity.

10. When the plants are 4-6" tall weeding and earthing up is to be carried out. At earthing up, the neck of plants should be fully covered with soil or else emerging stolons would turn into stems and yield would be reduced.

11. Irrigate as and when required based on the nature of experiment.

12. Cut the haulms on the day specified as per requirement of the experiment.

13. Allow a week or 10 days for tubers to suberize in case of normal planted crop.

14. Harvest carefully so that tubers are not damaged and all tubers are exposed.

15. Grade the produce into 5 grades and count number and weight.

Data Collection

The success of experimental trials is dependent on the type, accuracy, and precision of data collection. The time of collecting data depends on the kind of trait, and the observations must begin at the earliest and must continue till 50% population of each plot reaches the trait. Foliar disease scoring must be timed according to the epidemiology of the disease. Observations made too early or too late, may give wrong data and result in wrong conclusions. For most leaf diseases, scoring at the middle of the period are appropriate. Data should be collected only on experimental plants and not on border rows and plants.

Observations Prior to Planting in Field

In potato, the seed tubers for all the experiments are produced at Seed Producing Unit at Modipuram and distributed to all the AICRP centres. Hence, the care must be initiated as soon as the seed tubers are received or tubers are taken out from the cold stores.

Taking out of seed from stores

In the plains, the cold stored seed is used for planting.

- ✓ The time of removal of seed from cold stores, the duration given for pre-sprouting and the stage of sprouting at the time of planting would affect the growth and development and hence they have to be carefully recorded.

- ✓ Take out the seeds from the cold stores and keep for pre-sprouting in well ventilated stores with diffused light. The time of seed taking out depend on the planting season i.e. in early and late planting the seeds needs to be taken out by more than 20 days before the planting time while for the main season crop about 15 days for pre-sprouting would be sufficient.

Observations to be recorded

- ✓ Date of removal from cold stores
- ✓ Maximum and minimum temperature and RH of the store where the seed is kept for pre-sprouting.
- ✓ Total number of seed tubers, damaged and good tubers fit for planting
- ✓ Date of beginning of sprouting i.e. the date when sprouts are visible (> 1 mm).
- ✓ Weigh the seed sample received from the SPU and variety wise data may be maintained
- ✓ Plot wise sample size and number of tubers may be counted and maintained in the data register which should be separate for different experiments.

Crop Improvement
(F_1C_1- F_1C_4 stages at CPRI centres)

Varietal development and selection of superior clone for proposing in AICRP

Conventional potato breeding broadly involves selection of desirable parents having the trait of interest, hybridization to get a segregating population in F1 generation (potato is highly heteozygous), elimination of undesirable genotypes in F1 and in the subsequent clonal generations, preliminary and replicated yield trials against standard varieties over locations and years to select a superior genotype. Depending on the requirement, various breeding methods, viz., bi-parental matings, back crossing and recurrent selection cycles, etc. are adopted.

Selection of Parents: The choice of parents in hybridization depends on the objectives of the breeding programme. Breeders prefer to work with parents having good agronomic attributes, adaptation and other desired traits. Characters with high heritability and monogenic dominant control are the easiest to transfer. Evaluation of germplasm for selection of parents, in order of preference is

✓ *S. tuberosum* sub sp. *tuberosum* collection involving commercial varieties, breeding lines, stocks in on-going breeding programmes and old land races,

✓ Sub sp. *andigena*,

✓ Primitive cultivated species,

✓ Wild tuber-bearing species, and wild non-tuber bearing species.

Andigena collection in India possesses wide wealth of valuable traits such as resistance/tolerance to biotic and abiotic stresses, high starch and protein content, good keeping quality and response to fertilizers. The potato is a highly heterozygous crop wherein most of the economic characters are governed by both additive as well as non-additive gene actions. Consequently, the breeding value of a genotype can't be assessed from phenotypic expression alone and knowledge of combining ability is important. In potato, since non-additive gene action is important for several economic characters, identification

of specific crosses with high mean value is important for selecting desirable segregants. This identification of promising crosses is usually done by 'Progeny tests'.

Important Points for Consideration

✓ Presence of trait of interest in at least one of the parents.
✓ Genetic divergence among parents to produce heterotic effects using genetic distances for planning crosses with ideal parents.
✓ Agronomically improved and widely adaptable clones of short day adapted *S. tuberosum* sub sp. *andigena* serve as better parents for breeding varieties for plains.
✓ The crosses involving subsp. *tuberosum* and sub sp. *andigena* are known to exhibit heterosis for tuber yield and its components, though late maturity.
✓ Wild species such as *S. demissum, S. acaule, S. chacoense, S. spegazzinii, S. stoloniferum,* and *S. vernei* can be utilized as diverse genetic resources. In India, wild species *S. verrucosum* and *S. microdontum* have been used as donors of durable resistance (horizontal) to late blight and *S. vernei* as source of resistance to cyst nematodes.
✓ Good general combining ability of parents for economic, biotic and abiotic stress resistance characters should be identified. At least one good general combiner should be involved as a parent in crosses.
✓ Selection of superior crosses based on progeny means in seedling and/or early clonal generations have been found to be effective for a number of characters including resistance to potato cyst nematodes, late blight and tuber yield. Progeny test should be applied in seedling stage or in clonal generations. In general, 80 seedlings (genotypes) per progeny is grown in four replicates sample of 20 are considered desirable.

Hybridization

In potato variety improvement programme, availability of ample flowers at proper time with functional male and female parts is an indispensable requirement for hybridization. Important factors that determine flowering behaviour are the genotype, day-length and temperature. Though, floral primordial of potato can arise in total

darkness, a photoperiod of 14-18 hours and temperature of 15 to 20°C favour flower production and berry setting. In tropics and sub-tropics, such conditions are available only at high altitudes (>1500m above sea level) where the potato crop is grown during summer season. The *S. tuberosum* sub sp. *andigena* clones generally flower profusely but sub sp. *tuberosum* varieties are often shy flowering and in some cases no flowers are formed.

Planting of parents in the Hybridization Block

✓ The parents selected for hybridization are planted in hybridization block to obtain flowering under natural day-length conditions in the hills.

✓ Flowering under short day conditions can, however, be induced by extending the photoperiod to 14-18 hrs./day through artificial illumination (one 250 W sodium vapour lamp erected at height of 3.6m above the ground per 100 m^2 area) and application of growth regulators (three weekly sprays of 50 ppm Gibberellic Acid + 10 ppm Indole-3-Butyric Acid + 2ppm Kinetin) starting from 30 days after germination.

✓ The quantum of seed tubers of male and female parents planted in hybridization block depends on the amount of hybrid seed to be produced. Uniform seed sized tubers (30-40 g) should be used.

✓ Normal agronomical practices of the region viz., spacing (60x20 cm), fertilizer, pesticide and weedicide application needs to be followed for raising the plants in the hybridization block.

✓ More space (about 1m) is provided in between two beds to facilitate free movement for effecting hybridization.

Floral selection and pollen collection

✓ Peak flowering time in high hills is generally July while in plains flowering starts in the last week of November and the peak flowering is reached in December.

- As pollen fertility is genotype dependent, pollen fertility is estimated by staining the pollen grains in 2% aqueous solution of Aceto-carmine and pollen germinability in 15% sucrose solution. Normally, the quantity of pollens in anthers is highly correlated with fertility.

- Pollen grains are collected from about to open buds or freshly opened flowers are used both for fertility and germinability estimation and for effecting hybridization.

- At least 100 pollen grains are counted in five microscopic fields and the stained pollens are considered as fertile and the unstained and shriveled ones as sterile. Generally, the parents with about 40% or more pollen fertility are used as male parents, whereas, pollen sterile or low pollen fertile genotypes are used as females.

- Parents having greater fertile parents are being used as female parents by emasculating the flowers before anther dehiscence.

- In female parents, the inflorescence is trimmed to remove very small buds, open flowers and berries if any, so as to retain only 4-5 large size flower buds per bunch. Using a pair of fine tip foreceps the buds are carefully opened and the anthers are removed. The petals and sepals are also trimmed to facilitate pollination. Care is taken not to injure stigma during emasculation.

- Flowers from male parents are collected in the evening of the preceding day of pollination.
- Freshly opened flowers with anthers that are about to shed pollen or the large size buds that would open next day, are collected and spread on a sheet of paper placed at room temperature.
- The wet flowers and low temperature conditions, these flowers are dried under a light bulb.
- In the morning of next day, the stigma of male flowers is removed and the pollen is extracted by shaking the anthers of the flower with needle under the condition of small amount of pollen is required.
- Requiring large quantities of pollen, anthers are detached from the flowers and pollen is extracted by shaking anthers in nylon sieve.
- The collected pollen grains are stored in a refrigerator at 6-8°C and can be used for a few days too, however, use of fresh pollen is recommended for better success rate.
- The flower buds about to open, freshly opened flowers emasculated earlier are pollinated by dipping the tip of stigma in pollen.
- Repeated pollination of receptive stigma twice or thrice at interval of 8 hours is known to produce higher percentage of berries per flower bunch and more seeds per berry.
- The pollinated buds are tagged with label bearing information on cross code, bunch number, number of flowers pollinated and date of pollination. The other relevant information like cross details (male and female parents), total number of bunches/buds pollinated and date of pollination is recorded in the hybridization register.

Berry formation, Seed Extraction and Storage

- Berry formation can be seen just 4-6 days after hybridization. At this stage, staking/support with sticks is provided to ensure that the flower bunches and berries do not touch the ground.
- Once the berries are formed, bunches are covered with thin muslin cloth bags of about 8 cm x 12 cm size to protect them from damage.
- Berries are harvested after about 6-7 weeks of pollination and allowed to ripe at room temperature till they are soft and pale yellow in colour.

- ✓ Since the number of berries per cross is usually small, these berries are macerated by hand, washed in running water and true potato seeds are collected and dried in shade for about 72 hours before being packed.

- ✓ A large number of berries in TPS programme, the seeds are extracted using a screw type juice extractor or a low speed blender for crushing the berries into pulp. The seed and pulp mass is treated with 10 per cent hydrochloric acid and stirred for 20 minutes to separate seeds from debris. Thereafter, the seeds are washed with water 3-4 times to remove acid. Clean seeds are dried in shade on a stretched muslin cloth for 72 hours followed by half an hour drying under sun to reduce moisture content to 5-6 per cent.
- ✓ True potato seeds are packed in polythene lined aluminum foil covers or double polythene bags, sealed and stored in desiccators containing calcium chloride and kept in refrigerator at 6-10°C.

Evaluation of TPS/ Seedling stage

The breeder's task is to pick superior genotypes by eliminating undesirable ones as quickly and efficiently as possible, without losing the one in million that may become potential new variety. Selection among thousands of genotypes is a multiphase process which spread over several generations, the number of genotypes are reduced each year. Simultaneously, the selected ones are multiplied for larger evaluation trials in the subsequent years, both at and over locations, to draw valid conclusions. Size of population and selection procedures varies with the objectives of the breeding programme. Since potato is vegetatively propagated, each plant within a clone has the same genotype (barring an occasional somatic mutation) and the variation within a clone is purely environmental, selection within a clone is, therefore, not practiced except when eliminating virus infected plants. The seedlings are raised in low aphid conditions to prevent loss of material due to virus infection.

Seed Treatment

✓ Seeds of various crosses are treated with Gibberellic acid (1500 ppm) for 24 hours to break the dormancy and achieve uniform germination.

✓ For plains, these seeds are sown in nursery beds (1 x 3m^2) usually in the 1st week of October.

✓ For easy sowing of minute sized seeds, the substrate consists of a mixture of sandy loam soil and well rotten farm yard manure (1:1) is done and sown at 0.5 cm deep furrows drawn 10 cm apart across the breadth of the bed.

✓ For hills, the seeds are sown in seedling boxes in 2nd week of February. Adequate moisture is maintained in the beds/boxes during the growing period.

✓ After about 25-30 days after sowing, and the seedlings reached at 4-6 leaf stage are transplanted in the main field at 20 cm intra- and 60 cm inter-row distance (plains) as against the seedlings are transplanted in earthen or plastic pots to mature in the hills.

✓ Seedlings of poor vigour are rejected prior to transplanting in the field itself as they would likely have low yields in later generations.

✓ Seedlings showing wild type, long stolons or viral infection are rogued out.

Screening for Late Blight resistance

4-6 weeks old seedlings are screened under controlled conditions against complex races of *Phytophthora infestans* and resistant seedlings are transplanted in the earthen pots in the glass house/ field and retained for further evaluations.

Screening for Heat Tolerance

The seedlings are screened by exposing them at >20°C night temperature and seedlings with ability to form tubers after 4-8 weeks of treatment are selected for further evaluations.

Dehaulming

✓ The standard manurial and cultural practices are followed during the crop season for healthy crop.

- ✓ Plants are dehaulmed at 90 days after transplanting in the plains and after about 110-120 days in the hills.

Harvesting

- ✓ Each seedling is harvested separately and the genotypes showing irregular tuber shape, deep eyes and undesirable tuber colour are rejected.
- ✓ At seedling stage, tuber yield does not form a criterion for selection as yield is greatly influenced by environment hence selections based on single plants are not reliable.
- ✓ Clones with undesirable tuber colour, tuber shape, eye depth and tuber cracking are rejected from seedling stage onward, as these characters have a high repeatability over generations.
- ✓ Five tubers of selected clones are retained for evaluation in short rows in the next year.
- ✓ No rejection should be done on the basis of tuber yield, average tuber weight or number of tubers in the seedling generation.
- ✓ The subsequent clonal generations of F_1 seedlings are labeled as C_1, C_2, C_3 to Cn.

Evaluation of Early Clonal Generations

In potato breeding programmes, the major constraint in effective evaluation of genotypes in early generation is non-availability of sufficient number of plants per clone. The assessment is done based on un-replicated plots with just a single plant in the seedling (F_1), five in F_1C_1, 30 in F_1C_2 and 120 plants in F_1C_3 generation. Further, the seed tubers of clones used for planting often vary in size due to location and year effect. These factors are the bottleneck to make assessment of quantitative characters like tuber yield in early generations. Hence rejection of undesirable clones (rather than selection of desirable clones), based on stable characters that have no or very little environmental effect (tuber colour, shape, depth of eyes and tuber cracking) is practiced in early generations.

Important Points for Consideration

- ✓ Negative selection (rejection of poor phenotype) for tuber yield and tuber weight can be initiated from the first clonal generation

onwards, whereas, number of tubers can be considered as a basis for the rejection of undesirable types from second clonal generation onward.

✓ In subsequent generations, average tuber weight should be used as a selection parameter after fixing a standard for minimum number of tubers required in selected types.

✓ For screening of late blight, cyst nematodes, wart, etc. the susceptible genotypes in seedling/ early evaluation stages are discarded.

Screening for PCN resistance

The selected F_1C_2 clones are screened for PCN resistance in the glass house. 5 tubers of the selected clone are planted in the pots containing a population of 200-250 cysts of both species of *Globodera* per 100 ml soil. Clones showing 0-5 females/ root balls after 60-70 days of planting are selected.

Screening for processing

✓ Four tubers of each clone in the in F_1C_1 generation are dipped in brine solution for specific gravity estimation and clones with <1.080 specific gravity are rejected. Selected clones in F_1C_2 generation are tested for chips/ French fries colour.

✓ The breeding population is subjected both to resistance and quality tests are performed and rejection is done based on unacceptable tuber characters. Clones showing poor keeping quality in the form of high shrinkage, rottage, weight loss and excess sprouting after cold store are rejected.

Evaluation in Advanced Clonal Generations

In advanced clonal generations (F_1C_4 onwards), clones are evaluated in replicated trials of adequate plot size having with one or two standard varieties as checks. With advancement of clonal generations, plot size and number of replications are increased. The plants in each plots are dehaulmed at different dates to ascertain the potential of clones at various crop durations and requirement of thermal time. The clones are also evaluated for their suitability of planting on different dates in order to judge their potential for inclusion in different cropping sequences operating in a region.

Important Points for Consideration

✓ The tubers of selected clones in yield trials are graded, weighed and their tuber characters, viz., specific gravity, flesh colour and internal defects such as hollow heart and internal necrosis are recorded.

✓ Tuber samples are boiled and their texture, degree of sloughing is estimated. The tubers are also tested for after-cooking darkening, flavour, suitability for processing and for storage at ambient temperature.

✓ Cold storage of potatoes tends to promote conversion of starch to reducing sugars resulting dark colour in chips and French fries. Advanced clones for processing are tested for bulk frying at industrial level and evaluated for total solids (%), undesirable colour (%), internal defects (%), external defects (%), total potato defects (%) and hunter colour. The clones performing better than the controls are selected.

✓ To identify potential genotypes for processing, tests are conducted after different periods of storage, at more than one temperature and after various periods of reconditioning.

✓ Three years' data from replicated trials is considered adequate to identify superior clones for introducing in multi-location trials under All India Coordinated Research Project on Potato (AICRP-Potato).

✓ The hybrids identified for evaluation under AICRP (P) multilocation trials are characterized morphologically with DUS (distinctness, uniformity and stability) descriptors and molecularly with SSR markers.

Maintenance of Seed Stocks

Maintenance and multiplication of selected clone is done under disease or virus free conditions, during the course of evaluation over years. The seedlings and initial clonal generations are grown in low aphid (virus vector) zone and precaution is taken to minimize introduction and spread of virus diseases. To avoid aphid transmitted viruses, insecticides are used and vines are killed before aphid population reaches a critical level. Plants with visible virus symptoms are rogued out to reduce possibility of secondary infection. The seed production of advance clonal generation is done in separate seed-maintenance field following seed plot technique.

Table 1: Minimal criteria followed for selecting potato varieties for different purposes

Characters	Purpose of selection				
	Table potatoes		Processing potatoes		
	Boiled	Baking	French fries	Chips	Canned
Tuber shape	Long-oval/ round	Long-oval/ round	Long-oval (>3 inch)	Round 2.5-3.3 inch)	Round to oval (2.5-3.3 inch)
Tuber size, mm	40-80	40-80	>75	45-80	20-35
Eye depth	Shallow/ medium	Shallow/ medium	Shallow	Shallow	Shallow
Texture	Waxy	Mealy	Mealy	Mealy	Waxy
Uniformity	High	High	High	High	High
Dry matter (%)	18-20	>20	>20	>20	<18
reducing sugars (% fresh wt.)	-	-	<0.15	<0.10	0.5
Glycoalkaloids (% fresh wt.)	<0.15	<0.15	<0.15	<0.15	<0.15
After cooking discoloration	Slight	Slight	-	-	Nil

Table 2: Steps to be followed at different clonal generation

Sl. No	Characters	Early generation stage	Advance generation stage
1	Seed wt. per plot (Kg).		
2	Plant emergence (%) at every 3rd day till complete emergence (>95%) or up to 30 days after planting.	✔	✔
3	Plant vigor 60 days after planting (1-5 scale).	✔	✔
4	Foliage senescence (%) at haulms cutting		
5	In late blight & PCN resistance breeding, the disease incidence at 10 days interval after 1st appearance of disease	✔	✔
6	Incidence of any major diseases, final score.	✔	✔

Sl. No	Characters	Early generation stage	Advance generation stage
7	Tuber rottage in the plot (weight) at the time of harvesting.	✓	✓
8	Total and marketable tuber yield (t/ha) at harvest at 60, 75, 90 days harvest in plains and at 90 and 120 days harvest in hills	✓	✓
9	In processing breeding, total, process grade yield (t/ha) and tuber dry matter (%) at harvest at 75 & 90 days in processing for chips and Total and French Fry grade yield (t/ha) at harvest 110 days in processing for French Fry.	✓	✓
10	Total weight loss at 75 days after storage at ambient temperature	✓	✓
11	Organo-leptic test (1-5 scale) 1=V Poor to 5=V Good by about 20 volunteers at harvest at each location.	✓	✓
12	Meteorological data.	✓	✓

Base temperature of advanced hybrid

The information about the base temperature of advanced hybrid is very important so as to calculate its thermal requirement. After harvest, keep a set of 5 uniform tubers (60-80 gm) each at 6 different temperatures ($2^{\circ}C$, $3^{\circ}C$, $4^{\circ}C$, $5^{\circ}C$, $6^{\circ}C$, and $7^{\circ}C$, in BOD incubators and record the sprout initiation and weight loss data at 5 days interval.

Specific characters to be observed in crop improvement trials

Days to 1st flowering

✓ Flowering should be scored at every 7 - 14 days interval after planting

✓ Scoring such as 0 - 3 may be followed (scale: 0 = not flowering, 1 = flower buds present, 2 = flowers present, and 3 = post-flower)

✓ This information was used to calculate initial and final dates of flowering, as days after planting (DAP), and then used to determine the duration of the flowering period (in days).

Days to 50 % flowering

✓ The number of days taken from planting to the flowering in 50% of the population in each treatment and replication wise reckoned and expressed in days.

Number of flowers/cluster

✓ Record the number of flowers on each cluster of the plants from 1-5th cluster

✓ Buds should be included in flower count and expressed in numbers.

Flower color

✓ Five freshly opened flowers in 2-5th cluster of each plant should be chosen for color observation.

✓ 5-10 plants may be selected for observation

✓ Scoring should be given according colour present in most of the corolla (1= White/cream/yellow, 2= Pink, 3= Red, 4= Light blue, 5= Blue, 6= Purple, 7= Violet)

Flowering degree

✓ Five to ten healthy plants should be selected for flowering degree observation.

✓ 2-5th cluster of each plant should be chosen for observation at peak flowering period

✓ Scoring should be given according the degree of flowering which should be recorded at the peak of the flowering (0= No buds, 1= Bud abortion, 3= Scarce flowering, 5= Moderate flowering, 7= Profuse flowering).

Calyx length

✓ The calyx length should be measured from the tip to the point of attachment flower pedicle in ten flowers and an average is expressed in centimeter.

Corolla length

✓ The corolla length should be measured from the tip to the point of attachment flower pedicle in ten flowers and an average is expressed in centimeter.

Style length

✓ The style length should be measured from the tip to the point of attachment flower pedicle in ten flowers and an average was expressed in centimeter.

Pollen viability

✓ A mixture of pollen from flowers at anthesis of the same plant is collected. The pollens are collected by vigorously shaking the anthers with a needle over a microtube.

✓ The acetocarmine staining method is followed in which the proportion of pollen grain taking up the stain was measured (Fernandez- Munoz *et al.,* 1995).

✓ Non-staining pollen grains were classified as sterile and well stained pollen grains as fertile. The well stained pollen grains are counted and expressed as percentage.

Pollen germination (*in vitro*)

✓ Fresh flower are collected at the time of anthesis from 10 plants per genotype and immediately placed in polythene bags and carried to the laboratory.

✓ Pollen is sprinkled gently tapping a set of three flower directly above the surface of the medium consisting of 165 gL^{-1} sucrose, 12 gL^{-1} agar, $20 mgL^{-1}$ $Ca(NO_3)_2.4H_2O$, and 10 $mgL^{-1}H_3BO_3$ dissolved in distilled water.

✓ Pollen germination is determined by direct microscopic observation. The pollen germination is considered germinated when pollen tube

length is at least equal or greater than grain diameter (Tome. *et al.,* 2007).

✓ Germination per cent is determined by dividing the number of germinated pollen grains per field view by the total number of pollen per field view and expressed as percentage.

Fruit setting percentage

✓ The fruit setting percent is computed by the following formula given by Villareal and Lai (1978).

✓ Fruit setting percent= (No. of fruits per cluster / No. of flowers per cluster) x 100.

✓ The first five clusters were observed to represent fruit setting per cent (El-Ahmadi and Stevens, 1979).

Predominant tuber skin color

✓ Fully matured tuber of freshly harvested should be taken for color scoring

✓ 10-25 disease free, healthy tuber should be scored with the color which covers most of the surface of the tuber (1= White-cream, 2= Yellow, 3 = range, 4 =Brownish, 5 =Pink, 6= Red, 7 =Purplish-red, 8 =Purple, 9= Blackish).

Predominant tuber flesh color

✓ Fully matured tuber of freshly harvested should be taken for color scoring

✓ 10-25 disease free, healthy tuber should be scored with the color which covers most of the surface of the flesh of tuber (1= White, 2= Cream, 3 =Yellow-cream, 4= Yellow, 5= Red, 6= Violet, 7= Purple, 99= Other.

Tuber outline (shape)

✓ Fully matured tuber of freshly harvested should be taken for scoring of shape

✓ 10-25 disease free, healthy tuber should be scored with the shape using following score (1 Compressed (oblate) – major axis is the shortest axis

- ✓ 2= Round – an almost circular outline,
- ✓ 3 =Ovate – an outline resembling an egg. The broadest part is within 1/3 of the distance from the stolon end.
- ✓ 4= Obovate – an outline which is inversely ovate and broadest within 1/3 of the distance from the apical end (rose or eye end).
- ✓ 5= Elliptic – an outline showing the same breadth when measured at equal distance from both the stolon and apical ends. The outline is slightly acute at each end
- ✓ 6= Oblong – an almost rectangular outline with the sides nearly parallel but the corners are rounded. The length/breadth ratio should not be more than 3/2.
- ✓ 7 =Long-oblong – an oblong outline with a length/breadth ratio closer to 2/1.
- ✓ 8= Elongate – a long rectangular outline with a length/breadth ratio equal to or more than 3/1.

Table 3: Weightage scores used for hybrid introduction into AICrP (P)

Characters	Observations	Total Weightage
Table potatoes		
Tuber Eye depth	Deep-1, medium-deep-2, medium-3, shallow-medium-4, shallow-5	5
Tuber uniformity	Low-1, very high-5	5
Dormancy period (weeks)	Short-2, medium-3, long-5	5
Keeping quality Weight loss	Poor-0, Average-4, good-6, very good-8, excellent-10	10
Disease resistance	Sus.-0, field reistance-15	15
Dry matter (%)	15%-1, 16%-2, 17%-4, 18%-6, 19%-8, 20%-10	10
Tuber yield	At par 35, 5% higher-40, 10% higher-45	45
Additional character	Tolerance to hopper/mite and productivity under heat stress conditions	5
Total		**100**

Characters	Observations	Total Weightage
Processing Potatoes		
Tuber Eye depth	Deep-1, medium-deep-2, medium-3, shallow-medium-4, shallow-5	5
Tuber uniformity	Low-1, very high-5	5
Maturity type	Early-5, Early medium-4, Medium-3, Medium late-2, late-1	4
Dormancy period (weeks)	Short-2, medium-3, long-5	4
Keeping quality/ Weight loss	Poor-0, Average-4, good-6, very good-8, excellent-10	10
Disease resistance	Sus.-0, field reistance-15	15
Dry matter (%)	15%-1, 16%-2, 17%-4, 18%-6, 19%-8, 20%-10	10
Tuber yield	At par 35, 5% higher-40, 10% higher-45	45
Chip colour	1-10 scale	2
Total		**100**
Disease Resistant (Late blight/PCN) potatoes		
Tuber Eye depth	Deep-1, medium-deep-2, medium-3, shallow-medium-4, shallow-5	5
Tuber uniformity	Low-1, very high-5	5
Maturity type	Early-5, Early medium-4, Medium-3, Medium late-2, late-1	5
Dormancy period (weeks)	Short-2, medium-3, long-5	5
Keeping quality/ Weight loss	Poor-0, Average-4, good-6, very good-8, excellent-10	10
Disease resistance	Sus.-0, MR-5, R-15, HR-25	25
Dry matter (%)	15%-1, 16%-2, 17%-4, 18%-6, 19%-8, 20%-10	10
Tuber yield	At par 10, 5% higher-25, 10% higher-35	35
Total		**100**

(Check varieties for Table, Processing and LB resistant is K Bahar, K. Chipsona-1/K. Frysona and Kufri Girdhari/ K. Swarna, respectively).

Depth of tuber eyes

✓ Fully matured tuber of freshly harvested should be taken for scoring of shape.

✓ 10-25 disease free, healthy tuber should be scored with the depth of tuber eye using following score (3 =Shallow, 5 =Medium, 7 =Deep, 9 =Very deep).

Tuber Sprout Color

✓ Fully matured and freshly sprouted tuber should be taken for scoring of sprout color (5-10days after sprouting).
✓ 10-25 disease free, non shrunken healthy tuber should be scored with the sprout color (1= White-cream, 2= Yellow, 3= Pink, 4= Red, 5= Violet, 6 =Purple,7= Blackish, 99= Other.

Experiments at CPRI and AICRP Centres

AICRP experiments involve varietal trials, agro techniques and pest and disease management. Hence morphological observations to be recorded have some similarity while some traits are specific according to the nature of experiments. In general the common observations to be recorded are described below.

Emergence

✓ Record the date of start of emergence i.e. when the stems break through soil surface.

✓ Emergence starts after about 10 days after planting in the case of the crop planted at the optimum time in the northern plains. In the early and late planted crop as well as in the hills emergence is delayed.

✓ Record the number of plants emerged every alternate day till 100% emergence is achieved.

Juvenile growth habit

✓ Juvenile growth habit may be measured from the angle of the main stem observed from the entire plot prior to the maximum lodging stage and accordingly the plants are classified as .

✓ a. Erect – main stem at an angle of 30° or less from the perpendicular.

✓ b. Spreading – Main stem have leaning more than 60" from the perpendicular.

✓ c. Intermediate – main stem angle is intermediate between erect and spreading

Leaf development

✓ The main stem (the first emerged robust stem) on 5 uniform plants selected at random are tagged and the dates of unfolding of the leaves on them is recorded as below:

✓ date of first leaf of main stem unfolded (> 4 cm) ,

- date of second leaf of main stem unfolded (> 4 cm).
- This is continued till no further increase in leaf number is observed.

Basal Side Shoot

- Five uniform plants selected at random are tagged and observations as below are recorded
- The date when the first basal side shoot is visible (> 5 cm) on them is recorded.
- The date when the second basal side shoot is visible (> 5 cm).
- This is continued and the dates of appearance of subsequent side shoots are recorded till no further increase is observed.

Plant Height

- Plant height is the shortest distance between the upper boundary of the main photosynthetic tissues on the plant and the ground level, expressed in m or cm.
- Select the plants at centre portion of treatment plots (avoid plants at corner areas).
- Measure the height of the main plant from the soil level to the top foliage of the main plant stem (not the flower or seed etc).
- Ensure that the soil level not changes due to irrigation or any other cultural activities (if possible make a color mark at the soil line on the selected plants).
- Measure plant height at uniform interval to assess growth rate (10, 20, 30 days….etc).

Specific Leaf Surface Area

- Is the one sided surface of fresh leaf divided by its one dry mass expressed in m^2/kg or mm^2/mg.

- ✓ Relatively young and preferably photo-synthetically active leaf, but fully expanded, hardened leaf free from pest and diseases should be selected.
- ✓ Any petiole or rachis and all veins are considered part of leaf for SLA measurements.
- ✓ **Method 1:** Trace the leaves on graph paper and count the squares covered to give you an estimate of the surface area for each leaf. Repeat this for each leaf on a plant and for each plant in your experiment.
- ✓ **Method 2:** Trace out each leaf on paper. Make sure to use the same type of paper every time and make sure that the paper is not wet. Cut out the leaf tracings and weigh them. Weigh the cutouts and divide the total weight by the number of leaves to give you the average leaf area for each plant. Repeat this for each of the plants in your experiment.
- ✓ **Method 3:** Digital image analysis: Using a digital camera capture an image of a plant. Using special software, analyze the surface area of the leaves.

Number of Branches per plant

- ✓ The branches that arise from the mother tuber main stem of the plants were taken as primary branches.
- ✓ Number of primary branches should be counted from all the tagged plants during harvesting stages and mean number of branches per plant is worked out.

Plant Canopy Cover

- ✓ The space between 2 rows is considered for this trait and divided in to 5 parts and the width of the canopy extending to the furrows is rated. Since the spacing between the rows is 60 cm about 12 cm width on each side would represent 20%.
- ✓ Similarly for each 12 cm extension of the canopy 20% is accrued to the total canopy cover.

Grid Method

- ✓ The proportion of ground covered with green leaves is to be measured at ten days interval using a grid. It consists of a wooden

frame divided into 36 equal sections (internal dimensions of frame is 60x60 cm) of dimensions 10 cm x 10 cm- a multiple of the planting pattern for potato.

✓ The grid should be held on top of the potato crop at one metre from the ground and only those sections more than half filled with green leaves (haulms underneath) are counted by observing from vertically above to avoid parallax error.

✓ In example fig below 7 grids have canopy underneath occupying more than 50% of the grid area. The rest marked x are ignored though there is canopy underneath them but since their area is less than 50%. The canopy cover observations may be started from 30 or latest from 40 days after planting.
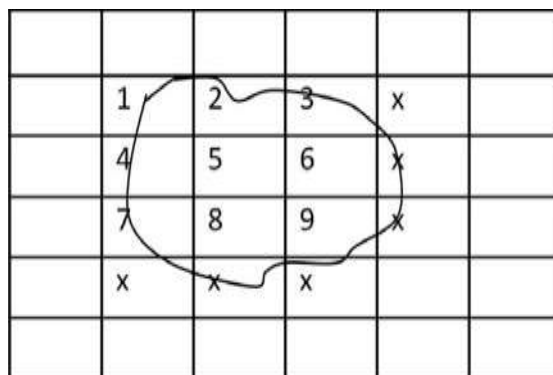
Digital Camera Method

✓ Hold the camera with its lens oriented directly to the top of the plant in automatic mode with No zoom, No flash, Iso 100 and at Maximum image resolution.

✓ The camera should be held parallel to the ground. The camera should be held about 1 meter from the top of the plant canopy at the same height at every observation.

✓ Measure the ground area covered at that height on bare soil and then shoot pictures of the canopy.

✓ The digital images are analysed using software to get the canopy cover.

Maturity time

- ✓ The time taken for natural senescence of 80-100% is genotype specific and its growing condition. Hence natural senescence time is to be recorded from date of planting.
- ✓ Maturity should be calculated from the day of halum cutting or natural complete senescence of a crop to the tuber harvested. It has been classified as follows.
- ✓ Very late (later than 120 days), (3) late (between 111 and 120 days), (5) moderate (between 101 and 110 days), (7) early (between 80 and 100 days) and (9) very early (shorter than 80 days).

Harvesting

- ✓ When the plants have attained 75 to 80 per cent senescence of the haulms or as per treatment haulms cutting is done and date of haulms cutting is recorded.
- ✓ Before haulms cutting the number of plants in the net plot (excluding border rows and border plants in each row) is to be counted.
- ✓ After haulms cutting the plants are allowed to suberize for about 7 to 10 days and then harvested.
- ✓ After harvesting the tubers are graded in to 5 grades (0-25g, 25-50g, 50-75g, 75-100g and > 100g).
- ✓ The number and weight of tubers in each grade is then recorded.

Post harvest characters

Dormancy

- ✓ Dormancy is calculated from the day of haulm cutting to sprouting of harvested tubers and the days are classified based on the below mentioned grades.
- ✓ Very short (shorter than 30 days), (3) short (between 31 and 60 days), (5) medium (between 61 and 90 days), (7) long (between 91 and 120 days) and (9) very long (longer than 120 days).
- ✓ Tubers of sufficient quantity should be kept at ambient temperature in open condition and sprouting in all the tubers should be seen.

Weight loss

- ✓ 10-20 tubers of freshly harvested tuber selected are weighed in soil free condition
- ✓ And the same is repeated at 10days interval.
- ✓ The rotten tubers are separated and weighed at 10 days intervals.

Crop Production

Cropping Systems

Experiments on cropping systems involve evaluation of crop sequences and intercropping systems in terms of their productivity, resource use efficiency, complimentary use of resources, benefit cost ratio etc. The data on yield, nutrient uptake by the different crops and the cost of the inputs and outputs are used to calculate various efficiencies some of which are listed below.

The various competition indices as given by different workers for intercropping studies:

Land equivalent ratio (Mead and Willey, 1980)

$$LER = \frac{Yab}{Yaa} + \frac{Yba}{Ybb}$$

Competition ratio (Cr) (Willey and Rao,1980)

Where

Yaa = Yield per unit area of species 'a' as sole crop

Ybb= Yield per unit area of species 'b' as sole crop

Yab= Yield per unit area of species 'a' intercropped with species 'b'

Yba= Yield per unit area of species 'b' intercropped with species 'a'

Zab= Proportion of intercropped area initially allocated to species 'a'

Zba= Proportion of intercropped area initially allocated to species 'b'

Multiple Cropping Index or Multiple Cropping Intensity (MCI) (Dalarymple, 1971)

It is the ratio of total area cropped in a year to the land area available for cultivation and expressed in percentage.

$$MCI = \frac{ai}{A} \, X \, 100$$

Where i = 1, 2, 3, n, n= total number of crops, ai = area occupied by crop and A= total land area available for cultivation. Or MCI is the

sum of area planted to different crops and harvested in a single year divided by total cultivable area and expressed as percentage. Or MCI means the sum of areas under various crops raised in a single years divided by net area available for that cropping pattern multiplied by 100. It is similar to cropping intensity.

$$MCI = \frac{\text{(Total number of crops + their respective area)}}{\text{(Net cultivable area)}} \times 100$$

Cultivated Land /Utilization Index (CLUI) (Chuang, 1973)

$$CLUI = \frac{aidi}{A \times 365} \times 100$$

It is calculated by summing the products of land area to each crop, multiplied by the actual duration of that crop divided by the total cultivated land times 365 days.

Where, I = 1, 2, 3, n, n = total number of crops. A1 = area occupied by the ith crop, di = days that the ith crop occupied ai and A = total cultivated land area available for 365 days. CLUI can be expressed as a fraction or percentage. This gives an idea about how the land area has been put into use. If the index is 1 (100%), it shows that the land has been left fallow and more than 1, tells the specification of intercropping and relay cropping. limitation of CLUI is its inability to consider the land temporarily available to the farmer for cultivation.

Crop Equivalent Yield (CEY)

The yields of different intercrops are converted into equivalent yield of any one crop based on price of the produce.

CEY = (yield of 'a' crop X price of 'a' crop + yield of 'b' crop X price of 'b' crop) / price of 'a' crop

Nutrient Management

Studies on nutrient management aim at determining the dose of nutrient to be applied to ensure a given uptake of the nutrient for a proportionate yield increase. This would involve determining the

nutrient present in the soil, the proportion of nutrient taken up from that present in the soil, the dose of nutrient added and the proportion taken up from that applied. Therefore, nutrient management studies would require proper sampling of the soil as well as plants so that their chemical analysis would give reliable results.

Collection and Preparation of Soil Samples for Analysis

✓ Only a minute fraction of huge soil mass of the field is used for the analysis in the laboratory to find out the relevant physical and chemical characteristics. Therefore, for collecting soil samples the following aspects should be considered carefully.

✓ The soil sample collected should be representative of the area sampled.

✓ Variation in slope, colour, texture, crop growth and management should be taken into account and separate sets of composite samples should be collected from each of such area.

✓ Recently fertilized plots, bunds, channels, marshy tracts and spots near trees, wells, compost, or FYM/piles or other non representation locations must be carefully avoided during sampling.

✓ Soil sampling should be done well in advance and not just before planting.

✓ Ten to fifteen sub samples of approximately equal weight should be drawn in a zig zag pattern from an apparently homogenous plot. However, where the area within the field looks different in appearance or topography, divide the field into parts and take sample of each portion (constituting a sample unit) separately.

Sampling Procedure

✓ To obtain a composite sample, small portions of soil are to be collected upto the desired depth. Generally the samples may be drawn to the plough depth (0 – 15 cm) by means of suitable sampling tools from at least 10 – 15 spots after scrapping off the surface litter, if any.

✓ For sampling soil, the tube auger, spade or khurpi is quite satisfactory. If a spade or khurpi is used, a V shape cut may be

made upto the plough layer and a uniform 1.5 cm thick slice is taken out.

✓ The soil collected in this manner should be thoroughly powdered and mixed by hand on a clean piece of cloth or polythene sheet or thick paper. The bulk is reduced by quartering and about 500 g of the composite sample is retained. The soil must be quickly dried in shade at room temperature and put in cloth or polythene bags with suitable description and identification marks.

Preparation of sample for Analysis

✓ **Drying:** Samples are generally air dried (25–35ºC), at low relative humidity and stored. Results of soil analysis are expressed on oven dry weight basis. This necessitates determination of moisture percentage by drying a small quantity in an oven at 105ºC for 2 hours. However, the sample used for estimation of moisture content should be different from that used for chemical analysis.

✓ **Sieving:** Field moist samples prior to drying can be made to pass through a 6 mm sieve (about 4 mesh per inch) by rubbing with fingers. Soils in the right moisture condition can even be passed through a 2 mm sieve (about 10 meshes per inch).

✓ **Mixing:** Sample should be thoroughly mixed by rolling procedure. Place the dried and sieved sample on piece of cloth. Grasp the opposite corners and then holding one corner down pull the other corner across the sample. Now the process is repeated in the reverse direction. Use the other two corners and roll the soil from one corner to another repeatedly. Continue this until thorough mixing is assured.

✓ **Storing:** Store the soil in paper carton (soil sample box) using a polythene bag as an inner lining.

Plant Sampling and Sample Preparation

Sampling Technique

✓ In plant sampling every alternate row should be sampled In small plots and in big plots every 5th to 50th row (depending upon the size of field) should be sampled. For small experimental plots (100 sq m) about 10 plants would serve the purpose but for bigger fields about

25-50 plants should be collected. It is always advisable to collect a larger sample in the field and then reduce it in the laboratory as it helps in getting a representative sample. For working out the plant nutrient uptake by crop, entire plants are to be sampled. Different tissue should then be separated (their dry weight must be recorded) and analysed separately.

Sample Preparation

✓ After sample collection, the fresh tissue should be decontaminated from dust and other foreign material.

✓ The fresh tissue should be washed in sequence in detergent solution (0.2% teepol), and then in dilute HCI (0.1N) and finally with deionized water. The liquid detergent will remove waxy coating on leaf surface and any soil particles. N/10 HCI will remove metallic contaminants and deionized water will wash the previous two solutions. The extra moisture is wiped out, the sample is placed in new paper bags and dried in an oven at 60+5 °C. In the case of haulms (composite including stems and leaves) about 200 g fresh weight materials should be kept for moisture content estimation and drying at 60+5 °C in an oven so that at least 20 g dry sample is obtained.

Nutrient uptake

✓ Two tubers of each grade should be cut into small pieces (5 mm size), mixed and a 100 g sample is drawn and dried at 60+5 °C for approximately 48 hours for working out the water content and nutrient analysis. The samples are ground and stored for analysis. The tuber samples should be prepared within a week after harvest otherwise driage takes place and higher dry matter content would be observed.

Water management and Irrigation scheduling

Experiments on water management aim at determining the water content of soil, the amount of water taken up by the plants, water use efficiency etc. Therefore, such studies involve periodic soil sampling and estimation of the soil water content.

Soil moisture content estimation Procedure

Soil samples are collected by tube or auger from a number of points (0-15 cm) within the experimental site and mixed thoroughly. The composite sub samples of about 50 g to 100 g of soil are placed in moisture cans and closed with tight fitting lids. The moist samples are weighed immediately after bringing to the laboratory and dried to constant weight in an oven at 105 to 110 ºC (for about 24 hrs) and reweighed after cooling in a desicator. The calculation of the soil moisture content is done by determining the loss in weight on drying and the weight of the oven dry soil as follows:

$$\text{Soil moisture content by weight } (\%) = \frac{(\text{Weight of wet soil} + \text{tare}) - (\text{Weight of dry soil} + \text{tare})}{(\text{Weight of dry soil} + \text{tare}) - (\text{tare})} \times 100$$

$$\text{Mw } (\%) = \frac{\text{Loss in weight on drying}}{\text{Weight of oven dry soil}} \times 100$$

The soil moisture content is estimated before each irrigation in case of irrigated crop.

Water Use Efficiency

✓ The Water use efficiency is computed by dividing potato yield with total water applied (cm).

✓ In water management studies the water content in the plants is also sometimes used for scheduling irrigation. The relative water content is estimated as below.

Relative Water Content (RWC)

✓ The relative water content of leaf should be estimated using physiologically active leaves.

✓ 25 leaf discs of one cm diameter is cut using punching machine and their fresh weight is recorded.

✓ The leaf discs are then immersed in water at constant temperature for 4 hours. Then, the turgid weight of the discs is recorded.

✓ The leaf discs should be oven-dried in hot air oven for 8 hours at 80°C, and the dry weight of the discs is finally taken.

✓ Using the formula proposed by Barrs and Weatherley (1962), the RWC is estimated and the values are expressed in percentage.

✓ RWC= {(Fresh weight- Dry weight)/(Turgid weight-Dry weight)} x 100

Weed Management

Weeds compete with the crop for space, nutrient and water. Therefore, it is necessary to estimate the competition offered by weeds in weed management trials. In such studies weed number and dry weight of the weeds is recorded and these samples can then be analysed for the their nutrient content to compute the competition for nutrients by the weeds.

Weed number and dry matter estimation

Weed population is estimated by using a quadrat of 50 x 50 cm^2 or 25 x 25 cm^2. The quadrat is thrown randomly at different number of places in a plot (depends upon size of field and weed incidence). Weeds of different species are counted and uprooted. For nutrient uptake estimation, preparation of sample is done in the similar way as for the potato tissues detailed elsewhere in the manual.

Plant Protection

Assessing and Scoring Diseases

✓ Plants are counted to determine disease infection at regular intervals in a randomized pattern throughout the field.

✓ It should be ensured that the inspection results are representative of disease levels in the field.

✓ At each observation preferably about 100 consecutive plants in a row should be observed.

✓ The actual number of diseased plants per observation is recorded and used to determine the percentage.

Late blight and early blight

Late blight and early blight are two of the most common potato diseases in India. Studies under AICRP (Potato) involves monitoring their appearance, and rating their severity. Another activity is the collection and sending the pathogen samples for analysis of the race spectrum, mating types, fungicide resistance and other advanced studies.

✓ Late Blight (*Phytophthora infestans*) and early blight *(Alternaria solanii)* begin to spread once the environment is congenial.

✓ Initial symptoms usually appear in warm and wet or humid weather.

✓ Once late blight begins to spread, it can be extremely destructive, destroying entire fields if left unchecked.

✓ Both Late blight and early blight spreads through mechanical means as well as through rain and wind.

✓ The data to be recorded in field experiments are disease appearance, incidence and disease severity.

Disease incidence and disease severity

Disease incidence is measured by counting the number of diseased plants/individuals divided by total number of plants/individuals multiplied by 100. This method of recording disease is most useful

and reliable in diseases like damping off, seedling blight, wilts and viral diseases. Disease severity on the other hand is the proportion of the diseased tissue out of the total healthy tissue.

✓ Disease incidence and severity are assessed on the experimental rows every week. Incidence of late blight is assessed by counting the number of plants on the experimental rows and expressed as percentage of total plants.

✓ As regards severity five plants are selected randomly from each treatment of each replicate and then five leaves of each plant are used to determine the disease severity.

$$\text{Disease Incidence} = \frac{\text{No of diseased plants}}{\text{Total Number of plant inspected}} \times 100$$

Two scales are most widely used in literature to rate the late blight severity and they are given below.

Scale for recording late blight severity

Score	9	8	7	6	5	4	3	2	1
Area infected (%)	Trace	10	11-25	26-40	41-60	61-70	71-80	81-90	Collapsed

(Source: Malcolmson, 1976)

Score	1	2	3	4	5	6	7	8	1
Area infected (%)	>90	81-90	71-80	61-70	41-60	26-40	11-25	d"10	Collapsed

(Source: Cruickshank *et al.*, 1982)

The accessions are categorised into highly resistant, resistant, moderately resistant and susceptible on the basis of following scale.

Area infected (%)	Category
Up to 5.0	Highly resistant (HR)
5 to 20	Resistant (R)
21 to 40	Moderately resistant (MR)
>40	Susceptible (S)

Collection and preparation of Late Blight infected samples for further analysis

For sending the late blight isolates from leaves or stem it is necessary to inoculate and create artificial infection in fresh potato tubers because infected leaves cannot be sent as such for long destinations. For this purpose, following steps are followed:

Collect late blight infected leaves (preferably having one lesion only) from the field and bring it to the laboratory.

- ✓ Wash the sample to make it free from dust, soil etc.
- ✓ Take the clean tubers of any late blight susceptible cultivar and surface sterilize them with alcohol.
- ✓ Cut small leaf pieces (bits) from the lesion margin containing some dead and some living tissue (40% dead, 60% living).
- ✓ Make a small cut on the tuber with the help of a scalpel/knife/ blade and insert the cut leaf piece into the cut portion with the help of forceps or needle.
- ✓ After inserting the leaf piece, press the cut portion of the tuber with the help of thumb and forefinger and seal it with wax/petroleum jelly/candle/parafilm tape. The wax should not enter inside the cut portion.
- ✓ Incubate the inoculated tubers at 18 ± 1°C for 3-4 days and after that send it to the desired laboratory for further studies.

To avoid wound/injury in transit, the inoculated tubers should be sent by keeping them in small boxes having sufficient number of holes for aeration.

Assessing Symptoms of Mosaic

- ✓ Mosaics are caused due to infection of potato plants with certain viruses.
- ✓ The symptoms may range in severity from slight mottling to severe crinkling of leaves and stunting of plants.

- ✓ Depending on the variety and/or the weather conditions it may also affect symptom expression. Cool, cloudy weather may make the symptoms more pronounced.
- ✓ The presence of more than one virus in the plant may affect the types of symptoms and increase the severity. Symptoms of the same virus can be differently expressed on different varieties.
- ✓ In general, leaves of virus affected plants are mottled often with some areas of light green to yellow and some darker green than normal.
- ✓ Mottled areas may vary in size and occur both on and between the leaf veins. Leaf margins may be wavy, and the leaves may appear slightly rugose where the veins and interveinal areas are raised.
- ✓ Symptoms caused by different viruses shown below can vary as below

Potato Leaf Curl New Delhi Virus

- ✓ The affected plants show curling/ crinkling of young leaves in the terminal region. In severe cases the plants completely wilt and dry. The virus is transmitted by whiteflies and the infection is more common in crops early planted in the northern plains due to large whitefly population.

Potato Leaf Roll Virus (PLRV)

- ✓ Primary infection results in a pale discoloration and in-rolling of leaflets starting at the leaflet base in youngest leaves.
- ✓ Some purple discoloration of affected leaflets may occur.

Collection and preparation of virus infected samples for DNA testing

✓ Collect leaf samples from top and middle parts of the plant at 30, 45 and 60 days after planting

✓ Avoid free water on leaf surface during sample collection

✓ Preserve them in between blotting papers and place them in polythene bags

✓ Make holes in the polythene bags for aeration and label them properly

✓ Pack them with some aeration (don't pack tight) and send them immediately for virus testing so that it can reach within a week's time.

Assessing Symptoms of Wilts and Scoring Method

✓ Bacterial wilt is the major wilt disease on potato in parts of Karnataka and foot hills of north India. Wilt symptoms may develop under conditions of warm temperatures, low soil moisture and low fertility. At first the lower leaves wilt, yellow and later turn brown.

✓ The vascular bundles in the lower part of the stem show a brown discoloration that can best be seen if the stems are cut near ground level.

✓ Symptom development is evaluated daily until the fourth day, then at 7, 10, 14, 21, 30 and 45 days after transplanting.

✓ **Scoring method:** A six point rating scale (0–5) modified from Winstead and Kelman (1952) is used, where: 0 = no wilt symptoms, 1 = one leaf wilted, 2 = two or more leaves wilted, 3 = all leaves except the tip wilted, 4 = whole plant wilted and 5 = death (collapse) of the whole plant.

- ✓ Disease incidence and severity index is assessed as percentage of wilted plants within each treatment. Area under disease incidence progress curve (AUDPC) and Area under percent severity index progress curve (AUPSiPC) for each treatment is calculated (Jerger and Vijanen-Rollinson, 2001; Ayana et al., 2011).

Insect Pest & Scoring Method

Aphids

- ✓ Aphids occur in both wingless and winged forms on lower side of the leaves.

- ✓ Aphid population under field conditions can be recorded by the leaf count method or yellow trap method:

- ✓ **Leaf count method:** Aphids can be easily counted on 100 fully expanded compound leaves from top, middle and lower leaves of 33 plants randomly in transverse direction. The threshold will be 20 aphids/ 100 compound leaves for seed crop.

- ✓ **Yellow water/sticky traps:** It is a tray prepared with yellow paint having clear water with 2cm height. Atleast 2 traps at 5 m distance are placed in the field and mounted them on platform 60cm above the ground level. The aphid populations are counted weekly (Musa, *et al.*, 2004). Installation of yellow sticky traps @ 60/ha and weekly data to be recorded from the traps.

Whitefly

Adultwhiteflies are 1mm long and powdery white. Both adults and nymphs are found on the undersides of leaves, the adults mostly on upper leaves and the nymphs on lower leaves. The white flies can be counted by the leaf count method or by the yellow trap method.

Leaf Count Method

Whiteflies are active under broad day light, therefore, observations to be recorded in early morning or in the evening when activity is less and they can be easily counted by turning the leaf very delicately.

Observations are recorded on 15 plants by counting their number on the upper most, middle & lower leaves

Trap Method

Installation of yellow sticky traps @ 60/ha is recommended and data from the traps is to be recorded weekly.

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