Appendix-4

Manual for Screening of Climate Resilient Apple Varieties

NMHS

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Climate resilient varieties: Climate resilient varieties are those varieties that have enhanced tolerance to biotic and abiotic stresses. Under changing climatic scenario in Kashmir valley, drought will be a common feature causing large annual fluctuations in apple production, especially in rainfed orcharding. Screening of new better quality and high yielding varieties tolerant to drought stress is one of the most efficient strategies to improve yields in unpredictable environments. However, this is complicated by the lack of fast, reproducible drought screening techniques due to the involvement of many physiological and morphological characters. Currently, efforts are directed to access new and cheap reliable indices that can help in selection of drought tolerant varieties /genotypes. The selection of apple variety with the best performance under water stress environments could increase the production under climate change scenario and rainfed orcharding. Different methods are designed for screening of apple varieties for drought tolerance and most reliable are leaf chlorophyll content, leaf total carbohydras content, leaf potassium content and leaf relative water content.

Leaf chlorophyll content

Photosynthetic pigments remain major drivers of plants photosynthetic capacity due to their crucial role in both absorption (chlorophyll) and dissipation (carotenoids) light energy. Chlorophylls are the critical pigments that capture the light to be transformed into carbohydrates during photosynthesis. Chlorophyll content contribute to increase light interception and efficiency of conversion, and therefore, to maintain/increase plant performance under stress. Varieties that maintain relatively higher chlorophyll content under drought use light energy more efficiently, which indicates higher drought tolerance.

Estimation of leaf chlorophyll content (mg g^{-1} fresh weight)

Leaf chlorophyll can be estimated by any of these two methods

i. Arnon method

Chlorophyll is extracted with a mixture of acetone and water at a ratio of 80% : 20% (v / v). 2 g of leaf tissue is homogenized with 25 ml acetone solution (80%), using a laboratory blender for 2 min. Filtration is followed, and then filtrate transferred to a volumetric flask of 100 ml covered with aluminium foil to avoid oxidation of chlorophyll from light and filled to the top with acetone solution (80%) solution. Absorption is measured at 663 and 645 nm using a Spectrophotometer.

Concentration of chlorophyll (a, b, total) is expressed as mg /g fresh weight. Determination of chlorophyll- a, b and total chlorophyll is carried out using the formula by Arnon, 1949:

Chlorophyll a (mg/g F.W) = $(12.7\ A_{663} - 2.69\ A_{645}) \times X/1000 \times n$

Chlorophyll b (mg/g F.W) = $(22.9\ A_{645} - 4.68\ A_{663}) \times X/1000 \times n$

Total chlorophyll (mg/g F.W) = $(20.2\ A_{645} + 8.02\ A_{663}) \times X/1000 \times n$

where: A_{645} = absorption value at 645 nm, , A_{663}= absorption value at 663 nm, X = total volume of filtrate, n = tissue weight.

ii. DMSO method

Samples of 15 representative leaves per tree are detached in the morning hours and are immediately placed in ice box and brought to the laboratory. The samples are then kept in the refrigerator below 0°C to avoid degradation of chlorophyll pigments. For estimation of total chlorophyll each sample is washed and chopped into fine pieces.

Total chlorophyll (chl-a and chla-b) and carotenoid present in different apple varieties are estimated by following the method of Hiscox and Israelestam (1979). 0.1 g of the fresh leaf sample is weighed and then crushed and put into the test tube and 10 mL of DMSO is to be added. It is then kept in oven for ½ an hour, and then transferred into test tube and absorbance measured at 480, 510, 645 and 663 nm in double beam UV -1800 Shimadzu spectrophotometer.

The quantity of photosynthetic pigments is calculated by using the standard formula:

Chlorophyll-a (mg/g)	$\frac{12.3\,(D663) - 0.86\,(D645)}{d \times 1000 \times w}$	$\times V$
Chlorophyll-b (mg/g)	$\frac{19.3\,(D645) - 3.60\,(D663)}{d \times 1000 \times w}$	$\times V$
Total chlorophyll (mg/g fresh weight) =	$\frac{20.2\,A_{645} + 8.02\,A_{663}}{a \times 1000 \times w}$	$\times V$

Leaf carbohydrates content

Carbohydrates produced from photosynthesis in plant leaves provide energy and building blocks for plant growth and productivity. These carbohydrates are known to act as important osmoregulation substances to maintain cell turgor under osmotic stress /drought.

Stress especially drought lead to the accumulation of carbohydrates in leaves, which play an important role in osmo protection under osmotic stress. Under stress conditions, a change in the sugar concentrations would be a result of the regulation of sugar metabolism and export. Accumulation of carbohydrates is regarded as an adaptive response of plants to drought stress.

Estimation of total leaf carbohydrates (mg g^{-1} fresh weight)

Total leaf carbohydrate content present in different varieties of apple is determined by using the method given by Thimmaiah (1999). 100 mg of the sample is weighed into a boiling tube, hydrolyzed by keeping it in a boiling water bath for three hours with 5 ml of 2.5 N Hydrochloric acid (HCL) and cooled to room temperature. It is then neutralized with solid sodium carbonate until the effervescence ceased. Volume is made up to 100 mL and centrifuged and supernatant is collected. About 0.2 to 1 mL of the sample is used for analysis. Standards are to be prepared by taking 0.2 to 1 mL of the working standards. 1 mL of water is serves as blank. Volume is made up to 1 mL in all the tubes with distilled water, then add 1 mL of phenol (5%) solution to each tube. Add 5 mL of 96 % sulphuric acid to each tube and shake well. After 10 minutes shake the content in the tube and place them in a water bath at 25-30 ℃ for 20 minutes and the green to dark green colour is then read at 490 nm in double beam UV -1800 Shimadzu spectrophotometer. A standard graph is then drawn by taking the concentration of glucose on X-axis and spectrophotometer reading on Y-axis. From the graph, the concentration of glucose in the sample is calculated. The amount of total carbohydrate is calculated in the sample solution using the standard graphs.

$$\% \text{ Carbohydrate} = \frac{\text{Conc. Sample from standard curve x Dilution factor}}{\text{Weight of sample}} \times 100$$

$$\text{Amount of carbohydrate present in 100 mg of leaf sample} = \frac{\text{mg of glucose}}{\text{Volume of test sample}} \times 100$$

Leaf potassium (K) content

Potassium is the essential macronutrient required by plants for optimum growth. K has role in biotic and abiotic plant stress tolerance. In plants, potassium remains the primary inorganic cation to accumulate in leaf tissues under water stress and is found in abundance as a solute in leaves.

Water maintains turgidity of cells which leads to enlargement and growth of cells. Potassium plays a central role as an osmolyte, aiding osmotic adjustment, where solutes accumulate in plant cells in response to reduced cellular water potential which enables continued growth under low water potential. There is positive correlation between osmotic adjustment and drought tolerance. Enhanced osmotic adjustment aided by adequate K enables maintenance of higher turgor pressure, relative water content and lower osmotic potential, thereby enhancing plants ability to tolerate drought stress.

Additionally, maintenance of a higher potassium concentration in plant tissues promotes root growth by increasing root elongation, consequently increasing root surface area that is exposed to soil as a result of increased root water uptake. K also improves cell membrane integrity which normally decline under drought stress. K regulates opening and closure of stomata.

Estimation of leaf potassium content

Collection of leaf samples

Leaf samples are collected from the middle of current season's growth around periphery of tree during July –August.

Preparation of leaf sample

After collection, the leaf samples are washed with tap water and later dipped in dilute HCl. Further, washing is repeated with single and double distilled water. The samples are then air dried on a filter paper and oven dried at 60°C for 24 hours **(**Chapman, 1964). Then these were crushed in a stainless steel blender to pass through 2-mm mesh and stored in polythene bags for chemical analysis.

Digestion of leaf sample

Digestion of processed sample is done in 10 ml di-acid mixture consisting of nitric acid and perchloric acid (9:4 ratio) taken in a 100 ml flask. The flask is kept undisturbed overnight and next day placed in hot plate at 115-118^{o}C for digestion till a watery transparent aliquot is obtained. The digested sample is diluted with double distilled water to make a volume of 50 ml and filtered through Whatman No.1 filter paper. The filtrate is ultimately used for estimation of the leaf potassium content.

Estimation of leaf potassium

Potassium is determined with flame photometry technique, using corning flame photometer and expressed in percentage.

Leaf Relative Water Content (RWC)

Relative leaf water content (RWC) is the most appropriate measure of plant water status in terms of the physiological consequence of cellular water deficit. Water potential as an estimate of the energy status of plant water is useful in dealing with water transport in the soil-plant-atmosphere continuum. For the same leaf water potential two different cultivars can have different leaf RWC. It estimates the current water content of the sampled leaf tissue relative the maximal water content it can hold at full turgidity. Normal values of relative water content ranges between 98% in fully turgid transpiring leaves to about 30-40 per cent in severely desiccated and dying leaves. All components of leaf water relations change during the day as irradiance and temperatures change. For not more than two hours at and after noon, the change is very small. This is the time "window" for leaf sampling.

Estimation of leaf Relative Water content

Leaf sample is placed in a pre-weighed airtight vial. These vials are immediately placed in a cooler (around 10^{0}C-15^{0}C) but not frozen on ice. These samples should reach the lab as soon as possible. In the Lab vials are weighed to obtain sample leaf weight (W), after which the sample is immediately hydrated to full turgidity for 3-4hours under normal room light and temperature. After hydration, the samples are taken out of water and are well dried of any surface moisture quickly and lightly with filter paper and immediately weighed to obtain fully turgid leaf weight (TW). Samples are then oven dried at 80^{0}C for 24 hours and weighed (after being cooled down in a desiccator) to determine dry weight (DW).

RWC (%) = [(W-DW) / (TW-DW)] x 100, Where,

W – Sample fresh weight(g), TW – Sample turgid weight (g), DW – Sample dry weight (g).

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References

1. Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. *Plant Physiol*., 24: 1-15.
2. Hiscox, J.D. and G.F. Israelstam. 1979. Different methods of chlorophyll extraction. *Can. J. Bot*., 57: 1332-1332.
3. Chapman, H. D. 1964. Suggested foliar sampling and handling techniques for determining the nutrient status of some field, horticultural and plantation crops. *Indian J. Hort*., 21 (2): 97-119.
4. Thimmaiah, S.R. 1999. Standard methods of biochemical analysis. Kalyani Publishers, New Delhi.

"Scientific Orchard Management Practices for Apple under Changing Climatic Scenario in Kashmir Valley"

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Introduction

Apple is the principal fruit crop of Jammu and Kashmir having high productivity, good eating and keeping quality. It has high nutritive value and better adaptability under different agroclimatic conditions. The weather data reveals that winter as well as spring temperature have increased from the last few decades. Due to rise in temperature and less availability of water in critical stages of growth, the yield and quality of apples in some areas of valley and mid temperate region of Jammu are fast deteriorating. Over the last few years, there has been distinct slow growth in production and productivity in rain-fed Kashmir's karewa areas. Due to unusual hail and windstorms in spring and summer, fruits like cherry, apple, plum, peach and apricot are getting damaged heavily. In recent years there is marked change in the pattern of snowfall in Kashmir which is affecting all the pome, stone and nut fruits. It has been observed that the snowfall and flowering in recent years is coinciding leading to great loss in quantity and quality of fruits. The changing climatic parameters have also caused loss of vigour and fruit bearing ability, reduction in fruit size, less juice content, low colour, reduced shelf life and increased attack of insects and diseases. The phenology, geographic distribution and local abundance of pollinators also appear to be affected by erratic weather behavior which in turn also affects flower production, flower size, timing of anthesis, floral scent, nectar and pollen production. Low winter chill results in uneven flowering. Thus horticulturists will have to play an important role in this changing climatic scenario and proper orchard management strategies have to be envisaged for saving fruit crops from further turmoil.

Soil and Climate

Most of the apple varieties require about 1500 hours below 7 °C to break the rest period. The average temperature should be 21 °C to 24 °C during growing season of the crop. High temperatures on fruit surface as a result of pronounced exposure to sunlight can increase ripening and other associated events. The apple can be successfully grown at an elevation of 1,200 to 2,250 m amsl. Sites having frequent frost and hail storms should be avoided for cultivation of apple. Loamy soils rich in organic matter are most conducive for apple farming. Water logged conditions should be avoided. Soil with slightly acidic to neutral reaction (5.5 to 6.5 pH) is most suited for cultivation of apple.

Selection of Root Stocks, Plants and Varieties

The most suitable root stock for low density apple orchards are crab apple and Maharaji (White Dotted Red) and if the objective is to go for high density plantation then rootstocks like M9, MM106, MM111, M9T337, M9T339 etc. can be used subject to provision of irrigation facilities. Healthy and disease free plants having well developed roots of the desired variety should be procured from a reliable source. Tree roots should be well developed. If it is a single stem plant at the time of transplanting, the main stem should not be less than pencil thickness. The union (of stock and scion) must be perfect and should be maintained at least 20-25 cm above ground level, at planting. Apple is a cross pollinated crop and sets better fruits and yield when cross pollination is assured. It is always desirable that more than one pollinizing variety should be planted in an orchard. The most important factor in the cultivation of apple is proper selection of varieties that have commercial value.

Apple varieties released by SKUAST-Kashmir: Sunhari, Akbar, Firdous, Shireen, Shalimar Apple-1, Shalimar Apple-2 and Lal Ambri.

Promising apple varieties: Royal Delicious, Mitch Gala, Gala Redlum, Gala Mast, Red Gold, Red Velox, Fuji Zen Aztec, Red Fuji, Silver Spur, Oregon Spur, Golden Delicious Reinder, Golden Clone B, Starkrimson, Red Spur, Richa Red and Super Chief.

Low chilling apple varieties: Topical Beauty, Maayan Michal and Schlomit.

Pollination: Majority of apple cultivars suffer from self-unfruitfulness and high level of female sterility. There should be 25 to 33 %

pollinizers in the orchard, 4-5 bee hives should be placed at sunny places in the orchards for better pollination.

Recommended Pollinizers

Variety	Pollinizers
Early Season	Tydemans Early Worcester, Black Ben Davis, McIntosh and Allington Pippin
Mid Season	Golden Delicious, Golden Delicious Reinders, Golden Clone B, Red Gold, King of Pippin, Yellow Newton, Spartan, Sunhari
Late Season	Rome Beauty, Granny Smith, Cortland
Spur type varieties	Golden Spur, Winter Banana

Rootstocks: Some important rootstocks are:

Very Dwarfing	M27
Dwarfing	M9, M9T337, M9T339
Semi-Dwarfing	M26, M7, MM106
Vigorous	MM104, MM109, MM111
Very-vigorous	M12, M16, M25

Training and Pruning

Apple trees should be trained to modified leader system and spindle bush system of training is generally followed for growing apple trees on dwarf rootstocks. Cut back one year old whip to a height of one meter. Shoots below the cut should be allowed to grow. Retain the bottom lateral 25 cm from bud/graft union. Among the laterals, the top most lateral is chosen as leader, giving it a slight cut. Select additional 2 laterals spaced at least 25 cm along the trunk and extending in different directions from the trunk. It is not necessary to select all main scaffold limbs at the start of second year. Additional strong laterals from the succeeding years should be chosen to complete the frame work. Remove all others laterals.

The laterals with weak crotches should be given slight bent with the help of rope tied with pegs inserted in the land to make the crotch angle wider. After four scaffold limbs are formed then very light pruning is done in the third and fourth year. Mostly thinning out is done to space out laterals. Remove narrow angled cris-crossing and interfering branches. Slight heading back is done to maintain balanced top.

For high density plantation on M9 root stock tall spindle system of training should be followed along with four wire trellis and drip irrigation system.

Pruning of bearing Trees

Light to moderate pruning should be done in bearing trees. The amount of pruning is regulated by yield and vegetative growth. Gradually moderate thinning out and heading back should be done. Cut the growing branches and large limbs if growing parallel.

- After pruning the pruned wood must be disposed in an eco-friendly manner.
- Don't burn the leaves in the orchard, instead dump the leaves properly.
- In the event of branch/limb breakage due to heavy snow fall, cut the damaged branch with sharp saw and paint the cut portion with Bordeaux/ Chaubattia paste.

Irrigation and Drainage

In case there is a prolonged dry spell, 4-5 irrigations should be given at fortnightly intervals during fruit development period. Areas having excessive rainfall and low lying should be provided with drainage channels so that excess of water is drained out to avoid damage caused by root and collar rot diseases. Prolonged drought adversely affects plant growth, fruit yield and quality.

Strategies to overcome adverse effect of drought

- Mulching with paddy straw/ dried grass (at least 10 cm thick).
- Apply balanced dose of fertilizer.
- Follow light hoeing in summer.
- Adopt rain water harvesting.
- Use of anti-transpirants (if possible)
- Whitewashing during spring.
- Use of drought tolerant rootstocks: MM-111, M-26 and MM-104.

Weed Management

The weeds can be controlled either chemically or mechanically. When the grasses are actively growing apply post-emergence weedicide. If weeds resume growth after cultivation, apply a second treatment of post-emergence herbicides.

Nutrient Management

The main sources of nutrients are organic manure and fertilizers. The application of organic manures at the following rates as per age of the fruit tress is recommended. The manure can be applied any time but most favourable is February- March.

Organic Manure (Fully decomposed FYM)

Age in Years	Quantity (Kg)	Time of Application
1-5	10-15	February – March
6-10	15-25	
11-15	25-40	
16 & above	40-60	

Inorganic Fertilizers

Application of inorganic fertilizers may be regulated on the basis of soil test report and leaf analysis, but in absence of soil test report the given recommendation may be adopted on an average:

- The fertilizers should be applied under the canopy of fruit trees and followed by cover with soil.
- Fertilizers should be applied 1-2 feet away from the tree trunk, 1 feet in young trees.
- Fertilizers should not be applied in too wet and too dry soils.
- Urea and MOP fertilizers are spread with in the tree canopy and mixed with the soil up to depth of 10-15 cm to active root zone and phosphorus should be applied in bands in root zone.
- Pre fall foliar application of urea @ 5% (5 g/lt) should be done for quick defoliation and decomposition of leaves.

Age in Years	Quantity of nutrients given to each tree (g)		
	Urea	DAP	MOP
1-5	50-250	25-125	80-400
6-10	350-750	175-375	550-1150
15 & Above	1500	750	2500

- Ist dose of fertilizers comprising 1/3rd dose of urea along with full dose of DAP and ½ dose of MOP should be applied 3 weeks before expected bloom as a basal dose.
- Second dose comprising 1/3rd of urea and remaining MOP may be applied three weeks after fruit set.
- 3rd dose of urea may be applied during June-July
- For better colour development of fruits 1.5 % potassium sulphate (15 g/ lt) should be sprayed at fruit development stage.
- Ethephon / etherel spray should be avoided for colour development as it decreases the shelf life of fruits and also results in barrenness in trees in the event of repeated applications.

Physiological Disorders

Water core of apple and bitter pit increases with the increase in temperature in areas where the soils are deficient in calcium.

Management:

- To avoid any calcium related disorders spray calcium chloride @ 3g/lit after 21 days petal fall and repeat the spray in case dry spell occurs and one spray @ 0.5 % (5g/lit) 30 days before harvest to improve the storability of fruits.
- To overcome boron deficiency, 3 sprays of boric acid @ 1.5 g/ ltr should be made at bud swell stage, after petal fall and 21 days after 2nd spray.

Maturity and Harvesting

- Fruits should be picked at proper stage of maturity.
- The mature fruits should be harvested once they develop proper size, colour, TSS, firmness, acidity etc.
- Apples picked on immature stage lack flavor and quality and also shrivel in storage.
- For packing use corrugated fiber boxes in place of wooden boxes.

Diseases:

With the increase in humidity and temperature the incidence of fungal and bacterial diseases (Scab, Alternaria leaf blotch, powdery mildew, Marssonina blotch, Sooty blotch, flyspeck, Collar Rot, Root Rot, cankers and apple Mosaic) increases.

Insect &Pests of Apple:

The incidence of insect and pests enhance with the rise in temperature (San Jose Scale, Wooly apple aphid (MM series of root stocks are resistant to wooly apple aphid), European red mite, Stem borer Shot hole borer, Codling moth etc.).

To manage disease and pests the farmers are advised to strictly follow the spray schedule recommended by SKUAST-Kashmir & Directorate of Horticulture, Kashmir

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