Practices & Research ON HORTICULTURE

Volume - 7

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Orchard Establishment, Orchard Planning and Layout

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Chapter - 1

Orchard Establishment, Orchard Planning and Layout

Dr. Anita Singh

Abstract

Orchard is a place where large scale intentional cultivation of fruits or nuts is done. Establishment of an orchard is a long-term investment and requires a very critical planning. The selection of proper location and site, planting system and planting distance, choosing the varieties and the nursery plants have to be considered carefully to ensure maximum production. The layout of the orchard is a very important operation. Under this, the arrangement of fruit plants in the plot is carefully done to put the plants at a suitable distance for proper development and for accommodating the requisite number of plants per unit area in addition to improving the aesthetic look of the orchard.

Hence, the factors which are considered important for proper layout of the orchard are

i) System of planting.

ii) Planting distance of individual fruit species.

An orchard's layout is the technique of planting the crops in a proper system. There are different methods of planting and thus different layouts.

Keywords: Orchard, planting system, fruits and tree

Introduction

Fruit Plants reduce heating and cooling costs, reduce pollution, take up carbon dioxide, produce oxygen, provide habitat for wildlife, hold water and reduce soil erosion. Orchards, mainly of citrus, olive and pomegranate trees, are located in the Mediterranean cities. Layout in general means making framework. It is the process of making outer look of lines in which we are going to plant different kinds of trees. External framework, Spacing, Planting spot, Road and irrigation are top 5 things that should be considered while designing layout of an orchard.

The mistakes that are done during the initial step establishment and designing an orchard cannot be resolved later and this will negatively hamper our production and our all investment and time will go in vain. The layout of the orchard is a very important operation which allows easy orchard operations like cultivation, intercropping, irrigation, spraying of plant protection chemicals and growth regulators, harvesting etc. Thus, it is necessary to know different layout design of an orchard before starting the work. This type of layout designing is very important in different types of farming such as Multilayer Farming.

Orchard establishment, orchard planning and layout

Principle of orchard establishment

- Establishment of an orchard is a long-term investment and requires a very critical planning. The selection of proper locations, planting system, planting distance and variety has to be considered carefully to ensure maximum production.
- Before going for transplanting one should follow the following things carefully to achieve maximum benefits.

A) Location and site selection for the orchard.

B) Planning of an orchard.

C) Laying out of the orchard.

A. Location and site selection for the orchard

Proper selection of the site is the basic need for growing of crops, as the phenotypic characters of plants are affected by the combining effect of the genotype and the environment. The grower must select the crop to grow according to the particular climatic conditions. Proper selection of site is very important.

Selection of site must be based on the following parameters

1) Location of orchard should be in well established fruit growing regions because one could get the benefits of experience of other growers, easy market facility etc.
2) Proper transportation facility must be there as horticultural produces are perishable.
3) Market area should be close to the orchard.
4) The climate of the region where, one wants to develop its orchard should be specific to the crop grown. There should be well

distributed rain fall, availability of optimum sunlight. The climate should not be more humid, which may allow the growth of diseases and pest e.g. in grape high humidity favours the growth of powdery mildew fungus.

5) Adequate supply of water throughout the year.
6) Suitability of soil, its fertility, soil depth and nature of subsoil must be observed. Soil with a pH at neutral range, along with loose sub soil is the best for the root environment. There should not be fluctuating water table, which may hinder the physiological activity of the root.
7) Site must have proper drainage during the rainy season.
8) Irrigation water must be of good quality.
9) Proper efficient analysis of seasonal gluts (demand of produce is more than supply).
10) The local demand on some specific crops must be looked and taken into consideration.
11) Land should be cheap and plenty available nearer to the site for future expansion of the orchard.
12) The grower must be aware of whether the orchard is a new venture or one already established one.
13) Sufficient availability of manure in cheap rate in the locality.
14) Site should free from natural disaster like flood, drought, heavy wind and frost etc.
15) The orchard crops being intensely cared, the availability of labour should be properly understood.
16) The orchards should not be located in hot spot area (pest/disease prone area) which makes the plant vulnerable to pest or diseases.

Preliminary operations

After selecting the suitable location and site, some preliminary operations have to be done. Trees are felled without leaving stumps or roots. The shrubs and other weedy growth are also cleared. Deep ploughing is essential to remove big roots. The lands should be thoroughly ploughed, levelled and manured. Levelling is important for economy of irrigation and preventing soil wash. In the hills, the land should be divided into terraces depending upon the topography of the land and the levelling is done within the terraces. Terracing protects the land from erosion. If the soil is poor, it

would be advisable to grow a green manure crop and plough it *in situ* so as to improve its physical and chemical conditions before planting operations are taken up.

Planning of an orchard

A careful plan of the orchard is necessary for the most efficient and economic management. Orchard buildings, roads, path, fencing, wind-breaks, layout, system of planting etc. are especially emphasized in planning. Generally 10 percent of the total area is left aside for building, roads, paths, tubewell, channels etc.

The following components should be given due attention

1. Fencing and Wind Break

- A strong fence with the use of barbed wire should be provided around the field meant for planting of Orchard. Months to a year before actual planting is done Karonda (*Carissa carandus*) or Jatti Khatti (*Citrus jambhiri*) can be planted near the barbed wire. This will provide impenetrable and strong fence, while protect the Orchard against the entry of wild/stray animals.
- Plant Eucalyptus or Jamun seedlings at a proper distance inside the provided hedge to provide the Orchard a strong wind break. Generally Wind breaks are planted at 5 metres intervals interference.
- Wind breaks are very effective in reducing the wind Windbreaks are planted normally in north-western side of the orchard. These sides are sensitive for cold and hot wind waves respectively.

2. Land preparation

- Before actual planting of the fruit plants the land should be cleared of all bushes and trees. Remove the complete root systems of the uprooted plants to check their resprouting later on. After levelling and making blocks green manuring by sowing dhaincha, sunhemp, guar or cowpeas should be done. This practice shall provide the land adequate organic matter and aeration, required for fruit plants.
- In arid and foot hills the lands are usually undulated. In such situations, the land may be divided into blocks before levelling. Level the individual block only, this shall avoid unnecessary lifting of upper soil from one point to the other.

3. Roads and Paths

- A well laid out network of paths and roads shall be useful throughout the life of the orchard. The paths should be provided with the plantation of Khabal grass. Such paths shall help in controlling the erosion of soil by wind and water.
- Roads approaching every corner of the orchard should be provided and preferably the cut roads should be at right angle. Generally, 10 to 15 feet inside the orchard serves the purpose of movement of carts, motors and machinery inside the orchard.

4. Tube-well/Tank

- Provision of irrigation in the orchard should be made properly. If it is a bore-well, it should be dug at a place suitable to feed the water requirement of entire orchard. Area for boring a tub-well or a tank may be marked at an appropriate place in the orchard land.
- Facility of water Supply is essential in view of installation of drip irrigation system also.

5. Buildings

- In a big-sized orchard some buildings are required, to store implements, place fruits after harvesting, for resting of labour and a rest house for the owner. These buildings should be prepared beforehand.
- Farm building should be located in the centre of the orchard. Provision of storage house, packing etc. Should be made while planning the orchard.
- At the very entry of the orchard, provision of watch –hut should be made.

Layout of orchards

Layout design of an orchard is heart of orchard as it gives ultimate look to our orchard garden which makes it aesthetically beautiful with easy to operate management practices. After Selection of the site, the orchard should be designed to make most efficient use of the fruit grower's assets. Arrangement of plants in a given block and the planting distance are the two most important factors influencing the efficient use of land.

The orchard is laid out as per following system of planting:

1) Square system
2) Rectangular System

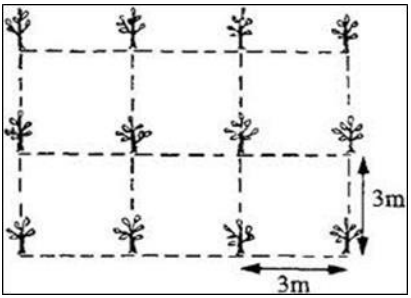
3) Hexagonal System
4) Quincunx System
5) Contour System

Intensive Planting system for Orcharding

1) High Density Planting
2) Meadow orcharding

a) Square system

It is the most easy and popular method of planting fruit plants. In this system, the distance from plant to plant and row to row is the same. The plants are planted exactly at right angle at corner, so that every units of four plants forms a square. This is the most common system and is easy to layout. This facilitates intercultural operations in both directions as the distance between trees and rows are similar. Adequate space is there to go for inter-cropping with vegetables.



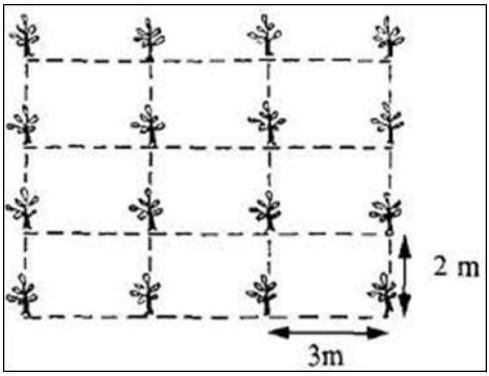
Merits of square system of planting

1) It is easy and takes less time to make.
2) Intercultural operation like irrigation, weeding, harvesting is easy in square system.
3) It facilitates interculture from both the direction from equidistant.
4) The middle area can be intercropped with quick maturing crops like banana which helps to generate extra income.

b) Rectangular system

The Field is laid out into rectangular shape plot keeping more space between row to row. The trees are planted in straight rows running at right angles on one side of the field. The distance from plant to plant and row to row is not the same and four trees joined at the base give a rectangle. Like square system, cultivation, irrigation and other intercultural operations can

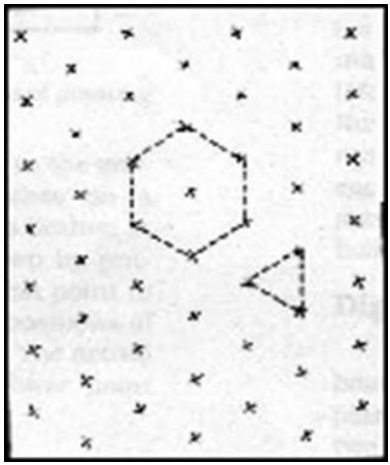
be done in two directions. Plants get proper space and sunlight for their growth and development.



c) Hexagonal system

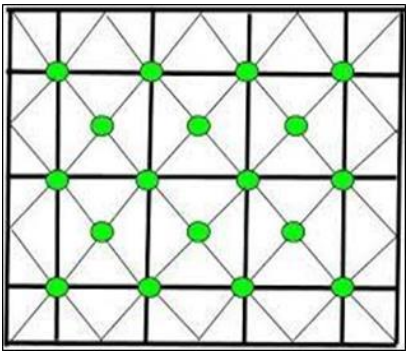
This system accommodates 15 % more plants than square system. This permits cultivation in three directions. In this system, the trees are planted in each corner of an equilateral triangle, thus six trees form hexagon and seventh tree in the centre.

Hence, this system is also called as "septule" as it accommodates seventh tree in the centre. This system differs from a square system in a way that the distance between the row is less than the distance between the plants/tree in a row, but distance from tree to tree in six directions remains the same. This system can be employed where the land is very fertile with assured irrigation. This is very intense method of planting.



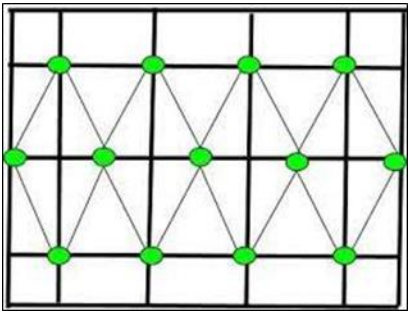
d) Diagonal or quincunx system

This system is similar to square system except one additional plant in the centre of a square. The central tree is usually not a permanent tree and is planted to fill the central space. This is known as filler. Filler serves as a source of additional income till the main trees come into bearing. Papaya in mango and peach in apple orchards can be planted as filler trees because of short juvenile period and smaller tree size. For laying out this system, the field is laid out in similar ways as in square system. Then, the rope is stretched through the diagonal points of the squares and additional pegs are fixed at the points where diagonals cross each other. Guava, Kinnow, Phalsa, Plum, Peaches, papaya etc. are important filler plants.



e) Triangular system

The trees are planted as in the square system except the plants which are in the even numbered rows are midway between, instead of opposite to those in the odd numbered rows. Every second row accommodates one plant less than the square system. For laying out an orchard according to the triangular system, a large triangle with a ring in each corner as used in hexagonal system. The sides of this triangle are equal to the distance to be kept for the plants in the orchard. Two of these rings are placed on the stakes of base line. The position of the third ring indicates the position of plant in the second row. This row is then used as a base line. The whole area is laid out in this manner. However, this system is not of practical importance.



f) Contour system

This system is usually followed in the hilly areas with undulating topography. The positions of plants are marked at various heights from mean sea level. The points having the same altitude are connected together by a line and trees are given spacing on this line. The rows are represented by line passing through the same contour. The main purpose of this system is to minimize land erosion and to conserve soil moisture so as to make the slope fit for growing fruits and plantation crops. The contour line is so designed and graded in such a way that the flow of water in the irrigation channel becomes slow and thus finds time to penetrate into the, soil without causing erosion. Terrace system on the other hand refers to planting in flat strip of land formed across a sloping side of a hill, lying level along the contours. Terraced fields rise in steps one above the other and help to bring more area into productive use and also to prevent soil erosion. The width of the contour terrace varies according to the nature of the slope. If the slope becomes stiff, the width of terrace is narrower and vice-versa. The planting distance under the contour system may not be uniform. In South India, tea is planted in contours either in single hedge system or in double hedge system. Double hedge contour planting system accommodates nearly 22 % higher population than single hedge system. Number of plant population that can be accommodated in this system is

1) $$\text{Plant population} = \frac{N \times \text{unit area}}{D\,(y + z)}$$

Where

N - Number of hedges.

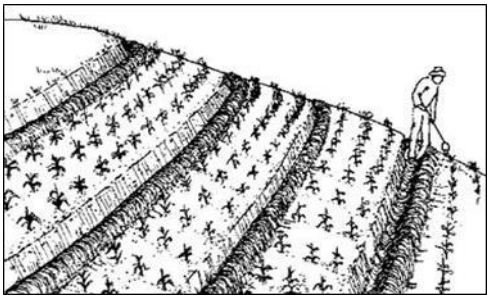
D - Distance between plants.

y - Distance between hedges.

z - Vertical distance between row.

This system in tea helps to get early, high yield, conserve soil and suppress weed growth.

1) In South Indian hills, peas and beans are sown under paired row system which is almost similar to double hedge planting system. The seeds are sown at 10 cm interval in each double row of 30 cm apart with the distance of 1.5m between each pair of rows.



Planting distance

The planting distance for a particular fruit tree is determined by various factors like kind of fruit tree and its growth habit, rootstock used, pruning and training needs, rainfall of the area and soil conditions etc.

The minimum vertical distance between any two trees or plants is referred as the planting distance and this varies depending upon many factors. The principles in deciding the planting distances are the following.

1) Trees when fully grown, the fringes of trees should touch each other but the branches should not interlock.
2) Trees root will spread over a much larger area than top and there should be proper room for the roots to feed without competition.

Factors which decide the planting distance are the following

1) Kind of fruit trees-mangoes are planted at a distance of 10m x 10m, guavas at a distance of 5m x 5m while papayas are planted at a distance of 2m x 2m.
2) Rainfall-wider spacing should be given in low rainfall areas than the high rainfall areas for a kind of tree.
3) Soil type and soil fertility-in heavy soils less spacing should be given because the top and root growth are limited.
4) Rootstocks-trees of the same variety grafted on different root stocks will grow to different sizes and as such require different planting distances. E.g. Apple.

5) Pruning and training-trees trained on head system requires closer spacing than the other type of training system.

6. Irrigation system

In general, if the spacing is too wide, it is obvious that the yield per unit area would be greatly reduced. Only in very, exceptional cases would this be justifiable. Ordinarily it is more profitable to plant the trees closer together and supply the needed water and food materials. If the trees are too close together, the trees grow tall rendering pruning, spraying and harvesting difficult. There is root competition and inadequate nutrition and the trees as such give fewer yields and produce smaller fruits of poor colour. Cultivation also becomes difficult in the closely planted orchards. Close planting results in a greater yield per unit area in the early life of the tree but less in the more important later years. Close planting .is therefore a false economy.

Table 1: The total number of trees per hectare for various important horticultural crops under square, hexagonal and triangular system of planting are given below:

Crop	Planting distance (m)	No. of trees per hectare		
		Square system	Hexagonal system	Triangular system
Mango	10 x 10	100	115	89
Sapota	8 x 8	156	118	139
Clove	6 x 6	277	320	248
Acid lime	5 x 5	400	461	357
Coconut	7.5 x 7.5	177	205	159
Litchi, Ber and Anola	8 x 8	156	179	139
Peach and Plum	7 x 7	204	235	182
Pomegranate and fig	5 x 5	400	460	356
Guava and sweet orange	6 x 6	277	319	247
Apple (Dwarf)	3 x 3	1111	1278	989

It may be seen that hexagonal accommodates 15% more number of plants while triangular system accommodates 11% lesser number of plants. The calculation of the number of trees per hectare when planted under square or rectangular system is very easy, and is obtained by dividing the total area 'by the area occupied by each tree (a x a in square system or l x b in rectangular system). The theoretical and the actual number of possible trees which can be planted in an orchard depend upon the shape of the field. In practice, in large fields, the percentage difference between the theoretical number and the actual number possible will be less.

The best time of planting of temperate fruits are January and February. Before planting, orchard site is properly laid out, after layout of an orchard, the pits of 1x1x1 m size are dug one month before the planting. The pits are filled with soils in which 40-50 kg well rotten FYM and 1 kg single super phosphate are mixed.

Intensive planting system for orcharding

1) **High density planting:** Accommodating more number of plants per unit area than what is accommodated under normal planting system is referred to as high density planting (HDP). It is one of the most efficient methods of hastening productivity per unit area of land. In Fruit crops, which are perennial in nature, HDP is more useful as it helps in efficient utilization of land and other resources, better canopy management, farm mechanization, convenient spray of pesticides, harvesting high yield of improved quality and consequently in getting higher net return. This technique was first established in apple in Europe during sixties and now majority of the apple orchard in Europe, America, Australia and New Zealand are grown under this system. Recently, super high-density plating system has been also established in apple orchards with a plant population of 20,000 trees per ha. In some orchards, still closer, planting of apple trees is followed (70,000 trees/ha) which is often referred as "meadow orchards".

HDP is achieved by

i) Using dwarf rootstock/inter-stock.

ii) Resorting to dwarf scion.

Advantages of high-density planting

2) Best utilization of land and resources.
3) Increase in yield per unit area.
4) Quality production of fruit crops.
5) Easy for intercultural operation, plant protection and harvesting.
6) Low labour costs and better orchard management.
7) Higher harvest index as well as early economics returns.

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Chapter - 2
Broccoli & Beet Root-Origin, Classification, Improved Varieties, Package of Practices

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Chapter - 2

Broccoli & Beet Root-Origin, Classification, Improved Varieties, Package of Practices

Dr. Richa Pyasi and Dr. Rajkumar Deshlehra

Abstract

At present broccoli is the most demanded cool season vegetable all round the world because of its nutritional richness. Broccoli is an edible green plant whose large flower head, stalk and small leaves are eaten as a vegetable. It has high value for freezing and canning industry. In India Himachal Pradesh has largest area covered under broccoli production. Whereas, Beetroot is another important vegetable crop occupying a important position in Europe, North America, Middle East, and parts of Asia including India. It is popularly grown for its red fleshy roots that are used as vegetable, salad purpose and canning. It is popularly used for sugar making in USA. Therefore, in this chapter an attempt has been made to discuss the valuable cultivation prospects of these crops.

Keywords: Broccoli, italica, beetroot, dark red, spartan early, crimpson etc.

Broccoli (*Brassica oleracea* var. italica)

Introduction

- **Family:** Crucifereae or Brassicaceae, 2n=2X=18.
- Broccoli (Italian word) derived from Latin word brachium meaning an arm or branch.
- In Italy, used as vegetable from early times • In USA large part of the produce goes to canning and processing industry.
- Edible part comprises unopened green flower bud or florets clustered along with part of fleshy stem.
- All the species of Brassica contains glucosinolates (goitrogenic substance).

Broccoli is very rich in:

- **Vitamin-A:** 2500 IU.

- **Vitamin-C:** 113mg.
- **Calcium (Ca):** 103 mg.
- **Phosphorus (P):** 78 mg.
- **Potassium (K):** 382 mg.

Origin and Taxonomy

All cole crops originated from a common parent, the wild cabbage or 'Colewart', *Brassica oleracea* var. sylvestris somewhere along the coasts of Mediterranean Sea.

- Present day varieties developed in Italy in the past 2000 years. Till 1660 it was referred to as 'Colliflower' or 'Italian asparagus'.
- Broccoli is a cool season crop & sensitive to high temperatures.
- Grown during winters in plains and in summers in high hills.

Temperature requirement

- For seed germination: 25-30 °C.
- For growth and development: 10-20 °C.
- Optimum for its cultivation: 15-20 °C.
- Sandy loam soils, rich in organic matter having good drainage with pH 5.5-6.8 are well suited.

Varieties

On the basis of maturity

i) Early season (58-65 days)-Gypsy (F1, 58 days), Blue Wind (F1, 49-55 days), DeCicco (48 days) Amadeus (F1, 56 days) Arcadia (F1, 63-68 days).

ii) Mid-season (65-75 days)-Marathon (F1, 68 days), Belstar (F1, 65-75 days), Diplomat (F1, 68 days), Express (F1, 75 days), Waltham 29 (OP, 63-74 days).

iii) Late season (>75 days)-Green Sprouting, Late Palam Haritika.

On the basis of head type

a) Sprouting type

Palam Samridhi 150-200 q/ha.

Palam Haritika 175-225 q/ha Delayed maturity.

Pusa Broccoli (KTS-1) developed by IARI Regional Research Station, Katrain.

Apollo F1, 60-90 days.

Santee F1, 80-115 days-Purple sprouting broccoli.

b) Heading type

Palam Kanchan, 250-275 q/ha yield, Large yellowish green heads.

Palam Vichitra, 200-225 q/ha yield, Compact purple colour heads.

Heat tolerant varieties

Flash: Ready to harvest in 60 days of planting.

Green magic: Green blue heads, good for freezing.

Side shoot producers

- Green Goliath
- Bonanza
- DeCicco
- Waltham

Speciality broccoli varieties

- **Romanesco broccoli:** It has a distinct fractal appearance of its heads and is yellow green in colour. It is technically a Botrytis group cultivar.
- **Broccoflower:** Cross between Broccoli × Cauliflower e.g. Veronica.
- **Broccolini:** Natural hybrid between Broccoli × Chinese kale.

Sowing time

Plains/low hills: August to September.

Hills & dry temperate areas: from March-April.

Seed rate: 500g/ha.

Spacing

Transplanting should be done in evening hours.

60X60 cm or 60X45 cm.

At higher spacing, main and lateral head size is improved.

Irrigation

The amount and frequency of irrigation depends upon soil type, environmental conditions and stage of crop growth. However, irrigation is

must immediately after transplanting of seedlings and at regular intervals. Cole crops are shallow rooted crops, therefore they requires frequent and light irrigations.

Fertilizers

FYM: 200 q/ha P_2O_5: 50-60 kg/ha K_2O: 50-60 kg/ha N: 80-100 kg/ha Full doses at the time of land preparation as basal dose ½ N as basal dose Remaining ½ in 2-3 splits.

Weeds create a serious problem in the cultivation of the cole crops due to wide spacing, higher fertility and frequent irrigations 1-2 shallow hoeings in early stages should be done to remove weeds and facilitate aeration Pre-plant soil incorporation of herbicides like Fluchloralin (0.75-1.0 kg/ha) or pre-transplant surface application of Pendimethalin (1.0 kg/ha) controls the weeds effectively.

Harvesting and Yield

Broccoli may take 45-90 days for maturity after transplanting depending upon cultivar and environment. The head is harvested along with about 6-8-inch-long stem. Removal of primary head can extend the harvest period because of the proliferation of lateral inflorescences. Yield varies from 150-250 q/ha

Post-harvest handling and storage

- At temperature 0 °C and >95% RH, broccoli can be stored for 21-28 days.
- Broccoli should be cooled rapidly in the field packed cartons.
- Refrigerated transport is must for long distance transportation.

Insect-pests

Control measures.

Cabbage white butterfly (Pieris brassicae)-Spray of 0.05% Chlorpyrifos and 0.4% Thuricide.

Diamond back moth (Plutella xylostella)-Foliar spray of Permethrin (0.2 kg a.i.), Use of mustard as trap crop.

Mustard sawfly (*Athalia lugens* Proxima)-Dusting with sevin @20-25 kg/ha or spraying the same @5g per litre of water.

Mustard aphids (*Lipaphis erysimi*) and Painted bug (*Bagrada cruciferarum*) Spraying with dimethoate @1ml/litre of water, Soil application of phorate 10 G @ 15 kg/ha.

Diseases

Control measures.

Damping off (*Pythium* spp., *Phytophthora* spp.)

- Provide good drainage.
- Treat the soil with Formalin (5%).
- Seed treatment with Captan (2.5 g/kg seed).

Downy mildew (*Peronospora parasitica*) Spray Ridomil MZ @ 3g/L of water.

Black Spot (*Alternaria brassicicola* and *A. brassicae*) Follow phytosanitary practices and spray mancozeb/iprodione @ 0.25%

Black Rot (*Xanthomonas campestris* pv. campestris) Use healthy seed or give a hot water treatment to seeds at 50 C for 20 minutes. Disinfecting of seed beds with formaldehyde solution (1:7) is important.

Black leg (*Phoma lingam*) Spray the seedling with copper oxychloride (0.3 per cent).

Beet root-origin, classification, improved varieties, package of practices

S.N-Beta vulgaris. L

(2n = 2x = 18)

Family: Chenopodiaceae.

Introduction

Beetroot (*Beta vulgaris* ssp. vulgaris, 2n = 2x= 18) is an important vegetable crop in eastern and central Europe and belongs to family Chenopodiaceae. It is known as garden beet or red beet. Beetroot is a major vegetable crop globally and occupies a important position in Europe, North America, Middle East, and parts of Asia including India. It is popular grown for its red fleshy roots that are used vegetable, salad purpose and canning. Tender leaves and soft stems are also used as vegetable. It is popularly used in USA for sugar making. Beet root is a rich source of protein (1.8 g/100), carbohydrates (86 mg), calcium (190 mg), phosphorus (57 mg) and vitamin C (88 mg). Leaves are rich in iron (3.1 mg), vitamin A (2100 I.U.), thiamine (110 µ g) and ascorbic acid (50 mg/100g).

Origin

Beet root originated from *Beta vulgaris* L. ssp. maritima probably in Europe. Beet root, is cross compatible with sugar beet and palak.

Botany

Beet root is a biennial crop, producing a fleshy round hypocotyls and a rosette of leaves. Hypocotyls gives rise to upper portion of fleshy root whereas tap root forms basal part. Colour of beet root is due to presence of red violet pigments of β cyanins β xanthin. Tap root grows as deep as 3m. Inflorescence normally develops in second year is a spike. Flowers are bisexual, small, with green calyx.

Varieties: Some varieties popular in India are:

Detroit dark red: Roots perfectly round with smooth uniform deep red skin; flesh dark red. Duration of crops 80-100 days.

Crimson globe: Roots are round to flat. Outer skin is medium red and flesh is crimson red; duration 55-60 days.

Early wonder: Roots flat globular with dark red skin and dark red flesh and light red zoning.

Ooty-1: Round roots with red flesh colour; yields 28 t/ha, duration 120 days; it sets seeds under Nilgiri conditions.

Crosby egyptian: Roots flat globe dark red; duration 55-60 days.

Breeding objectives of beetroot

High root yield.

Round to globe shaped roots.

Dark red, uniformly coloured roots.

Improved colour and sweetness.

Uniform root shape.

Absence of internal white rings in roots.

Increased betalain concentration.

Slow bolting.

Mono-germ seed.

Resistance to downy mildew (*Peronospora farinosa* f. sp. betae), powdery mildew (*Erysiphe communis* f. sp. betae), Cercospora leaf spot (*Cercospora beticola*).

Climate

Beet root needs a cool climate and can withstand low temperature. High temperature causes root zoning or formation of alternate light red concentric rings in the root.

Soil

Deep well sandy loam soil is the best for beet cultivation. Heavy clayey soils result in formation of a soil crust after rains. Beet root is highly sensitive to soil acidity and the ideal soil pH is 6-7. It can be grown in saline soils.

Land preparation and sowing

Being a cool season crop, beet root is raised during winter in plains. Sowing is done in October-November. Land is ploughed to a fine tilth by ploughing. Apply well decomposed FYM at the time of field preparation. Seeds are sown in flat beds or ridges and furrows. 'seed balls' which contain 2-4 seeds are sown 2.5 cm deep in soil at spacing of 45-60 x 10 cm. 5-6 kg of seed is required for planting one hectare.

Manures and fertilizers

60-70 kg N, 100-120 kg P and 60-70 kg K/ha is recommended. Half dose of N and full P and K and FYM (25t/ha) should be applied as basal dose at the time of field preparation.

Irrigations

Usually 5-6 irrigations are sufficient during summer and three irrigations during winter in North Indian plains. Field is usually kept weed free by light hoeing at early stage of crop. Swollen roots are covered with soil by earthing up.

Harvesting

Medium sized tubers. 3-5 cm in diameter are of great demand. Harvesting is done 8-10 weeks after sowing by pulling the top with hand. Later tops are removed, graded and marketed. In European countries, where small sized bunches are in demand, tubers are tied in bundles of 4-6 with their tops. Over matured and oversized tubers become woody and crack.

Yield

25-30 t/ha yield is commonly obtained.

Pests and diseases

Insect pests like leaf miners, web worms, semi loopers; fungal diseases like Cercospora leaf spot and downy mildew and viral diseases cause damage in beet root cultivation.

Seed production

Tropical types are not found in beet root unlike other root crops. All cultivars in beet root are temperate biennial types and seed production is possible only in hills. A low temperature of 4.5 to 7.9C for 6-8 weeks is required for flowering. Common method of seed production is root to seed method. In this method, seeds are sown and well-developed roots are harvested during December. After selection of root tubers, top is cut. Then whole tubers of selected plants are transplanted at a spacing of 60 x 45-60 cm in well prepared fields and irrigated. Being a cross pollinated crop, an isolation distance of 1000 m for certified seed production and 1600 m for breeder and nucleus seed production is needed. Average seed yield of 2.0 t/ha is obtained.

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Chapter - 3
The Hidden Trove of Under-Exploited Leafy Vegetables in Tropical Regions

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Chapter - 3

The Hidden Trove of Under-Exploited Leafy Vegetables in Tropical Regions

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Abstract

Vegetables play a vital role in food and nutritional security, especially the greens which are packed with vitamins and minerals. Though the diverse agroclimatic conditions in India favours the cultivation of more than 60 cultivated and about 30 lesser known vegetable crops, many of the traditional greens still remain under exploited. They are inexpensive, easy to cook and are usually consumed in the rural areas, but lack national level recognition and appreciation. In India, there is a good number of such leafy vegetables which are embedded with proteins, vitamins, minerals and phytonutrients. Most of them are having medicinal values owing to the presence of pharmaceutical components in them. Lack of availability of planting material, lack of awareness on nutritional and medicinal importance and lack of information on production technique of these crops are the major reasons behind the poor utilization of these plants. This is high time to highlight the potentials of these hidden good greens to ensure food and nutritional security for future.

Keywords: Leafy vegetables, under exploited, nutrition

Introduction

Vegetables are considered as protective food for mankind and they form the key component of a balanced diet. Among the wide range of vegetables, leafy vegetables are considered as an exceptional source for vitamins, minerals and phenolic compounds. They are the richest source of minerals like iron, calcium as well as antioxidants and dietary fibres. Dieticians advocate an intake of 125 g leafy vegetables every day to make our diet balanced along with other diets. Besides the commonly used leafy vegetables, there are many traditional and less familiar greens which are used locally. Most of them are found as weeds in local environmental conditions where they thrive very well with least care. Often these good greens are not utilized to their potentials. More than 102 crore people (15% of the world population) are undernourished

and hungry [1]. The population of India will hit 1.6 billion by 2050, exceeding China. To develop a hunger free India we need to focus on under utilized and under exploited horticultural crops and highlight the plant wealth in our country. There are many leafy vegetables with untapped potentials to meet the nutritional requirements of at least the poor in developing countries who can not afford high value vegetables and animal products. Some of the under exploited leafy vegetables are and their importance are described here.

Amaranthus

Amaranth is one of the few duel-purpose plants which can supply tasty leafy vegetables as well as grains of high nutritional quality. Unlike cereals, most of which belongs to the grass family, amaranth is a broad leaf plant producing edible grains. Hence, they are classified as Pseudo-cereals. The genus Amaranthus consists of around sixty members which are widely distributed throughout the tropical, sub-tropical and temperate regions of the globe. They fall into four categories viz. ornamentals, weeds, grain amaranth and leaf amaranth. Almost all these species are edible. Majority of leafy cultivars in India belongs to *Amaranthus tricolor*.

Members of the genus amaranthus are short lived annuals, having an erect to spreading habit. Leaves vary in shape and colour. Inflorescence is a racemose spike, axillary or terminal. Flowers are small, unisexual and monoecious. Leaf amaranths are predominantly self-pollinated because of the presence of high number of male flowers where as grain types are cross pollinating.

They are relatively high temperature tolerant, but sensitive to frost and an optimum temperature of 22-30 ^{o}C is favourable for growth and development. They can be grown throughout the year in tropical regions. Amaranth comes up well in well drained loamy soil rich in organic matter. The ideal is pH is 5.5-7.5 but there are types which can come up in soils with pH as high as 10.0.

Normally sowing is done by broadcasting. As the seeds are small in size, often mixed with sand and then broad casted at the rate of 3-10g/m^2 (1.5-2kg/ha) on prepared beds. Thinning may be don eon later stages to maintain a spacing of 15-22cm on either way. Seedlings can also be raised on nursery beds and may be transplanted at a spacing of 20-30cmX10-20cm. leaf yield of amaranth increased quadratically when inter row spacing was decreased. Harvesting is done by either pulling or clipping. Pulling can be done at 30, 45, and 55 days after planting along with roots and clipping, first cut is done 25-35 days after sowing.

1. *Amaranthus caudatus*

Commonly known by the name, Kiwicha. *Amaranthus caudatus* is grown for its edible leaves, grain and also have ornamental value. It has long and pendent inflorescence. It has been reported for its antioxidant, anthelmintic, antinociceptive, antipyretic, anticancer, antiallergenic, antidiabetic, cardioprotective, hepatoprotective and antibacterial activities. In ayurvedic medicine system of India, *A. caudatus* is used to treat a number of ailments, namely goiter, urinary trouble, piles, pulmonary disorder, etc. [5]

2. *Amaranthus cruentus*

Commonly known as Red amaranth or blood amaranth. It is one of the grain producing species from Amaranthaceae genus. It has a high protein content and good amino acid composition. It is used as cholesterol lowering, antioxidant, anticancer, anti-allergic, and antihypertensive agent. It is rich in iron, calcium, magnesium, niacin, riboflavin, phytin, tannin etc. [4]

3. *Amaranthus spinosus*

It is commonly known as spiny amaranth, pig weed, Kate Wali Chaulai etc. and is commonly throughout the tropical regions across the world. It is an annual or perennial plant with varying color from green to purple and spines all over the stem. The leaves are used as a leafy vegetable and is a good source of vitamins and minerals. In Ayurveda, it used as febrifuge, antipyretic, laxative and diuretic. Also used for curing bronchitis, biliousness, galactagogue, nausea, flatulence, anorexia, blood diseases, burning sensation, leucorrhoea, leprosy and piles [11].

4. *Amaranthus viridis*

It is a cosmopolitan weed distributed throughout the warmer parts across the world. Leaves are edible and has got numerous medicinal values. The whole plant is medicinal used for relieving pain, fever and possess analgesic, anti-pyretic, antioxidantal, antimicrobial, antinociceptive, hepatoprotective, anti-inflammatory properties [12].

Alternanthera (*Alternanthera sessilis*)

Alternanthera belonging to the family Amaranthaceae, is a wild leafy vegetable is consumed by a predominat population in India, especially South India for its medicinal benefits. It is a herbaceous perennial or annual found wet and damp shady areas. It is reported to be used as galactagogue, cholagogue, febrifuge and in indigestion problems. Also used to cure eye diseases, cuts, wounds and antidote to snake bite; skin diseases [13]. Seeds are used for planting and first clipping can be done 1-2 month after planting.

Indian spinach (*Basella spp*.)

Basella, is a perennial, climbing creeper having succulent stem and fleshy leaves which are edible. It is known by various names such as Indian spinach, Malabar spinach, Indian spinach, vine spinach, Country spinach, Poi etc. It belongs to the family Basellaceae; there are three different types based on the colour of leaves and stem and shape of leaves.

1) ***Basella alba*:** Dark green, oval almost round leaves.
2) ***Basella rubra*:** Leaves and stem red colour.
3) ***Basella cordifolia*:** Dark green, cordate leaves.

Fleshy leaves and stem used as leafy vegetable. It has got antiviral, antiulcer, antimicrobial, anti-inflammatory, antidiabetic, antioxidant activities and wound healing power [3]. The red cultivar contains a red pigment which can be extracted and is reported to be used as a food colorant [2]. Basella prefers hot, humid climate and is a high temperature tolerant crop. It thrives hardly at lower temperatures and higher altitudes. It comes up well in all soils, but soils rich in organic matter will be best for its cultivation. Optimum soil pH is 5.5-8.0. seeds and stem or root cuttings are used for propagation. Seeds can be either sown directly or transplanted. In the case of using stem cuttings, cuttings of 20-25cm length with 3-4 internodes are recommended. Harvesting can be started around 30-45 days after planting.

Chekkurmanis (*Sauropus androgynus*)

Chekkurmanis, also known as vegetable of 21st century belongs to the family Euphorbiaceae. It is a glabrous perennial shrub of 2-3.5 m height. Leaves are ovate, sessile, alternate with dull green color and possess a waxy coating. Leaves are rich source of vitamins and minerals such as β-carotene, vitamin E, vitamin C, thiamine, riboflavin, calcium, iron, zinc and proteins [9]. Hence this unusual vegetable crop is popularly known as "multivitamin green" and "multi mineral packed leafy vegetable. The leaves are consumed either after cooking or as powder incorporated with other spice powders, especially in South India.

The plant is adopted to mild humid conditions and thrives well in almost all types of soils. Soft wood and semi hard wood cuttings are used for planting. Planting can be done on ridges or pits. It takes 5-6 months to reach harvest.

Water leaf (*Talinum triangulare*)

Water leaf is a traditional leafy vegetable belonging to the family Portulacaceae. This herbaceous annual leafy vegetable is widely grown in

tropical regions. It has ornamental (produces small violet-colored flowers), antioxidative and medicinal values [8]. It is rich in minerals viz. calcium, magnesium and potassium and vitamins like ascorbic acid and pyridoxine. Root extract is used against asthma [7]. It controls blood pressure and cholesterol levels, prevents heart diseases and cancer, also good for eyes [14]. Young fleshy stem and leaves are consumed with or without cooking and is also forms the constituent of vegetable soups. It can be grown even in shady areas. Comes up in all types of soils. Seeds or stem cuttings are used for propagation. Harvesting can be started within one month after planting.

Roselle (*Hibiscus sabdariffa*)

Roselle is a bushy plant, well adopted to tropical climate and growing up to a height of 2m, belongs to the family Malvaceae. Tender leaves and stem are used as vegetables. Calyxes (fresh or dried) are rich in acids and pectin and so used for making chutney, jellies, and sauces. Leaves are rich in carotene and vitamin C. it has many medicinal properties and used for treating abscesses, bilious conditions, cancer, cough, debility, dyspepsia, dysuria, fever, hangover, heart ailments, hypertension, neurosis, and scurvy [6]. This crop can also be considered as an allied fibre crop as it is used as a substitute for jute. Propagated by seeds or stem cuttings. Leaves and flowers may be harvested at 90-100 days after sowing.

Agathi (*Sesbania grandiflora*)

Agathi belongs to the family Fabaceae. It is a perennial tropical tree grown widely in India and has food, fodder and ornamental values. There are red flowering type and white flowering type. Leaves, flowers and green fruits are consumed as vegetable either raw or cooked. It is rich in vitamin A, B, C, calcium, iodine, protein and fat. It has diuretic, emetic, emmenagogue, febrifuge, laxative, tonic properties and is used for bruises, dysentery, fevers, headaches, smallpox, sores, sore throat, stomatitis and night blindness [10]. Agathi prefers humid tropical climate with an optimum temperature range of 26-35 ^{o}C and light to heavy soils. Black cotton soils are found best for growing Agathi. This tree can also tolerate water logging to some extent. Seeds are used for propagation.

Spreading hog wood (*Boerhavia diffusa*)

This prostrate, sparsely pubescent herb belongs to the family Nyctaginaceae. It is a rich source of Na, Mg, Ca, Vit-C, B3, dietary fibre, antioxidants [20] and therapeutically important chemicals such as boeravinone, punarnavine etc. Leaves are eaten as vegetable. Leaves and roots possess several medicinal properties (used against blood purifier, cough, asthma, dropsy, chest pain, piles, snakebite, jaundice etc.)

Lettuce tree (*Pisonia alba*)

It belongs to the family Nyctaginaceae. It is an ornamental tree with greenish yellow leaves which are edible. *P. alba* plants used for preparation of several folk medicines. They possess antidiabetic [18], anti-inflammatory, analgesic and wound healing properties [15].

Water spinach (*Ipomoea aquatica*)

Also known as swamp cabbage and belongs to the family, Convolvulaceae. It is a popular leafy vegetable in South East Asian countries. Young leaves and shoots are consumed as vegetable and vines are used as a cattle feed. Roots are also eaten after cooking. It is a rich source of proteins, carbohydrates, crude fibres, vitamins like A, B, C, E and U and minerals like Mg, Mn, K, Fe [21]. Used as emetic, diuretic, purgative agent and to cure liver problems [19]. Also good for anaemia patients as it is rich in iron.

Purslane (*Portulaca oleracea*)

Belongs to the family Portulacaceae and found in the warmer regions. Used as a salad vegetable or cooked vegetable and the whole plant is medicinal. High mucilage content makes it a constituent in stews and soups. It contains considerable amount of Fe, Ca, Zn, Vit-A, riboflavin, folic acid, flavonoids etc. [17]. It has diuretic, sedative, analgesic, cardiotonic properties and also used to treat rheumatism, gynaecological diseases, renal and colorectal diseases.

Reasons for under-utilization

- The un-assessable area, geographical isolation, complete unexplored status of agronomic practices of production and ultimate too little institutional interest for these plants are different issues for low production standards of these vegetables [16].
- Even though these plants are diverse in types, morphological and anatomical variations, adaptations, reactions to diseases and pests, they still remain under explored because of lack of awareness about their nutritional and medicinal properties among people.
- Poor recognition of these crops in extension and popularization programmes.
- Inadequate facilities for processing and value addition.
- Lack of sufficient research on under-utilized leafy vegetables.
- Weak policy support from different organizations.

- Insufficient marketing facilities, infrastructure facilities for transportation and storage and poor supply chain.
- Unavailability of desirable seeds and planting material.
- Innovative and novel technologies such as biotechnology, tissue culture for enhancing productivity are lacking in these crops.

Ways forward

- Encourage homestead cultivation of potential wild species.
- Ensure the sufficient supply and distribution of planting materials.
- Research and development efforts on these crops should encouraged as they are nutritionally rich, require less inputs, but contribute substantially to food security.
- Most of the under explored crops are managed and cultivated by ethnic communities. Ethnobotanical studies should be encouraged so that documentation of indigenous knowledge is given increased focus.
- Promising selections/varieties should be made available by overcoming the problems in the production of quality planting material.
- Local crop planning may be done according to the agro-climatic conditions of regions.
- Improving market, transport storage and processing facilities.
- Make the farmers aware about the nutritional importance of these crops via campaigns, exhibition, etc.
- It would also provide employment opportunities to the rural folk.
- Moreover, encouraging the cultivation of these under exploited plants will also help to protect them from extinction.

Conclusion

Underexploited greens are the richest source of nutraceuticals and pharmaceuticals along with ability to grow under low input farming. They will go a long way in fighting malnutrition and hidden hunger.

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Chapter - 4
Classification of Vegetable Crops

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Chapter - 4

Classification of Vegetable Crops

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Abstract

The crops used for vegetable purpose in the world belong to 1200 species under 78 families and of them, more than 860 species under 59 families belong to dicotyledoneae and about 340 species under 19 families belong to monocotyledonae. In the tropical and subtropical parts of the world, about 90 species of the vegetable crops are cultivated, but hardly 20 of them are commercially important. Classification of vegetables is grouping of vegetables based on certain similarities, which is useful to researchers, cultivators and students.

Keywords: Classification, vegetable, dicotyledoneae, monocotyledonae

Introduction

There are different ways of classifying vegetable crops. Broadly there are twenty methods of classifying vegetables.

These include;

1) Botanical classification.
2) Classification based on Cultural Requirement.
3) Classification based on Hardiness.
4) Classification based on Parts used.
5) Classification based on Photoperiodism.
6) Classification based on Season of Cultivation.
7) Classification based on Response to Transplanting.
8) Classification based on pH Requirement.
9) Classification based on Tolerance to soil salinity.
10) Classification based on Tolerance to soil acidity.
11) Classification based on Photosynthesis.
12) Classification based on Temperature requirement.

13) Classification based on Respiration rate.
14) Classification based on Respiration.
15) Classification based on Root depth.
16) Classification based on Water requirement.
17) Classification based on Tenderness.
18) Classification based on Nutrient content.
19) Classification based on seed longevity in normal storage condition.
20) Classification of male sterility in vegetable crops.

1. Botanical classification

This scientific classification is based on morphological and cytological similarities. Plants are first divided into four sub communities. They are Thallophyta, Bryophyta, Pteridophyta and Spermatophyta. All the vegetables belong to Spermatophyta; which include two divisions Gymnospermae and Angiospermae. No vegetables belong to division Gymnospermae. Angiospermae has two classes: Monocotyledonous and Dicotyledonous. These are further divided into families, genus, species, sub-species and botanical varieties. The grouping of vegetables based on botanical similarities is as follows.

Monocotyledoneae

Family-Amaryllidaceae/Alliaceae

Onion	*Allium cepa* L.
Multiplier onion	*Allium cepa* var. *aggregatum* L.
Top onion	*Allium cepa* var. *viviparum*
Leek	*Allium ampeloprasum* L. var. *porrum*
Garlic	*Allium sativum* L.
Welsh onion	*Allium fistulosum* L.
Shallot	*Allium ascalonicum* L.
European chive	*Allium schoenoprasum* L.
Chinese chive	*Allium tuberosum* Rottler ex Spreng

Family-Araceae

Taro	*Colocasia esculenta* (L.) Scott
Eddoe type taro	*Colocasia esculenta* var. *antiquorum*
Dasheen type taro	*Colocasia esculenta* var. *esculenta*
Giant taro	*Alocasia macrorrhiza* (L.) Scott
Swamp taro	*Colocasia esculenta* var. *stolonifera*

Giant swamp taro	*Cyrtosperma chamissonis*
Tannia	*Xanthosoma sagittifolium* (L.) Scott.
Amorphophallus	*Amorphophallus paeoniifolius* (Dennst.) Nicolson

Family-Dioscoreaceae

Greater yam	*Dioscorea alata* L.
Aerial yam	*Dioscorea bulbifera* L.
Lesser yam	*Dioscorea esculenta* L.
White yam	*Dioscorea rotundata* L.

Family-Gramineae (Poaceae)

Sweet corn	*Zea mays L.* var. saccharoda

Family- Liliaceae

Asparagus	*Asparagus officinalis* L.

Dicotyledoneae

Family-Aizoaceae

New Zealand Spinach	*Tetragonia expansa* (Murr.)

Family-Amaranthaceae

Leafy Amaranth	*Amaranthus tricolor* L., *A. Blitum* L *A. viridis* L., *A. tristis* L., etc.

Family-Basellaceae

Basella (green type)	*Basella rubra* L. var. *alba*
Basella (red type)	*Basella rubra* (L.) var. *rubra*

Family-Chenopodiaceae

Beetroot (Garden beet)	*Beta vulgaris* L.
Spinach beet (Palak)	*Beta vulgaris* L. var. *bengalensis*
Spinach	*Spinacia oleracea* L.
French spinach (orach)	*Atriplex hortensis* (L.)
Pigweed	*Chenopodium album* L.

Family-Compositae

Lettuce	*Lactuca sativa* L.
Head type lettuce	*Lactuca sativa* var. *capitata* L.
Leaf type lettuce	*Lactuca sativa* var. *crispa* L.

Cos type lettuce	*Lactuca sativa* var. *longifolia* L.
Asparagus type lettuce	*Lactuca sativa* var. *asparagine* L.
Globe artichoke	*Cynara scolymus*
Jerusalem artichoke	*Helianthus tuberosus*

Family-Convolvulaceae

Sweet potato	*Ipomoea batatas* (L.) Lam
Water spinach	*Ipomoea aquatica* Forsk

Family: Brassicaceae (Crucifereae)

Kale/Collard	*Brassica oleracea* L. var. *acephala*
Cauliflower	*Brassica oleracea* L. var. *botrytis*
Cabbage	*Brassica oleracea* L. var. *capitata*
Red cabbage	*Brassica oleracea* L. var. *capitata f. rubra* (L.) Thell
Savoy cabbage	*Brassica oleracea* L. var. *sabauda*
Brussels sprout	*Brassica oleracea* L. var. *gemmifera*
Sprouting broccoli	*Brassica oleracea* L. var. *italica*
Knol-khol	*Brassica caulorapa* L.
Turnip	*Brassica campestris* L. var. *rapa*
Chinese cabbage	*Brassica chinensis* L. var. *pekinensis*
Horse radish	*Armoracia rusticana* L.
Radish	*Raphanus sativus* L.

Family-Cucurbitaceae

Summer squash	*Cucurbita pepo* L.
Winter squash	*Cucurbita maxima* L.
Red pumpkin	*Cucurbita moschata* L
Watermelon	*Citrullus lanatus* L.
Round melon	*Praecitrullus fistulosus* (stocks) Pang.
Muskmelon	*Cucumis melo* L.
Netted melon	*Cucumis melo* L. var. *reticulatus*
Tinda	*Praecitrullus fistulosus* L.
Cucumber	*Cucumis sativus* L.
Gherkin	*Cucumis anguria* L.
Chow-Chow	*Sechium edule* L.
Ridge gourd	*Luffa acutangula* L.
Sponge gourd	*Luffa cylindrica* Roem
Bottle gourd	*Lagenaria siceraria* (Molin) Stadl.
Bitter gourd	*Momordica charantia*

Kartoli	*Momordica dioica* Roxb.
Pointed gourd	*Trichosanthes dioica* Roxb.
Snake gourd	*Trichosanthes cucumerina* L.
Little gourd	*Coccinia cordifolia* L.

Family: Euphorbiaceae

Tapioca	*Manihot esculenta* Crantz.

Family-Labitae

Chinese potato	*Solenostemon rotundifolius* (Poir)

Family-Leguminosae (Fabaceae)

Garden Pea and snow pea	*Pisum sativum* L. sub sp. *hortense*
French bean	*Phaseolus vulgaris* L.
Lima bean	*Phaseolus lanatus* L.
Broad bean	*Vicia faba* L.
Cowpea	*Vigna unguiculata*
Yard long bean	*Vigna unguiculata* var. *sesquipedalis*
Dolichos bean	*Dolichos lablab*
Winged bean	*Psophocarpus tetragonolobus* L. Taub.
Fenugreek (methi)	*Trigonella foenum-graecum* L.
Kasuri methi	*Trigonella corniculata* L.

Family-Malvaceae

Okra	*Abelmoschus esculentus* (L.) Moench

Family-Polygonaceae

Rhubarb	*Rheum rhaponticum* L.
Sorrel	*Rumex vesicarius* L.

Family-Solanaceae

Potato	*Solanum tuberosum* L.
Brinjal	*Solanum melongena* L.
Tomato	*Lycopersicon esculentum* L.
Chilli	*Capsicum annuum* L.
Sweet pepper	*Capsicum annuum* L. var. *grossum*

Family-Umbelliferae

Carrot	*Daucus carota* L.

Celery	*Apium graveolens* L.
Coriander	*Coriandrum sativum* L.

2. Classification based on cultural requirement

It is an important classification of vegetable as it is based on cultural practices. It is more advantageous to farmers because the vegetables requiring the same cultural practices (but not same season for cultivation) are grouped together, though they may be divergent botanically. These groups are as follows.

Group 1: Potato.

Group 2: Solanaceous vegetables (Tomato, brinjal, chilli, sweet pepper).

Group 3: Cole crops (Cabbage, cauliflower, sprouting broccoli, Brussels sprout, knol-khol, Chinese cabbage).

Group 4: Cucurbits (Pumpkin, summer squash, winter squash, muskmelon, watermelon, snap melon, long melon, round melon, cucumber, bitter gourd, bottle gourd, ash gourd, ridge gourd, snake gourd, sponge gourd, little gourd).

Group 5: Root crops (Radish, carrot, beetroot, turnip).

Group 6: Bulb crops (Onion, garlic, leek).

Group 7: Salad crops (Lettuce, celery, parsley, sorrel).

Group 8: Leafy vegetables/greens and pot herb (Amaranth, palak, spinach, fenugreek).

Group 9: Peas and beans (Pea, cowpea, French bean, cluster bean, broad bean, asparagus bean, lima bean).

Group 10: Tuber crops other than potato (Taro, Giant taro, yam, cassava, elephant foot yam, Chinese potato).

Group 11: Sweet potato.

Group 12: Okra.

Group 13: Pointed gourd.

Group 14: Chow-chow.

Group 15: Tropical perennial vegetables (Drumstick, agathi, curry leaf).

Group 16: Temperate perennial vegetables (Asparagus, rhubarb, globe artichoke).

3. Classification based on hardiness

This classification determines the ability of vegetable crops to withstand frost and low temperature. It will be useful to know the season of cultivation of vegetable crop. Based on hardiness, vegetables are classified as hardy, semi hardy and tender. Hardy vegetables can tolerate frost and low temperature. These hardy vegetables are called as temperate vegetables or cool season vegetables. Those vegetables cannot tolerate frost are considered as tender vegetables and also known as warm season or tropical and subtropical vegetables. Temperate vegetables, in general, can be stored for longer period under low temperature. Tropical vegetables are bulky and more perishable compared to temperate vegetables. In general, temperate vegetables can tolerate frost and low temperature, biennial in nature, plant spread is relatively smaller, surface root system, more response to frequent application of split nitrogen dose, require frequent light irrigation and most of the temperate vegetables have edible part other than fruit. Temperate vegetables require low temperature for seed production. Tropical and subtropical vegetables have plant spread relatively larger, relatively deep root stem and most of the vegetables have edible part is fruit.

Hardy	Semi-hardy	Tender
Asparagus	Carrot	Amaranth
Cruciferous	Celery	Okra
Garlic	Beetroot	Brinjal
Leek	Globe artichoke	Chilli
Globe artichoke	Lettuce	Cowpea
Onion	Palak	Cluster bean
Parsley	Parsnip	Cucurbits
Spinach	Potato	Tomato
Pea		Colocasia
Rhubarb		Amorphophallus
Radish		Yams
		Sweet potato

4. Classification based on parts used

Vegetables are classified as per the plant part used for vegetable purpose. However, this classification is not suitable for farmers as season of cultivation as well as cultural requirements may not be same.

Leaves	Flower	Fruits	Modified stem	Under ground (plant parts)	Corm
Cabbage, palak, fenugreek, amaranthus, lettuce, celery, parsley	Broccoli, Globe artichoke	Tomato, brinjal, chilli, beans, okra, and all cucurbits	Knol Khol, cauliflower, asparagus	Carrot, turnip, beet, radish, potato, sweet potato, taro, ginger, garlic, onion, elephant foot yam, cassava	Colocasia, elephant foot yam

5. Classification based on photoperiodism

Long day plant	Short day plant	Day neutral crop
Potato	Sweet potato	Tomato
Onion	Indian spinach	Brinjal
Cabbage	Dolichos bean	Chilli
Cauliflower	Cluster bean	Okra
Radish	Winged bean	Cucurbits
Lettuce		Amaranthus
Spinach		French bean
Palak		Cowpea
Turnip		Sweet pepper
Carrot		
Beet root		

6. Classification based on season of cultivation

It is an important classification from the farmers or vegetable grower's point of view. Because majority of vegetables are season-bound and specific to particular temperature range for growth and development of edible plant part and seed. Vegetables are classified as summer, rainy and winter season; based on growth and production during particular seasons. Summer season starts from February- June/July under north Indian plains, January-May/June in south Indian plains. October-January is winter season, experiencing chilling temperature, in most parts of the country. However, in some part of the South India, severe winter is lacking. Rainy season starts from June and extends up to September. Season bound crops are as follows.

Winter season crops: Cole crops, radish, carrot, beet, turnip, potato onion, lettuce, garden peas, etc.

Summer season crops: Tomato, brinjal, chilli, cucurbits, cowpea, cluster bean, amaranth, okra, etc.

Rainy season crops: Okra, cucurbits, tomato, brinjal, chilli, etc.

However, in southern part of the country most of the vegetables are grown all the year-round due to mild climatic conditions.

Vegetables crops can also be classified based on crop growth and flowering as annual, biennial and perennial. Mode of pollination is also considered for vegetable classification as self-pollinated, often cross pollinated and cross pollinated. Soil, pH, salinity, response to transplanting, etc. are also considered for vegetable classification.

7. Classification based on response to transplanting

Plants easy to transplant: Tomato, cabbage, Brussels sprout, beet, broccoli, lettuce, etc.

Plants transplanted with moderate care: Brinjal, chilli, onion, pepper, leek, cauliflower.

Plants transplanted with difficulty, requiring special care: Cucumber, watermelon, sweet corn, legumes, squash, gourds.

8. Classification of vegetables based on pH requirement

Class	pH	Crops
I	pH 6.0-6.8 (Slightly tolerant)	Cabbage, cauliflower, beetroot, lettuce, muskmelon, okra, onion, palak, cowpea
II	pH 5.5-6.8 (Moderately tolerant)	Beans, brinjal, carrot, cucumber, garlic peas, pepper, radish, tomato, pumpkin, turnip
III	pH 5.0-6.8 (Very tolerant)	Potato, sweet potato, watermelon

9. Classification based on Tolerance to soil salinity

Soil salinity is the major constraint affecting vegetable production. In coastal areas, salinity occurs due to saline water and high-water table. In arid and semiarid climatic conditions, evaporation exceeds rainfall causing rise of salt rich water up to soil level. In saline soil, soluble salts like chloride, sulphate and bicarbonates of sodium, calcium and magnesium will be very high. Soil salinity leads osmotic and toxic effects on metabolic processes of cells. Saline soils can be reclaimed by elimination of salts from soil by leaching. Based on salt tolerance, vegetables can be classified as

Class		Crops
I	Less tolerant	Celery, peas, beans, radish, potato, sweet potato, snake gourd, sweet pepper, brinjal
II	Moderately tolerant	Tomato, chilli, watermelon, cucumber, pumpkin, muskmelon, bottle gourd, amaranth, cabbage, cauliflower, carrot, onion
III	Tolerant	Beetroot, palak, ash gourd, bitter gourd, kale, lettuce, asparagus

However, none of the classification, except botanical classification is hard and fast, since the same crop fall in different groups or can be accommodated in more than one class.

10. Classification based on tolerance to soil acidity

Class		Crops
I	Less tolerant	Okra, onion, cabbage, cauliflower, broccoli, Chinese cabbage, musk melon
II	Moderately tolerant	Brinjal, tomato, chilli, radish, carrot, summer squash, winter squash
III	Very tolerant	Potato, sweet potato, rhubarb

11. Classification based on photosynthesis

C4 plant	Amaranthus, Globe amaranth (Artichoke)
C3 plant	Lettuce, Sugarbeet, Carrot, Tomato, Potato, Sweet potato

12. Classification based on temperature requirement

18-25 ^{o}C	Cabbage, Cauliflower, Radish, Carrot, Lettuce, Potato
20-25 ^{o}C	Tomato, Brinjal, Pepper, Onion, Garlic, Cucumber, Pumpkin
25-27 ^{o}C	Okra, Chilli, Sweet potato, Water melon, Muskmelon

13. Classification based on respiration rate

Very low	Potato, Onion
Low	Sweet potato, Cabbage, Cabbage, turnip
Moderate	Tomato, chilli, Carrot, beet, sweet pepper
High	Peas, Beans, radish, lettuce
Very high	Leafy greens, Green onion, Musk melon, Water melon, Cauliflower, Asparagus, okra, broccoli, Brussels sprout

14. Classification based on respiration

Climacteric	Tomato, Musk melon, Watermelon
Non-climacteric	Cucumber

15. Classification based on root depth

Very shallow rooted (15-30 cm)	Onion, lettuce, small radish
Shallow rooted (30-60cm)	Cole crops, potato, radish, garlic, cowpea, celery, leek, palak, spinach
Moderately deep rooted (60-90cm)	Cucumber, muskmelon, brinjal, french bean, carrot, beetroot
Deep rooted	Summer squash, chilli, pea, turnip

(90-120cm)	
Very deep rooted (120-180cm)	Winter squash, pumpkin, sweet potato, tomato, artichoke, lima bean

16. Classification based on water requirement

High	Sweet pepper, cole crops, radish, ridge gourd, turnip, beet root, leafy greens
Moderate	Tomato, brinjal, chilli, cucumber, onion, carrot, potato
Low	Peas and beans
Very low	Watermelon, muskmelon, pumpkin, ash gourd

17. Classification based on tenderness

Tender crops	**Very tender crops**	
Cowpea	Cucumber	Bitter gourd
New Zealand spinach	Brinjal	watermelon
French bean	lima bean	Ridge gourd
Soybean	Cluster bean	Muskmelon
Sweet corn	Winged bean	Cassava
Tomato	Amaranth	Pointed gourd
Sweet potato	Hyacinth bean	Indian spinach
Sweet	Okra	Elephant foot yam
Summer squash	Chilli	
Pumpkin	Bottle gourd	

18. Classification based on nutrient content

Calories	Immature seeds of lima bean, broad bean, peas, tapioca, sweet potato, yams, colocasia, potato, garlic, Brussels sprouts and onion.
Protein	Peas, double bean, winged bean, garlic, Brussels sprouts, cowpea, lima bean, lima bean seeds amaranth leaves, drumstick leaves and Mathi.
Precursor of vitamin-A (β-Carotene)	Carrot, spinach, turnip green, palak, mustard green, amaranth, coriander, colocasia leaves, sweet potato (yellow), pumpkin (yellow) and tomato.
Vitamin-B complex	Peas, broad bean, lima bean, garlic, asparagus, colocasia corms and tomato.
Vitamin-C (Ascorbic acid)	Turnip green, green chillies, Brussels sprouts, mustard green, amaranth, coriander, drumstick leaves, cauliflower, knol-khol, spinach, cabbage bitter gourd and radish leaves.
Calcium	Curry leaves, amaranth leaves, drumstick leaves, methi, turnip green, mustard green, coriander and palak.
Iron	Drumstick leaves and fruits, amaranth, methi, mint, coriander, spinach, palak and mustard green leaves.

Roughages	Spinach, lettuce, cabbage, amaranth and root vegetables.
Vegetable milk	Pea pods and cabbage leaves.

19. Classification based on seed longevity in normal storage condition

Up to 1 year	Onion, French bean.
Up to 2 years	Okra, carrot, chilli, sweet pepper, cauliflower, cabbage, broccoli.
Up to 3 years	Radish, beet, peas, ridge gourd, turnip, palak.
Up to 4 years	Tomato, brinjal, pumpkin, summer squash, bottle gourd, bitter gourd, watermelon, muskmelon, cucumber, spinach.

20. Classification of male sterility in vegetable crops

S. No.	Types of male sterility		Inheritance	Crops in which identified
1	Pollen sterility	(a)	Cytoplasmic	Carrot, sweet pepper, turnip, chinese cabbage, cucumber (in cauliflower, cabbage and broccoli this male sterility has been introduced from sterile cytoplasm of radish)
		(b)	Genic	
			i) Single recessive gene	Tomato, brinjal, pea, muskmelon, watermelon, chilli, lima bean, pumpkin, cucumber, cabbage, cauliflower, Brussels sprouts, broccoli, chinese cabbage
			ii) Duplicate dominant genes	Cauliflower
		(c)	Cytoplasmic genic	
			i) Single recessive gene	Onion, radish, sweet pepper
			ii) Two recessive gene	Beet
			iii) Single dominant gene	Carrot
2	Staminal sterility		Genic (single recessive gene)	Tomato
3	Functional male sterility			
	a) Positional sterility		Gene (single recessive)	Tomato, brinjal. Sweet pepper
	b) exerted stigma sterility		Gene (single recessive)	Tomato

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Chapter - 5
Post-Harvest Handling of Cut Flowers and Its Application

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Chapter - 5

Post-Harvest Handling of Cut Flowers and Its Application

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Abstract

Flowers always remain as an integral part as they are very important in the traditional way of life, with the increasing standard of living. The demand for flowers has been increasing and for meeting these demands increase in the production and also proper postharvest handling is essential. In the floriculture industry postharvest losses are the major loss and also the worldwide problem with flowers. Postharvest handling is to be done immediately after the harvest and it includes cleaning, cooling, sorting and packing. Flowers remain alive even after detaching from the parent plant and continue the metabolic activities causing depletion of carbohydrates, rise in temperature and respiration. All these activities lead to deterioration of the harvested flowers which in turn decrease the longevity of the fresh produce. Different factors affect the vase life of cut flowers are chemical factors like carbohydrate status, ethylene, pathogens and physiological factors like stored foods for the flowers, humidity, water, light, and temperature. Water uptake is a major factor affecting the postharvest life of cut flower, such as air embolism and duration of vascular occlusion contribute to cut flower senescence. Less life in harvested flowers may be due to nutrient deficiency, bacterial and fungal infection, water stress induced wilting, vascular blockage and the action of ethylene in plant cells. Wilting, food depletion and bluing is the main cause in the termination of storage and vase life of cut flowers. Vascular occlusion is a mechanism, as a result of water stress that induces senescence in flowers.

Keywords: Senescence, vase life, chemical solution

Introduction

Cut flowers are highly perishable and most of the cut flowers can last from 10 to 28 days in vase with holding solution. Compared to other horticultural or agricultural produce, the nature and extent of postharvest loss on flowers are larger for each variety. Cut flowers that needed long distance transport requires proper harvesting, it must be keep out of direct sunlight and

changing of water with an expert handling. The postharvest life and quality of cut flowers after harvest depends on the way they are handled. Different factors affect the vase life of cut flowers are chemical factors like carbohydrate status, ethylene, pathogens and physiological factors such as the content of stored foods for flower, humidity, water, light, and temperature of the place. Cut flowers are to continue remain fresh with reserved carbohydrates, proteins and fat for their longevity. Vase life of cut flowers can be prolonged by the addition of chemical preservatives. By applying various chemicals the postharvest life of cut flowers can be extended.

The major reasons for less vase life may be due to nutrient deficiency, infection caused by microorganisms, water stress induced wilting, vascular blockage and also the action of ethylene in plant cells. Ethylene regulates the aging of cut flowers and remains as a hormone in plants by stimulating and regulating the opening of flowers and the shedding of flowers. Cool temperatures are important for maintaining good quality and shelf life of cut flowers. After harvesting, flowers need to be moved to a cool area, placed in chemical preservatives with nutrient solutions depending on the specific need of the flower. Postharvest life of cut flower depends upon the turgidity of the cells, dried stem ends and bacterial growth restricts water uptake, causing flower stems to droop and flowers to wilt. Cut flowers will start decaying when they are not able to draw water and nutrients from the vase solution because of the clogging of vascular tissues of the stem by phloem, blocking the absorption of water. Factors affecting water uptake such as air embolism and duration of vascular occlusion results in the senescence of cut flowers.

Sugar has an important role in the longevity of cut flowers. Sugars can be provided to cut flowers by dissolving in to the vase solution. The optimum concentration of sugar varies depending on different flowers. Most flowers requires a supply of 2 to 5% sugar in the vase solution. After harvest they cannot receive any nutritional and hormonal support from the mother plant, stored foods helps the flowers to remain fresh during the vase life. Proper harvesting, care of flowers and postharvest handling are important to maximize the vase life. Harvested cut flowers should also be protected from mechanical damage during the process. Mechanical damage to any part of the flower will reduce the aesthetic value of the flowers and could lead to bacterial infections and the flowers turns black and decay. The weather conditions and environment where plant grown will affect the postharvest longevity and also the plants were not water stressed prior to harvesting flowers. Plants should be properly watered before harvesting flowers so that the cell walls are turgid.

Stages of harvesting

The correct stage of harvesting for each plant species is very much important and this ensure good quality and postharvest behaviour of cut flowers. To ensure maximum vase life, cut flowers are to be harvested at proper stage. Studies revealed that harvesting of flowers too early and too late significantly reduces the vase life. If the harvesting is before or after the optimum stage the developing flowers use the carbohydrates that will be used for the development of smaller flower buds and slowing down the growth of other flowers in the bunch. Every species of plants have proper maturity stage and time in which flowers can be harvested without affecting the postharvest quality. Some plant species, flowers can be harvested at the bud stage with no reduction in quality and vase life. In some other plants improper harvesting of flowers leads to disorders such as bent neck, improper development of pigmentation, abnormal opening of the buds. If flowers are to be stored for longer time, for minimizing mechanical injury during handling and need to transport to longer distances then they are usually harvested at an earlier stage.

Pre-harvesting care for flowers

1) Plants should be healthy and turgid.
2) Storage containers after harvesting and cutting tools should be cleaned and sanitized. Cutting tools should be sharp, blunt cutting tool results in crushed stems that reduces water uptake.
3) Storage containers should be cleaned and disinfected regularly using a solution containing 5% hypochlorite.

Storage solution should contain biocide or germicide to the water. These chemicals that can prevent the growth of microorganisms. Microorganisms and the substances that they produce can plug the xylem, the water conducting tissue of the plant and thus blocking the uptake of water. Research works have shown that there is a strong action of microbes in the water on the vase life of cut flowers. Commonly used biocides are calcium or sodium hypochlorite, aluminium sulphate, silver nitrate, silver thiosulphate, hydroxycitrate and 8-hydroxy quinolone were added in the water at time of harvest or in the vase solution. It is important to follow the recommendation for the correct dilution for the specific flowers. The pH of the water holding flowers should be 3 to 5.5, as research findings that flowers absorb more water in acidic solution. Chemicals such as citric acid, 8-hydroxyquinoline citrate, aluminum sulfate etc. are generally used to lower the pH of the water. The requirement of chemical needed to acidify will depend on the alkalinity of the water. The pH and the alkalinity of the water source should be analysed to determine the

proper amount of chemical needed to lower the pH. High levels of sodium, fluoride or sulphate in the holding solution can be toxic.

Care on harvesting flowers

1) Harvest flowers in the morning or evening.
2) After harvest place immediately in cold water.
3) Reduce foliage on the flower stems.
4) Slant cuts will be given at bottom of the stem to increase water uptake.
5) Disinfect cutting tools and storage containers.
6) Grade, bunch and transport flowers immediately after harvest.
7) Avoid over filing of containers with flowers.

Reasons for flowers less storage life

1) Food depletion
2) Bacterial and fungal infection
3) Maturation and aging of flower
4) Water stress and xylem blockage
5) Bruising and crushing of flower
6) Temperature variation during storage and transport
7) Bluing of flowers
8) Accumulation of ethylene
9) Poor water quality
10) Cultural practices

Care for cut flowers after harvesting

Pre-cooling

Precooling is very essential to remove the field heat after harvest. This is done by air cooling, hydro cooling or by ice cold water, to bring down the temperature to 1 degree Celsius in a very short period.

Conditioning

Conditioning is done with demineralised water supplemented with germicides, sucrose, growth regulators and acidifying with citric acid.

Pulsing

Treating the flowers with high concentration of sucrose and germicide for a short time to increase the shelf life. Pulsing is to be done immediately after harvesting.

Water relations

The postharvest life of harvested flowers depends on water uptake and the capacity of flower tissue to retain water. The rate of water uptake of cut flowers depends on the transpiration pull, temperature, fungicide and nutrients. Disruption of water columns in stem vessels is by air embolism and resistance to water flow in stems.

Respiration

The rate of respiration depends on the quality of carbohydrates available in the harvested flowers, with high temperature there will be faster rate of respiration and burning of tissues and also the life of flower is shortened.

Relative humidity

Humidity is the concentration of water vapour in air and higher the relative humidity in the air the transpiration rate of cut flowers will be less. By reducing the transpiration rate vase life can be extended.

Growth regulator

Postharvest life of flowers can be regulated by the application of growth regulators. Cytokinins delay senescence in some cut flowers. Depending upon the concentrations, Gibberellic acid also promotes longevity of flowers. Auxin can also delay senescence and abscission in some flowers.

Preservative solutions

Preservatives in the form of powder or liquid are prepared with a mixture of sugars, germicides, salts and growth regulators. Anti-ethylene compound in the solution reduce the action of aging in cut flowers.

Impregnation

Loading of flowers with high concentration of silver nitrate or cobalt chloride for a short period of time is known as impregnation. This is used to reduce the action of microbes and synthesis of ethylene.

Grading

The flowers should be graded after harvest as per specification for different market requirements. Grading varies from growers, it is better to keep colours separate. Grade criteria varies for each type of flower. Some grades commonly followed are length of the flower stem, stem girth, stem deviation, flower diameter and flowers per stalk or petals per flower.

Storage

There are two types of storage methods commonly used, wet and dry. Wet method is used for short term storage, in which cut flower stems are dipped in water. In dry storage the cut flowers are placed in controlled atmosphere to reduce the respiration, transpiration loss, conserve energy and to delay the ethylene action. It involves the use of increased level of CO_2 and decreased level of O_2.

Packing

Packing ensures the freshness of flower till it reaches the consumers. Low rate of transpiration and respiration and cell division during transportation are essential for long life and keeping quality. Packing must ensure protection from the flowers against physical damage, water loss and external conditions during transportation. The flowers should be transported at low temperature and relative humidity should be maintained at 95 -98 %. Lack of light during transportation causes yellowing of leaves in many flowers.

Transporting

For short distance cut flowers may be transported in insulated trucks without refrigeration after pre-cooling and proper packing. Air shipment is the quickest method followed. After harvesting, flowers are moved to a cool area and the stems are recut and placed in solutions depending on the specific need of the flowers. Most flowers will fully recover from wilting if recut and placed in nutrient solution. Stems are recut by removing about an inch of the stem end under water before placing them in the solution.

Supplying food

Harvested flowers should be kept indoor in vases where light requirement will be low and photosynthesis will be minimum. Photosynthesis is a biological process forming carbohydrates that are needed for the cut flowers to continue to develop and remain alive after harvest. Carbohydrates needed for flowers come from starch and sugars stored in the stem, leaves and petals but the levels may not be adequate. In addition to clean water with germicide and growth regulator, continuous supply of food is needed for achieving maximum postharvest life of the cut flowers. Water and the addition of preservatives to the solution will result in the best performance of cut flowers. Numerous brands and mixtures of floral preservatives and flower care products are commercially available and each is formulated for a different purpose. The basic types of flower care products are hydration solutions, holding Solutions, anti-ethylene treatments and specialized care solutions.

Role of ethylene

Ethylene affect the postharvest life of cut flowers and it accelerates flower abscission and leaf yellowing. Ethylene is an odorless and colorless gas, it is a natural plant growth hormone that affects many physiological processes ranging from seed germination to senescence of plants. Flowers generate ethylene as part of the normal aging process. Cut flowers are very sensitive to ethylene and very small amounts can be very harmful.

Maintaining straight stems

Flower stems naturally bend away from gravity and this phenomenon is called geotropism. Flowers such as gladiolus, snapdragon, gerbera, tulip, and anemone bend upward when placed horizontally. This bending of the stem away from gravity results in curved stems when they are later placed in a vertical position. These flowers should be handled upright.

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Chapter - 6
Intercropping in Tomato with Short Duration Vegetable Crops

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Chapter - 6

Intercropping in Tomato with Short Duration Vegetable Crops

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Abstract

Intercropping is a production technique in which the land is utilized for growing more than one crop at a time for efficient utilization of farm resources to increase the profitability of the farming system. The benefits of the intercropping system is maximised through selection of companion crops and planting geometry. Usually plant species from different families with variation in shoot morphology and rooting pattern are chosen under intercropping. Tomato is a versatile crop grown throughout the world under different farming systems. This crop is widely chosen by small and marginal farmers to sell their produce easily in the market, as most of the consumers visiting the vegetable market will buy tomatoes. It is a highly perishable vegetable and there is a drastic reduction in price of the produce during the market gluts. Adoption of intercropping in tomato with short duration vegetables like radish, Aggregatum onion and vegetable cowpea will increase the income of farmers besides reducing the loss if any, under tomato farming.

Keywords: Intercropping, tomato, radish, small onion, vegetable cowpea

Introduction

Tomato which belongs to family Solanaceae is one of the most popular and widely grown nutritious vegetable all over the world. In India, tomato is grown in an area of 7, 89, 000 ha with a production of 1, 97, 59, 000 metric tonnes and productivity of 25.04 t/ha during 2017-18. The leading tomato growing states in India are Andhra Pradesh, Madhya Pradesh, Karnataka, Gujarat and Odisha (Anonymous, 2019). Intercropping system becomes more productive and profitable by selecting appropriate crops, population density and planting geometry of component crops (Begum *et al*., 2015). Intercropping with some companion plants could increase tomato quality, suppress nematodes, and improve soil environment without decreasing tomato yield (Tringovska *et al*., 2015). Intercropping practice that allows more

efficient use of on-farm resources are among the agricultural practices associated with sustainable crop production. Intercropping provides year-round ground cover, or at least for a longer period than monocultures, in order to protect the soil from desiccation and erosion. By growing more than one crop at a time in the same field, farmers maximize water use efficiency, maintain soil fertility, and minimize soil erosion, which are the serious drawbacks of mono-cropping. It also reduces seasonal work peaks as a result of the different planting and harvesting times of intercropping crops. Moreover, it could serve to increase output per unit area, particularly with low levels of external inputs since a mix of species makes better use of available nutrients and water in the soil. Intercropping gives higher yields in a given season and greater stability of yields in different seasons compared with sole cropping. The two major advantages of intercropping are higher productivity and greater production stability through efficient utilization of solar energy, moisture, nutrients and human resources. Intercropping with some companion plants could increase tomato quality, suppress nematodes, and improve soil environment without decreasing tomato yield. (Liu *et al*., 2014; Tringov-ska *et al*., 2015).

Studies on intercropping with tomato as a main crop

A field study conducted at Horticulture Department, Agriculture and Forestry College, Mosul University, Mosul, Iraq by Abdel (2006) in tomato cultivars super lady and castle rock suggested that mitigating heat stress through intercropping by evaporative cooling and shading appeared to be the most effective way to improve fruit set and consequently a yield of perfect quality. Tomato-corn intercropping resulted in 70% yield increases in relation to check. Moreover, the highest yield (3.4kg m^{-2}) was obtained from super lady cultivar intercropped with sunflower and irrigated at 25% soil AWC depletion level to a depth of 40 cm. The results of a study by Rodriguez *et al*. (2007) on intercropping tomato (*Lycopersicon esculentum* Mill.) with marigold (*Tagetes erecta* L.) at Mexico, revealed that tomato plants intercropped with marigold or pigweed grew longer stems (26 to 33%), thinner leaves (33 to 35%), and lower specific leaflet area (61 to 69%) than plants grown alone. Also, tomato plants intercropped with marigold had a higher net photosynthetic rate (48 and 64%) and chlorophyll content (4 and 9%) than tomato pigweed intercropping and tomato alone, at 10 weeks after tomato transplant (WATT). They also found that tomato-marigold intercropping produced more fruits (100 and 148%, at 8 WATT), inflorescences (46 and 25%, at 6 WATT) and fruit yield (100 and 148% at 15 WATT) compared to treatments tomato pigweed intercropping and tomato alone.

Research work conducted at Research Farm, NWFP Agricultural University, Peshawar, Pakistan by Hussain *et al*. (2008) on tomato, intercropped with okra, potato, corn, chilli and eggplant showed that the maximum LER of 2.41 was recorded in intercropping of tomato with okra. This indicates that these two summer vegetables are the best companions and resulted in maximum income of Rs. 1, 96, 393 ha^{-1} due to maximum yield of tomato (27.61 t ha^{-1}) compared to that of as a sole crop (20.34 t ha^{-1}) and high market price of both vegetables. Tomato + corn combination resulted in the second highest income of Rs. 1, 80,700. A study carried out by Carvalho *et al*. (2009) on tomato intercropping with (*Foeniculum vulgare* Mill.), (*Mentha piperita* L.), (*Ocimum basilicum* L.) and (*Ruta graveolens* L.) observed that the intercropping with *Ruta graveolens* L. resulted significant increase in commercial production of fruits, of about 26%. The relative yield of tomato was greater in intercropping with rue (13.6 t ha^{-1}), followed by intercropping with peppermint (9.8 t ha^{-1}) and with *Ocimum basilicum* L. (9.1 t ha^{-1}) and lesser on intercropping with fennel (6.4 t ha^{-1}).

The reports given by Adeniyi (2011) at Department of Agricultural Economics and Extension, Bowen University, Iwo-Nigeria on tomato intercropping with cowpea and okra at varying levels of cropping densities showed that the tomato + okra (2:1) showed significant values for yield (12,729.17 kg/ha) in 2007 and yield of 10,957.93 kg/ha in 2008. This system gave increased net returns of US$ 214.97 (21.04%) and US$ 865.49 (233.18%) over the sole cropping of tomato and cowpea respectively. This also increased net revenue of US$ 309.51 (33.38%) above the intercrop of a pair row of tomato with one row of okra and US$ 655.47 (112.78%) above the intercrop of a pair row of okra with one row of cowpea. Research work conducted at Horticulture Department, Faculty of Agriculture, Assiut University, Assiut, Egypt by Nassef and El-Gaid (2012) on tomato intercropped with garlic reported that the land equivalent ratio (LER) of tomato/garlic intercropping was more than unit. Also, Land equivalent coefficient (LEC) was the same for both seasons. Moreover, the highest net profit was obtained from (one tomato plant: three garlic plants).

Shrestha (2012) conducted an experiment on intercropping with five tomato varieties Pusa Ruby, CL-1131, BARI-4, BARI-5 and Bio Rakshya in a newly established mango orchard. The results revealed that the highest tomato fruit yield and economic benefit was obtained from variety Pusa Ruby and recommended it for intercropping in young mango orchard. At FCAV/Sao Paulo State University, Jaboticabal, SP, Brazil a research work on five intercropping ratios (tomato: slender amaranth with 100:0, 75:25, 50:50, 25:75

and 0:100 ratio) by Silva *et al*. (2013) reported that the tomato exhibited a higher competitive ability for resources than slender amaranth, and the intraspecific competition was more important than interspecific competition for the cultivated plants. An experiment on tomato (*Lycopersicon esculentum* Mill cv. Super strain B) as a main crop intercropped with different plant population density of common bean (*Phaseolus vulgaris* L. cv Bronco) as intercrop by El-Gaid *et al*. (2014) reported that the highest LER in 1:3 tomato and common bean planting system as 1.26 and 1.25 in first and second season, respectively. It is recommended to use this pattern to improve farmer's income and LER under New Valley conditions.

An experiment on tomato cultivar 'Difenni' with garlic intercropping system under plastic tunnel organic medium cultivation by Liu *et al*. (2014) at College of Horticulture, Northwest A&F University, Yangling, China revealed that the plant height of tomato intercropped with garlic was higher in comparison to the control (monoculture) at 25 days after transplanting. They found that the net income of the intercropping mode (\$ 22886 ha^{-1}) was 12.3% higher than that of the monoculture mode (\$ 20378 ha^{-1}). In an experiment conducted at Department of Agronomy, Faculty of Agriculture, Federal University, Kashere, Nigeria by Degri and Samaila (2015) on intercropping tomato (base crop) and maize (component crop) observed the higher fruit weight and total fruit yield in intercrop tomato than sole tomato.

A field experiment conducted at Department of Horticulture, Wolaita Sodo University, Wolaita Sodo, Ethiopia by Gebru *et al*. (2015) on three component populations of tomato (T) and maize (M) i.e. (100T:50M, 67T:33M and 50T:50M) and five maize intercropping dates (30 days before tomato transplanting, 15 days before tomato transplanting, just at tomato transplanting, 15 days after tomato transplanting and 30 days after tomato transplanting) revealed that the highest total LER value of 2.06, Gross Monetary Values of 3,98,611.11 rupees/ha and Monetary advantages of 2,05,110.57 rupees ha^{-1} was obtained from component populations of 100T:50M. Intercropping lettuce with tomatoes is 40% more efficient than growing the two crops individually in the same area within the high tunnel. When the relative yield of each crop was totalled, the Land Equivalency Ratio or Relative Yield Total (RYT) was equal to 1.40, or the intercropping treatment was 40% more efficient use of the high tunnel environment when compared to a monoculture system of all crops individually. During next year, the combined RYT of both tomato crops and the lettuce/basil was 1.83, indicating that intercropping was 80% more efficient within a high tunnel relative to monoculture production of each crop. (Jett *et al*., 2015).

The result of a study by Demir and Polat (2016) at Akdeniz University on tomato (*Solanum lycopersicon* L. 'Selin' F_1) intercropped with broccoli (*Brassica oleracea* L. var. *italica* 'Chief' F_1) showed that the highest N (2.52%), P (0.37%), K (3.42%) and Mg (0.16%) contents in tomato fruits were obtained from tomato-broccoli intercropping system in the autumn period. The analysis of tomato leaves showed the highest K content (2.51%) in intercropping while N (3.02%), K (1.41) and Mg (0.46%) contents were analyzed more in intercropping. Research work conducted at Department of Horticulture, Northeast Agricultural University, Harbin, China by Wu *et al*. (2016) on tomato intercropping with potato onion, revealed that the root dry weight, shoot dry weight, and P uptake in the shoot of tomato seedling at 37 DAT were significantly higher in intercropping system than those in tomato mono cropping system. However, the root dry weight, shoot dry weight, and P uptake in the shoot of potato onion seedling at 37 DAT were significantly lower than those in the potato onion mono cropping system. The RYT values were 1.93, 1.76, and 1.76 at 23, 30, and 37 DAT, respectively. Research work conducted at University of Nairobi, Kenya by Ndukhu *et al*. (2017) on tomato (*Lycopersicon esculentum* L, variety Rio Grande) and maize (*Zea mays* L, var.H513), intercropped or in rotation with chickpea revealed that the tomato in season IV in rotations with chickpea showed the highest fruit yield of 3.95 Mg ha^{-1}

An investigation was carried out by Soniya *et al.* (2021a) to study the effect of intercropping on growth and yield of tomato (Solanum lycopersicum L.)" at a farmer's field at Sorakayalnatham, Natrampalli taluk, Tirupattur district, (Tamil Nadu) during January-May 2019. The treatments comprised of three intercrops viz., radish, small onion and vegetable cowpea, and three levels of recommended dose of fertilizers (RDF) viz., 100, 125 and 150% along with sole crop of tomato under 100% RDF. The results indicated that the maximum values for growth attributes viz., plant height at 30, 60 and 90 DAT (48.5, 63.5 and 92.1 cm, respectively), primary branches/plant (11.5), leaf area index (3.58) and yield components like fruits $plant^{-1}$ (35.5), single fruit weight (82.9 g) and weight of fruits $plant^{-1}$ (2.9 kg) were recorded in the plots which received 25 t FYM ha^{-1} + 150% RDF in tomato + small onion intercropping system. This was followed by the tomato + vegetable cowpea intercropping system which received 25 t FYM ha^{-1} + 150% RDF. Soniya *et al.* (2021b) reported that higher fruit yield (99.16 t/ha), tomato equivalent yield (210400 kg/ha), land equivalent ratio (1.63), area time equivalent ratio (1.54) and production efficiency (1753.33 kg/ha/day) with benefit cost ratio of 4.08 and income equivalent ratio of 1.63 were recorded under application of FYM @ 25 t/ha + 150% recommended dose of inorganic fertilizers in

tomato + small onion intercropping system. In view of the above facts, they concluded that by adopting the above said treatments, farmers can effectively utilize their land area and time and increase their income and production efficiency besides minimizing soil erosion, which are the serious drawbacks of mono-cropping. It also reduces seasonal work peaks as a result of the different planting and harvesting times of intercropping crops.

Performance of radish-a short duration vegetable as an intercrop with other vegetables

Asaduzzaman and Hussain, (1990) reported that the intercropping of jute + radish gave a benefit cost ratio of 9.38. Research work conducted at Himachal Pradesh Krishi Vishwavidyalaya, Lari Spiti, by Malhotra and Kumar (1995) in different vegetable crops as intercrop with potato (*Solanum tuberosum* L.) revealed that the highest land-equivalent ratio of 1.31 was in potato + radish (*Raphanus sativus* L.) and followed by potato + cabbage (1.13). Rezende *et al*. (2006) suggested the potential of cabbage + pepper + radish in reducing operational cost (20.8 to 34%) in inter cropping system than their sole cultivation. A study carried out by Filho *et al*. (2007) on cultivars Taia (lettuce) and Crimson Gigante (radish) in intercropping system at UNESP, in Jaboticabal observed that the radish yield was the highest (518.8 g m^{-1}), in intercropping when compared to sole crop. In an experiment conducted at Department of Horticulture College of Agriculture, Raichur, Karnataka, India by Suresha *et al*. (2007) on different intercrops viz., radish, carrot, onion, garlic, cluster bean and dolichos bean on chilli reported that radish + chilli intercropping system results in realization of significantly the highest (72.05 q/ha) yield in chilli followed by chilli + carrot (70.77 q/ha). An experiment was carried out by Rodge and Yadlod (2009) at Department of Horticulture, Marathwada Agricultural University, Parbhani, Maharashtra to study about intercropping in solanaceous vegetables with onion, radish, coriander and palak. They observed that the intercropping of tomato with radish had recorded a yield of 136.65 q ha-1 of tomato and gave the maximum net profit of Rs. 34,432.

A field experiment conducted at NRCSS, Ajmer in intercropping system *viz.,* fennel + radish (1:1), fennel + radish (1:2), fennel + radish (2:2), fennel + carrot (1:1), fennel + carrot (1:2), fennel + carrot (2:2) by Mehta *et al*. (2012) reported that the highest yield of fennel, radish and carrot was obtained with the application of 120 kg N and 50 kg P_2O_5 which was significantly higher over lower levels. Economic yield of radish was obtained higher as compared to carrot in all the intercropping ratios. A field study conducted at Department of Agronomy, Tamil Nadu Agricultural University, Coimbatore on drip

fertigation in maize based intercropping system by Fanish *et al*. (2013) suggested that radish intercropped with maize registered a higher maize grain equivalent yield of 11,153 kg ha^{-1}. The results showed positive values for radish yield (5,101 kg ha^{-1}), higher net returns (Rs. 56,858) and B: C ratio (3.24) under drip fertigation with 150 per cent recommended dose of fertilizer and radish as an intercrop.

The reports given by Choudhary *et al*. (2014) at the agriculture research farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi on winter maize (*Zea mays* L.) intercropped with various vegetables like radish (*Raphanus sativus* L.) spinach (*Spinacia oleracea* L.) and carrot (*Daucus carota* L.) revealed that under sole winter maize treatment maize equivalent yield decreased significantly than its equivalent yield under maize based intercropping association *viz.*, maize + carrot, maize + radish and maize + spinach. All the intercropping systems showed superiority over sole cropping of maize. Maximum maize equivalent yield (282.46 q ha^{-1}) was recorded under maize (paired) + carrot followed by maize (paired) + radish (164.25 q ha^{-1}) and minimum in maize (paired) + spinach (116.21 q ha^{-1}). Inter cropping of carrot with winter maize registered higher B: C (3.86) followed by maize + radish (2.41) and minimum in maize + spinach (1.19). At Farming Systems Research and Development (FSRD) Site, Kushumhati, Sherpura research work by Ali *et al*. (2015) in maize intercropping with radish, potato, coriander, bush bean, spinach, lalshak and maize sole revealed that the highest grain yield was obtained from maize + radish (9.92 t ha^{-1}). The results showed maximum maize equivalent yield of 10.81 and 10.66 t ha^{-1} from maize + radish and maize + coriander, respectively.

The results of a study by Begum *et al*. (2015) at the farmers' field of Dori Bakkhali, Mymensingh on intercropping chilli with radish, onion, carrot and garlic reported that the maximum chilli equivalent yield was obtained from chilli + garlic (25.01 t ha^{-1}) followed by chilli + radish (24.05 t ha^{-1}) and chilli + carrot (22.38 t ha^{-1}) combination. A field experiment conducted at the Multi Location Testing (MLT) site Bhuapur, Tangail in hybrid maize (BARI Hybrid maize-7) intercropped with radish (BARI Mula-1), potato (Diamant), spinach (local), red amaranth (BARI Lalsak-1) by Rahaman *et al*. (2015) suggested that the highest vegetable yield was recorded from maize + radish intercropping combination (22.03 t ha^{-1}) followed by spinach combination (19.65 t ha^{-1}). The maize equivalent yield from maize + potato, maize + spinach, maize + red amaranth and maize + radish intercrops combination showed 77.92, 140.89, 45.72 and 39.36% higher yield over the sole maize respectively.

A study carried out by Pal and Tarai (2015) at Regional Research Station, Jhargram, Bidhan Chandra Krishi Viswavidyalaya, West Midnapur, West Bengal, India on intercropping combination in between mosambi and some profitable vegetable crop such as leafy radish (*Raphanus sativus* L.), guwar (*Cyamopsis tetragonoloba* L.), groundnut (*Arachis hypogaea* L.) suggested that the intercrop, leafy radish gave a yield of 45 q/ha and net return of Rs. 27,000. An experiment on okra intercrop with radish, cowpea, amaranthus, palak in 1:1 row ratio at the Instructional Farm of Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal by Choudhuri and Jana (2016) observed that the maximum shoot to root ratio was recorded in okra+ radish intercropping system. The reports given by Kumari *et al*. (2016) at Agricultural Research Institute, Lohiya Nagar, Patna, Bihar in Mango intercropped with onion (Patna Red), radish (Snow White) garlic (local variety), pea (Azad P-1), carrot (Pusa Kesar), palak (All Green), coriander (Pant Haritima) and French bean (P-44) suggested that intercropping of mango with radish had recorded an yield of 188 q ha^{-1} and net profit of Rs.1,26,565.

A field study conducted at Farm Science Centre, VPKAS, Almora, Bageshwar, Uttarakhand, India on potato intercropped with radish (*Raphanus sativus* L.) or spinach (*Spinacia oleracea* L.) by Singh *et al*. (2016) suggested that the intercropping radish and spinach with potato increased potato equivalent yield over monocropped potato. Potato + spinach had a higher land equivalent ratio (1.78) and area time equivalent ratio (1.29) than the potato + radish intercrop, which had a higher relative net return (3.28) and B: C ratio (6.38). Potato + radish had higher energy input and output and energy intensiveness than other cropping systems. Radish and spinach can be intercropped with potato for efficient use of land and higher economic return. The potato equivalent yield (PEY) increased by 228% in intercropping radish with potato over mono cropped potato. The result of a study by Begum and Kader (2018) at Farm Research Division, Bangladesh Agricultural Research Institute, Mymensingh, Bangladesh on coriander green, red amaranth, radish green, mustard green, jute green, and spinach intercropped with pumpkin revealed that the highest number of fruits $plant^{-1}$ (3.48), vine length (637.5 cm), fruit length (21.6 cm), fruit circumference (57.9 cm), Average fruit weight 2.75 kg and fruit yield 23.93 t ha^{-1} was recorded in pumpkin + radish green intercropping. This intercropping had also recorded the maximum system productivity 37.2, Land Equivalent Ratio (1.73), Area Time Equivalent Ratio (1.13) and BCR (5.8). A field experiment conducted at Department of Agronomy, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Maharashtra, India on sweet corn intercropped with coriander, spinach, amaranthus, mustard and radish by Chaudhari *et al*. (2018) revealed that the

sweet corn + radish intercropping gave significant values for yield (107.09 q/ha), LER (1.64), SEY (255.47 q/ha), total cost (2, 66, 957.28), gross income (5, 05, 627), net income (238669.72) and B:C ratio (1.89).

Performance of small onion-a short duration vegetable as an intercrop with other vegetables

The reports given Mahadevaswamy and Martin (2003) at Department of Agronomy, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu on sugarcane intercropped with aggregatum onion (*Allium cepa* var. *aggregatum* L.) revealed that the intercropping of aggregatum onion significantly increased the total productivity as well as gross and net returns as compared to sole crop of sugarcane. Adopting 120 cm wide row spacing for sugarcane and planting high density intercropping of aggregatum onion (1:4 ratios) recorded the maximum productivity and economic returns. The total productivity and monetary benefits under 150 cm wide row spaced cane was also superior compared to productivity under normal row spaced (90 cm) cane. The economic indices like Relative Net Return Index and the Monetary Equivalent Ratio were enhanced by 6.4 to 8.2% and 12.3 to 17.0% respectively, indicating the economic efficiency of aggregatum onion intercropping, especially under wide row spaced cultivation of sugarcane.

In an experiment conducted at Department of Horticulture College of Agriculture, Raichur, Karnataka, India by Suresha *et al*. (2007) on different intercrops viz., radish, carrot, onion, garlic, cluster bean and dolichos bean on chilli suggested that chilli + onion intercropping system showed significant values for LER (1.32), ATER (1.19), gross return (80, 321), cost of cultivation (49, 564), net returns (30, 756), BCR (1.29). A field experiment conducted at Felin Experimental Station of the University of Life Sciences in Lublin on intercropping of root vegetables with carrot and parsley by Baaiewicz-Woiniak and Wach (2011) revealed that among the various combinations, intercropping with onion turned out to be most beneficial to both carrot and parsley. The ones cultivated together with onion created the highest yield of marketable roots, total yield and yield of leaves. Onion growing next to them had positive effect on the structure of root yield, especially in case of carrot, increasing commercial root share. Total marketable yield of root vegetables obtained from intercropping with onion was greater than that from homogenous cultivation. The highest yield was harvested from cultivation of parsley alongside onion (average 5.45 kg m^{-2}) and the lowest from cultivation with marigold (2.56 kg m^{-2}). On the average, after the 3-year research period, the highest vegetable yield was harvested from intercropping of carrot with onion (3.38 kg m^{-2}). Cultivation of parsley with onion increased total yield of

vegetables obtained in this combination up to average 2.38 kg m^{-2}, including yield of parsley root to 1.06 kg. Both carrot and parsley produced greatest mass of leaves in intercropping with onion (1.08 and 3.60 kg m^{-2} respectively).

The reports given by Mutiga *et al*. (2011) at Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Plant Science Building, Ithaca, USA on collards intercropped with chilli and spring onion showed that the higher yield in a unit of land when they intercropped collards with spring onions as indicated by a LER of 1.06. The results of a study by Baidoo (2012) at Department of Theoretical and Applied Biology Kwame Nkrumah University of Science and Technology, Kumasi, Ghana on 4 rows of cabbage to 1 row of onion revealed that intercropping did not significantly affect plant height. Mean canopy spread on the plots did not differ significantly. Mean fresh weight of cabbage heads were significantly heavier on the intercropped plots than on the sole cabbage plots.

Research work conducted at the State University of Montes Claros (UNIMONTES), Campus of Janauba by Mota *et al*. (2012) on five lettuce rows inter spaced with four onion rows suggested that the intercropping did not affect the agronomic performance of onion or lettuce. Higher plant densities (100% for both vegetables) resulted in higher lettuce and onion yields. The best economic results were observed using onion at 80% of plant density combined with lettuce at 40 and 100% and onion at 100% and lettuce at all densities (40 to 100%). Bulb diameter was the highest (3.68 cm) at a plant density of 40% lettuce and 100% onion and, the lowest (3.01 cm), at 100% lettuce and 40% onion. Onion total yield increased as lettuce and onion densities rose, reaching 9.79 t ha^{-1} with 100% of both onion and lettuce plants. A field experiment conducted at Soil and Water Management Research Farm, Navsari Agricultural University, Navsari by Mahant *et al*. (2012) inferred that intercropping in banana was more productive and profitable than their sole cultivation without loss in yield. Intercropping either with onion or garlic in banana at initial growth stage of planting increase the total profit without affecting the yield, they also observed that intercropping with garlic and onion with 60% coverage in banana under drip irrigation gave maximum gross and net returns as well as benefit cost ratio. They also noticed LER values in all the treatments were greater than one indicating the profitability of intercropping over sole cropping. Maximum LER was recorded in banana + onion (1.60) followed by banana + garlic (1.56) and banana + cauliflower (1.54).

In an experiment conducted at Crop Intensification Research Section, Field Crops Research Institute, Agriculture Research Center, Giza, Egypt by Abou-Keriasha *et al*. (2013) on intercropping faba bean on two crops (onion and wheat) and inoculated with bacteria reported that the highest values of land equivalent ratio "LER" (1.59) and monetary advantage index 'MAI' (3636.477) were observed when inoculating faba bean seeds with bacteria before sowing and intercropping on onion. While, the values of competitive ratio (CR) of faba bean were greater when intercropped with onion than those intercropped with wheat. An experiment on pepper (*Capsicum annuum* L.) intercropping with onion (*Allium cepa* L.) at The South Border College, Pan-American and Peripheral South Highway, Mexico by Alvarez-Solis *et al*. (2016) reported that Bokashi significantly increased the number of leaves and plant height in onion (37 and 62%) and jalapeno pepper (133 and 94%) compared with the control plants. In onion, Bokashi increased the polar and equatorial diameters and bulb weight by 28, 69 and 269%, respectively, and its yield increased from 6.4 to 21.0 t ha^{-1}. The land equivalent ratio (LER) was 1.34 to 1.55, which indicated that intercropping was advantageous on monoculture, regardless of the fertilization type.

A field study conducted at Entomology Division, Bangladesh Rice Research Institute (BRRI), Gazipur, Bangladesh on mustard with onion, garlic, radhuni and coriander intercropping systems by Afrin *et al*. (2017) observed that the highest number of pods/plant was recorded in mustard with onion intercropping system which was statistically similar in mustard with coriander and lowest number of pods/plant was recorded in mustard with gram. They observed that mustard with onion caused a significant increase in number of seeds/plant compared to other treatments. A study carried out by Sharmili and Manoharan (2018) on little millet based intercropping system with five intercrops *viz.*, radish, coriander, small onion, black gram and green gram in 8:2 ratio at Department of Agronomy, Agricultural College and Research Institute, Tamil Nadu Agricultural University, Coimbatore, revealed that improvement in plant height, DMP, productive tillers $plant^{-1}$, grains $panicle^{-1}$, thousand grain weight and little millet grain equivalent yield (LMGEY) were recorded in intercropping over sole little millet. Among the intercropping system, LMGEY (1,832 kg ha^{-1}), Net return (Rs 35,612 ha^{-1}) was found to be higher in little millet intercropped with small onion followed by little millet + green gram. Intercropping competition index (CI) was also higher in little millet + small onion (1.00).

Performance of vegetable cowpea-a short duration vegetable as an intercrop with other vegetables

The results of a study by John and Mini (2005) at Department of Olericulture, College of Horticulture, Kerala Agricultural University, Thrissur, Kerala on okra + cowpea intercropping system during the kharif and rabi seasons of 2000 showed that biomass production in the okra + cowpea intercropping system at lower spacing was 26328 and 27000 kg ha^{-1} during the kharif and rabi season respectively. As regards to yield levels, among the different treatments, okra in combination with cowpea at lower spacing gave the highest okra yield equivalent (7907 and 8709 kg ha^{-1} in the kharif and rabi season respectively). The highest net returns were also obtained for the okra + cowpea combination at 60x45 cm spacing (Rs. 33,456 and Rs. 43,329 ha^{-1} during kharif and rabi seasons respectively). A research work conducted at farmer's field Keezakundalapadi, Cuddalore District, Tamil Nadu on intercropping of leguminous vegetables in a pruned field of jasmine (*Jasminum sambac* L.) by Lakshminarayanan *et al*. (2005) indicated that intercropping of pruned jasmine with double rows of vegetable cowpea fetched highest land equivalent ratio (1.99), net returns (Rs. 1,44,113 ha^{-1}) and income equivalent ratio (IER) of (1.8). The net return per rupee invested was maximum in jasmine + vegetable cowpea at double row sowing system (1:3). As well as, they also suggested that intercropping of pruned jasmine with double rows of vegetable cowpea fetched the highest equivalent yield of jasmine (5393 kg/ha) as compared to sole jasmine (3049 kg/ha).

The results of a study by Nataraj *et al*. (2007) at Agriculture College, V.C. Farm, Mandya, Karnataka on baby corn intercropped with leguminous vegetables showed that vegetable cowpea intercropped with baby corn in 1:1 and 2:2 row proportions yielded 14.63 t ha^{-1}. In an experiment conducted at Bidhan Chandra Krishi Viswavidyalaya, West Bengal by Chattopadhyay *et al*. (2008) in elephant foot yam intercropped with vegetable cowpea, okra, cucumber and amaranth showed that the growth parameters viz., height of pseudostem, pseudostem girth and canopy spread were superior when elephant foot yam was grown as a pure crop followed by vegetable cowpea grown as an intercrop. When cowpea was grown as an intercrop sprouting of elephant foot yam was enhanced by 10 days. The mean corm yield of elephant foot yam was found to be maximum (61.27 t ha^{-1}) when grown as a sole crop followed by cowpea intercropping (57.28 t ha^{-1}). Among the intercrops, the highest elephant foot yam yield equivalent (9.80) was obtained from cowpea. The maximum net return (Rs. 2, 15, 400/-) and the income per rupee investment (1.80) were obtained from elephant foot yam + cowpea

intercropping closely followed by sole crop of elephant foot yam (Rs. 1, 96, 350/- and 1.78, respectively). The results clearly indicated that vegetable cowpea can be profitably intercropped with elephant foot yam.

At Sri Lanka a research work on capsicum intercropped with vegetable cowpea at Agronomy farm, Eastern University by Brintha and Seran (2009) revealed that the LER was higher than unity in all treatments. The treatment of sole capsicum (40 cm × 40 cm) gave high return per rupee invested (9.43) followed by 30/60 cm paired row planting of capsicum and vegetable cowpea (6.83). Monetary equivalent ratio, the economic superiority of cropping system was observed in capsicum (60 cm × 40 cm) and vegetable cowpea and 30/60 cm paired row planting of capsicum and one row of vegetable cowpea performed well (1.09). The Net return (Rs 47, 818/ ha) was high in 30/60 cm paired row planting of capsicum and vegetable cowpea. Research work conducted at Agronomy farm, Eastern University of Sri Lanka by Seran and Jeyakumaran (2009) on capsicum (*Capsicum annum* L.) variety, CA 8 intercropped with vegetable cowpea (*Vigna unguiculata* L.) variety, bushido revealed that the economic cost-profit was high in intercropping system than the mono cropping. In 30/60 cm paired row planting of capsicum (30 cm space between rows in the pair and 60 cm interspaces between two pair of capsicum); one row vegetable cowpea (two seedlings per hill) between two pairs of capsicum (paired row planting system) recorded high profit (Rs 345.377/per plot) and yield of (17.92 kg per plot) among other treatments. The additional yield of vegetable cowpea also achieved in intercropping system. Further, the LER of all intercropped systems was greater than one.

A field experiment conducted at Department of Horticulture, Faculty of Agriculture, Annamalai University, Annamalai Nagar in jasmine intercropped with vegetable cowpea by Anburani and Priyadharshini (2011) showed that the vegetable cowpea intercropped at a spacing of 45 × 15 cm recorded the highest number of leaves 293.59, leaf area of 73.25 m^2, the maximum number of productive shoots (235.98) and plant spread (2.55 m^2). A study was carried out by Krishna *et al*. (2011) on the production potential of vegetable cowpea intercropped in jatropha based cropping system under dryland conditions. Among the three different tree spacings of jatropha, growth and yield of cowpea was higher when grown in the interspace of jatropha trees at 4.0 m x 3.0 m spacing. Research work conducted at the experimental farm area of National Research Centre for Litchi, Mushahari, Muzaffarpur by Kumar *et al*. (2012) on intercropping of vegetables with pre-bearing litchi plants revealed that the maximum cumulative net profit was Rs. 349672 in litchi + (Okra - Gladiolus) followed by Litchi + Cowpea – Potato – Onion (Rs. 2,37,193 per ha).

A field study conducted at research farm of Michael Okpara University of Agriculture, Umudike on vegetable cowpea (*Vigna unguiculata* (L.) Walp) and Maize (*Zea mays* L.) intercropping system by Muoneke *et al*. (2012) suggested that the highest number of fresh pods per plant in cowpea (25.9 and 27.5 in 2006 and 2007 cropping seasons, respectively) and number of grains per cob in maize (415.5 in 2006) were produced with the lowest component density combination (10,000 maize + 10,000 vegetable cowpea per hectare). The component density combination, 30,000 vegetable cowpea + 10,000 maize plants per hectare however produced the highest fresh pod yield per hectare (4.9 t ha^{-1}) from the cowpea in 2007. In all the combinations, the land equivalent ratio (LER) was greater than unity, with 20,000 vegetable cowpea + 10,000 maize combination enhancing 61% yield and gave the highest LER 1.61 advantage of the intercropping system. The reports given by Choudhuri and Jana (2016) at Department of Vegetable Crops, Faculty of Horticulture, BCKV, Mohanpur, Nadia, and West Bengal revealed that the maximum okra equivalent yield ha^{-1} (15.67 t) was observed in okra + cowpea intercropping system. This was found to be the best user of biological resources as it recorded the maximum values for LER (1.56). Economic analysis also showed that okra + cowpea intercropping system was most remunerative as it recorded the highest net return and B: C ratio (1, 35,891.50 and 2.61, respectively).

A research work conducted at ICAR-Indian Agricultural Research Institute, New Delhi, India on four combinations of two cotton establishment methods and two planting geometries (PGMs) by Rajpoot *et al*. (2016) found that transplanted cotton with 90 × 60 cm planting geometry in Bt-cotton + vegetable cowpea intercropping system exhibited maximum seed-cotton equivalent yield as well as gross and net returns and other economic indices, followed by Cotton + Okra and sole cotton. In vegetable cowpea, the highest number of pods per plant of 16.7, pod length of 18.5 cm, single pod weight of 7.3 g, pod yield of 1523 kg/ha was recorded under 90 × 60 cm planting geometry. The same geometry also observed the maximum gross return of Rs. 1,38,448, net return of Rs. 94,674 and IER of 1.45.

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Chapter - 7
Scientific Cultivation of Tuberose
(*Polianthes tuberosa*)

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Chapter - 7

Scientific Cultivation of Tuberose (*Polianthes tuberosa*)

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Abstract

Flowers are connected with human's history from Neolithic age and the part in human life from birth to death. Flowers are traditionally used by humans for expressing or exhibiting their innermost feelings to God and deities or presenting it to beloved ones or complementing anyone or convey any conceivable emotion. Thus, importance of flowers is increased in socio-cultural and religious life of the humans that hardly exaggerated. Normally flowers are used as loose flower, cut flower and dry flower in humans day to day life. Marigold, jasmine, rose, crossandra and china aster etc are popular as loose flower, whereas Rose, tuberose, gerbera, dahlia and orchids are post popular as cut flower. Among these cut flower tuberose is most commonly used as oil, perfume and cosmetic industry because it contains essential oil, because of fragrance it gains popularity among those cut flowers. Tuberose is a popular and very important fragrant ornamental bulbous flowering crop. The long flower spikes are excellent as cut flower which is useful in vase decoration and bouquets. The spikes are generally lasts for 7-12 days in vase depending upon the room temperature. Individual florets are used for making *veni*, garland, button-holes or crowns; the last-named ornament is used during marriage or other religious ceremonies.

Keywords: Tuberose, cut flower, human, ornamental, popular etc.

Introduction

Tuberose is a half-hardy, perennial bulbous plant. It belong to family Amaryllidaceae and basic chromosome number (n=30). The word tuberose (*Polianthes tuberosa* L.) is derived from the Greek word '*Polios',* which means white or shinning and *'anthos'* meaning flower. Tuberose is one of the important cut flower among the top ten cut flowers. In India, tuberose is commonly known as *Rajanigandha* (Hindi), *Rajanigandha* (Bengali) *Gul-e-shabu* (Urdu), *Nishigandha* (Marathi) and *Sugandharaja* in Kannada, Nila Sampangi in Tamil Sugandaraja and Nelasanpengi in Telegu and

Sugandharaja in Kannada. Tuberose is suitable for both under protected cultivation or open field condition (Brundell and Steenstra 1985). The tuberose occupies a very selective and special position among the ornamental bulbous plants for its beauty, elegance and sweet pleasant fragrance (Sadhu and Bose, 1973). Tuberose can be successfully grown pot plant and bedding plants for it several uses. It commercially used for garland making, Aesthetic purpose, birthday ceremony, floral arrangement such as; rangoli, bouquets, boutonnieres, Potpourri. Tuberose also uses for table purpose because it has long spike length, long post-harvest life and extremely fragrance due to present of Geraniol, Nerol, Benzyl Alcohol, Eugenol and Methyl anthranilate. The flowers emit very sweet fragrance that ability to open the heart refers your mind and clam effect the nerves. Tuberose flowers extremely used in perfume and essential oil industry (Qureshi *et al*., 2018). Now a days tuberose also use for beverage industry food industry. Dried tuberose bulbs in powdered form are used for gonorrhoea treatment.

Area and Production

India is having tradition of growing flowers since time immemorial. Owing to steady increase in demand of flowers, floriculture has become one of the important commercial trades in agriculture. Tuberose occupies a prime position among commercial ornamental bulbous crops, because of its highly fragrant waxy flowers which can be used in various ways (Ranchana *et al.*, 2013a). All over the world, the floricultural sector is experiencing rapid changes. Besides traditional centers of production *viz.*, The Netherlands, Columbia, Israel and Kenya, new production centers are developing in Latin America, Africa and the production has increased many times, as compared to what it was a few decades ago. The Asian countries like India, China, Korea, Vietnam, *etc*. are moving in the direction of more intensive floriculture. Area under flower growing in India is about 324 thousand ha with 1962 thousand MT production of loose flowers and 823 thousand MT productions of cut flowers. In India, tuberose commercial cultivated to West Bengal, Karnataka, Tamil Nadu, Maharashtra, Uttar Pradesh and North Eastern part of India. At present scenery tuberose is cultivation in India 7. 95 lakh hectare and cut flowers is estimated to be 27.71 '000 MT and 1560.70 lakh No's respectively. (www.nhb.com).

Morphology/botany of tuberose

Tuberose inflorescences (spikes) bear 10 to 20 pairs of florets which open from the base upward. Commercially, spikes 2 to 3 feet long are harvested when the basal florets are open. Tuberose flowers basically funnel shaped and

perianth part highly fragrant and waxy white flowers, about (20-25) mm long, single or double and borne in a spike. Number of stamens are six, anthers dorsifixed in the middle, ovary 3 locular, ovules numerous and fruits type are capsule. The leaves are long, narrow, linear, grass-like, light green, and arise in rosette. It is propagated by bulb (25-30gm weight and 2-3cm diameter) and bulblets (1-1.5cm diameter). Bulbs are made up of small scales and leaf bases and the stem is a condensed structure which remains concealed within scales. Adventitious and shallows root mainly found in tuberose.

- The haploid chromosome number of tuberose is 30, among these 5 are large and 25 are small.
- The somatic chromosome number is 2n=2x=50.
- Single cultivars are fertile used in perfumery and seed setting erratic with 2n=2x=60.
- Double cultivars are fertile and used as cut flower. Seed setting is not observed in double cultivars.
- Non-availability of genetic variability has become a major constraint in conventional breeding of tuberose.

The first cross involving tuberose was reported in 1911 as *Polianthes* x *blissii*, a cross between *Polianthes geminiflora* and *P. tuberosa*.

Tuberose (*Polianthes tuberosa* L.) has the characters of dichogamy and self-incompatibility.

Crosses between single and double varieties produce fruits and seeds after 2–3 days after anthesis in the female parent.

Species and Cultivars

A. Species

Tuberose has around fifteen species under the genus of Polianthes but, twelve species are distributed from Mexico. Among of these flowers nine species have white, one is white tinged with red and two are red colour. Only *Polianthes tuberosa* L. are growing commercially and all the others are found growing wild. Different types of species of tuberose mention below

1) ***Polianthes tuberosa*:** It is commercially cultivated species and this is an erect herb, 70-130 cm high with stout and short bulbs; leaves basal, 8 to 10 in number, 30-50 cm long, about 1.5 cm wide, linear, bright green, reddish near the base, flowers star shaped, waxy white, the tube bent only near the base, filaments attached on upper part of corolla, fragrant in long terminal racemes.

2) ***Polianthes durangensis:*** This species bulb are small and the flowers arranged in one to six pairs are all sessile, erect at first becoming curved and then become purplish.

3) ***Polianthes montana:*** This species having oblong bulbs, the stem long and tender, and possessing 12 pairs of flowers all with pedicels. The flowers are short with lobes erect and rounded.

4) ***Polianthes graminifolia*****:** This species having deep red colour. This is closely resembles to *P. geminiflora*. The bulb shape is long and tuberous. Slendery stem, densely hairy below and glabrous above. The flowers are in 10 to 15 pairs. The lower ones often on peduncles, deep red, bent downward near the base and the filaments are slender.

5) ***Polianthes Gemini flora*****:** This species was originally described as Bravoa geminiflora. Stems are smooth throughout with several basal leaves. *P. geminiflora* also having saponaceous roots which is used as soap substitutes. Flowers colour is light orange-red and arranged in pairs of 7 or more.

6) ***Polianthes polustris*****:** Bulbs of this species are oval to oblong, erect stem, length 40 cm, flowers are 3 to 5 pair arising from single bracts, perianth slightly curved with segments short and spreading filaments short near the top of the tube, ovary free at tip.

7) ***Polianthes longiflora*****:** This species is tall and flowers in three to five pairs. Perianth is white tinged with purple colour.

8) ***Polianthes platyphylla*****:** In this species the flowers are white tinged with redish colour. The florets are arranged in fifth to seven pairs with tube of the floret which is bent at the base.

9) ***Polianthes pringlei*****:** Plants of this species have small bulbs and smooth stems. Flowers are sessile, in pairs of 4 or 5, curved, fragrant and white in colour which changes to slightly purplish on drying.

B. Cultivars

Tuberose has three types of cultivar, such as

i) **Single type:** This type of tuberose having single row of petals, flower white in colour and highly fragrance. Concrete yield 0.08-0.11 percent. Flowers basically use for loose flower such as garland making, flower arrangement and rangoli purpose. Examples-

- **Arka nirantara:** It variety is released by Indian Institute of Horticultural Research (IIHR), Bangalore. It having white colour with prolonged blooming.

- **Shringar:** This is hybrid variety of tuberose, cross between 'Single x Double' and was released by Indian Institute of Horticultural Research (IIHR), Bangalore. Flower is highly fragrance. Flower bud attractive due to pinkish tinge. More number of flowers per spike and florets are large in size.
- **Prajwal:** This hybrid also develops through hybridization, cross between 'Shringar' x 'Mexican Single'. The hybrid was released by Indian Institute of Horticultural Research (IIHR), Bangalore. Flowers are white and flower buds are slightly pinkish. This variety is most commonly used among single type of tuberose.
- **Rajat Rekha:** This variety is develop through induce mutation (gamma ray). This variety is released by National Botanical Research Institute (NBRI), Lucknow. Flowers are silvery white streaks along the middle of the leaf blade. Concrete content 0.089 per cent.

Mexican Single, Hyderabad Single, Calcutta Single, Phule Rajani, Kahikuchi Single, Pune Single etc some other single varieties.

ii) Double type: In this type of tuberose found more than three rows of petals. Flower are less fragrance than single type and concrete yield is 0.06-0.08 percent.

- **Suvasini:** It is multi whorled variety and release from Indian Institute of Horticulture Research (IIHR), Bangalore. It is develop by hybridization, cross between 'Single' and 'Double'. These varieties produce more number of flowers per spike, bold flowers and uniformity. Flower are white colour with fragrance. This variety is mainly used for cut flower purpose. Spike yield is higher 25% than Pearl Double cultivar.
- **Pearl double:** It variety have reddish tinged flower. In this variety flower yield is very high with quality flower production and used for flower arrangement and also used for oil extraction purpose.
- **Swarna Rekha:** It is developed through induce mutation (gamma ray) and release from National Botanical Research Institute (NBRI), Lucknow. The flowers are double with golden yellow steaks along the margins of leaf.

iii) Semi-double: This type of cultivar found two to three rows of petals. Flower spikes are straight and Flower colour is white with tinged pinkish red.

- **Vaibhav:** This is important variety suitable for cut flower purpose.

Climate requirement and Soil

1) **Climate:** Tuberose prefers to grow in an open sunny condition. In India, the commercial cultivation of tuberose is mainly confined in warm humid areas with average temperature range from 20 ^{o}C to 35 ^{o}C. For its luxuriant growth, it requires high humidity and a temperature 30 ^{o}C but above 40 ^{o}C reduces the spike length and quality of the flowers. Very low temperature and frost also affect growth and flowering of the plant.
2) **Soil:** Ideal soil tuberose cultivation is sandy-loam or loam soil. Soil should be well aeration and good drainage with pH (6.5-7.5) for quality flowers production (Sharga and Sharma, 1994).

Cultural practices

- **Site selection:** Tuberose is a sun-loving plant, so the site should be chosen, where the plants will get enough sunlight throughout their growing period.
- **Land preparation:** The field should be worked deep to a good plough and properly manure. Well rotten cow dung or farmyard manure @ (50-60) tonnes/ha, should be incorporated with the soil about a month before planting. Care should be taken to make the land free from disease, pest and weeds.
- **Bulb treatment:** At first Bulbs totally cleaned after that treated with Bavistin (0.2%) for 30 minutes to reduce fungal infection. Dry in shade before planting or storing. Before planting bulbs treated with GA_3, etherel or thiourea to break dormancy, promotes early appearance of flower spike and produces longer spikes with maximum number of florets. Before planting treat bulbs in systemic fungicide and before storing in contact fungicide.

Propagation

Tuberose is commercially propagated by vegetative methods like-Bulb, Bulblets and division of bulb but, rarely tuberose propagated by seed, in case of single tuberose varieties because seed propagated is very difficult. Now a day's tuberose also propagated by tissue culture to get virus-free planting material production.

A) Seed propagation

Seed setting of tuberose is observed under suitable climatic condition but, only single type cultivar. Seeds are sown in well prepared growing medium

under portrays nursery. Ideal soil temperature of 25 °C is fully effective for increase seed germination. Before transplanting bed should be prepared by digging and sufficient quantity of FYM is to be mixed before sowing. The seedling is sown in rows 10-12 cm apart and 5 cm deep in heavy soil and 2.0 cm in light soil.

B) Vegetative propagation

1) **Propagation by bulb:** Propagation by bulbs for the commercial multiplication of tuberoses. Ideal size bulbs is very important for quality production. In general, spindle shaped bulbs diameter between 2.0 cm to 3.0 cm are suitable for planting. About 1.30 - 1.60 lakh bulbs (10-12 tons of bulbs) are required for planting of tuberose one hectare.
2) **Propagation by division:** Tuberose can also be propagated by division of bulb. Bulbs normally cut into 2-3 vertical sections, each segment must be contain bud and a part of the basal plate and treated with fungicide and planted vertically in a rooting medium with their tips just showing above the surface.

C) Micro-propagation

It is hi-tech method for quality planting material production. The main purpose is nematode free planting material production.

Spacing, depth and time of sowing time

Planting density is highly effected for yield and quality flowers production. Bulbs are planted at an optimum spacing of 30 x 20 cm or 20 x 20 cm (Yadav *et al.*, 1984) or 30 x 30 cm (Nagaraja *et al.*, 1999) with 5.0 to 7.0 cm depth. Depth also depend upon size of bulbs. Generally depth should kept 2.5 time more than bulb. Tuberose is generally planted in January-March in the plains and in April-May in the hills. Planting of bulbs should be done in the month of April for getting the highest yield of spikes and flowers in the single tuberose cultivars.

Nutrient management

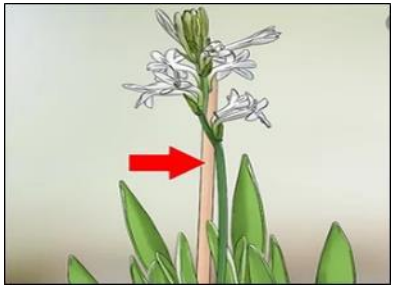
Organic manure has vital role for quality flowers production. Ideal fertilizer dose are FYM (20 tonnes/ha), a fertilizer dose of 120 kg N, 60 kg P_2O_5 and 80 kg K_2O per hectare is recommended for tuberose production. The dose should be apply half the N, the full dose of P and K has to be applied at the time of planting and the remaining half of N is apply two split dose such as 30 and 60 days after sowing.

Water management

Optimum amount irrigation should be apply before sowing so that better sprouting. The tuberose fields should be irrigated at interval of 10-15 days, if the weather is dry. It has been suggested that during summer months (April to June) the crop should be irrigated at weekly interval and during winter at 10 days interval.

Earthling up and Staking

When plant height is 20-25 cm earthling up should be done up to 10-15 cm high. Staking is done by bamboo stick or iron angles in beds and string or rope may be tied in three rows along the plant to avoid lodging of tuberose spikes.



Weed control

Yield losses through weeds more than (30-40) % in tuberose cultivation. Manual weeding is effective if done frequently. Generally, weeding should be done after each irrigation. It has been found that pre-emergence application of Gramoxone at 3 lit/ha or Diaron at 3kg/ha and ATP+CIPC mixture 2.0+1.5 Kg/ha, effectively controlled near about 70-80% weeds in tuberose field.

Effect of growth regulators

Growth regulators play vital role for acceleration of vegetative and reproduction in plants. Foliar application of GA_3 at 50 to 100 ppm thrice at 40, 55 and 60 days after planting is found to be beneficial for all flower and yield parameters. Application of both together CCC at 5000 ppm and GA_3 at 1000 ppm induces early flowering, increased flower stalk, number of flower florets production and improves the quality of flowers.

Disease and Pest controls

A) Disease

1. Stem rot or basal rot

The Fungi *Sclerotium rolfsii* cause stem rot. The most common symptom is a brown to black rot of the stem near the soil line. A course white cottony fungal growth, containing white spherical resting bodies covers the affected area. Drenching the soil with 0.3% Zineb is effective in controlling the disease.

2. Alternaria leaf spot

This Leaf spot disease is caused by fungus *Alternaria polyantha*. The disease is characterized by the appearance of brown spots with faint concentric rings on the mid-rib. The disease can be controlled by the spray of Bordeaux mixture (0.4%), Zineb (0.5%) or Mancozeb (0.2%) or Iprodione (0.2%) at 10 days interval.

Application of fungicide such as brassicol at 1% or Zineb (20%) at the rate of 25 kg/Ha.

3. Leaf blight

This fungal disease is caused by *Botrytis elliptica*. The disease appears during the rainy season. Infected flowers show dark brown spots and ultimately the entire inflorescence dries up. The infection also occurs on the leaves and stalks. Spraying the plants with Carbendazim @ 2g/litre of water, ammoniacal copper at 2gal/l00 gal of water or Greeno (0.5%) for 15 days intervals.

4. Flower bud rot

It is a bacterial disease and caused by *Erwinia* sp. It results in dry rotting of the buds with brown necrotic discoloration of peduncles. The diseased plants should be uprooted and destroyed. The disease can be controlled by the spray of Streptomycin (0.01%).

B) Pest

1. Aphids

These are tiny insects, soft bodied, green, deep purple or black in colour usually found in cluster feed on flower buds and young leaves. Aphids can be controlled by a 0.1% spray (1 ml/litre) of Malathion or Rogor at an interval of 15 days.

2. Thrips

Thrips attacks on leaves, flower stalk and flowers. These suck the sap flowers get deformed and damage the whole plant. Thrips are controlled by the spray of Rogor or Metasystox @ 1.75 to 2.0 ml / L or 0.1% Malathion also spray Nuvacron (0.1%).

3. Grasshoppers

These attacks on young leaves and flower buds. Affected plants with damaged foliage and flowers lose their elegance, especially during rainy season. Scraping of buds exposes egg masses to natural enemies. Netting prevents damage from hoppers to nurseries. Spraying of or Quinalphos @0.05% (0.5 ml/litre) or Malathion 0.1% or Carbaryl @0.2% protects foliage of newly planted crop.

4. Weevils (*Myllocerus* sp.)

The weevils are nocturnal in habit and they cause damage on shoots and leaves. Usually, they feed the edge of the leaves, producing a characteristic notched effect. Larvae feed on roots and tunnel into the bulbs. Applying BHC dust (10%) in the soil before planting controls larvae. The weevils can be controlled by the spray of Thiodon @ 2.0 mg/litre.

5. Red spider mites

Mites thrive well under hot and dry conditions, usually on the undersides of the leaves, where these make webs. Mites suck sap, which results in the formation of yellow stripes and streaks on the foliage, leaves become yellow, silvery or bronze and distorted. Spraying with Kelthane @1.2% concentration is effective to control the mites.

6. Bud borer (*Helicoverpa armigera*)

This pest mainly affects flowers eggs are deposited singly on growing spikes. Larvae bore on buds and flowers and affect the plats by making holes. Collection and destruction of damaged buds reduces the damage. Setting up of light traps helps to control population by attracting them. Spraying of monocrotophos (0.2%) or Thiodan (0.5-0.8%) or Methyl Parathion (0.05%) taken up at appearance of eggs on buds and tender foliage controls borer damage.

7. Nematodes

Root-knot nematodes (*Meloidogyne incognita* and *Meloidogyne javanica*), reniform nematode (*Rotylenchulus renioformis*) and greasy streak nematode (*Aphelenchoides besseyi*) causes damage to the crop, which leads by the stunted growth of the plants and extensive yield losses. The size of leaves is reduced and the flowers look sickly and, ultimately, the roots rot. Application of Thimet or Furadan (20 kg/ha), Furadon @ 2 g/plant or carbofuran @ 2-5 kg/ha, neem @ 1 tonne/ha controls nematode infestation.

8. Rodents

Rodents also affect tuberose plants in standing crop by making burrows/holes. Poison bait which is available in the market name, 'Roban' is helpful in checking rodent menace in the field.

Harvesting

Flower production of tuberose starts 3 to 4 months after planting, flowering time is July but, August-September is the peak period of flowering.

Tuberose should be harvest when lower pair of flowers is fully open as a local market and as distant market lower flower buds slightly open (Prasad. K. *et al.,* 2016). But ideal harvesting time at early morning or evening with the help of sharp knife. Tuberose bulbs are stored at proper stage of maturity is important for storage of bulbs and quality flower production. The bulbs reach maturity when and plant growth ceases, old leaves become dry and the flowering is over.

Ratoon cropping

About 3-4 ratoon crops can be taken from a single planting. After harvesting the main crop, the flower stalks are headed back (cut to the base) and the plots should be well- manured and irrigated.

For the proper growth and development of plants, fertilizer application to the main crop should be given in two equal split doses in January-February

and April. Compared to main crop there is early flowering in ratoon crop as. In ratoon crop production of more number of spikes but less number of florets, length of spikes and weight of flowers. Ratoon crop should be used only for loose flowers or oil extraction purpose.

In temperate climate, during November -December, when temperature drops. Digging of bulbs should be done when leaves of the plants turn yellow and die and plants undergo dormancy.

Yield

Yield of tuberose is depends on different time of planting, varieties, bulb size and plant population. Generally plant start flowering about 3 to 4 months after planting.

Loose flower production 14-15 t/ha and from cut flower 5-6 lakhs/ha spikes. The yield of concrete ranges about 8 to 10 kg/ha. In addition, 25-30 tonnes/ha of bulbs and bulb may be harvested at the end of 3rd year.

Post-harvest handling and packaging

After harvesting handling of tuberose flower spikes is very important because it is uses for various purposes like loose flower, cut flower and oil extraction purpose etc. In case of loose flower post-harvest handling loose flowers are packed in bamboo baskets containing 10-15 kg flowers for selling in the local market where flower are sold by weight. Care should be taken for post-harvest handling of cut flower for this grading should be done and it depends upon length of the spike, number of floret, weight of spikes, injury of the spike after grading and bunched in round bundles. Each bundle containing about 100 spikes.

The stem of the spike in bundles is wrapped in wet newsprint sheets for reducing damage of the spike, flowers and buds, then the whole bundle should be wrapped in white tissue paper or polythene. Before packing of spikes pulsing treatment should be given that solution containing sucrose 2% + 8 HQC (200 ppm) + $AgNO_3$ (50 ppm) for increase self-life (Sudagar *et al*., 2010) or solution containing 200 ppm silver nitrate ($AgNO_3$) and 4mm silver thiosulfate STS (Bakash *et al*., 1999) after that spike bundles packed in the card-board boxes and transferred by train or air to reach the destination quickly.

Storage of bulbs

Harvesting stage of tuberose bulb is important for storage of bulb and their growth. Bulbs are harvested when the flowering is over and plant ceases to grow. Bulb reach maturity at about 40-50 days after flowering. After

digging of clump adhered soil is removed and the off shoot is separated by rubbing off. Scales and roots should be removed and after that bulbs are graded into different sizes. Bulbs are kept in a cool, dry and shady place. After storage the bulbs should be stirred within 3-5 days to prevent the spread of storage diseases mainly mould and rot. The bulbs are ready for planting after 5-6 weeks of storage.

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Chapter - 8
Advances in Sampling Techniques of Fruit Crops in Relation to Nutrients

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Chapter - 8

Advances in Sampling Techniques of Fruit Crops in Relation to Nutrients

Pratibha, Ramdhan Chopdar, Alka and Jitendra Gurjar

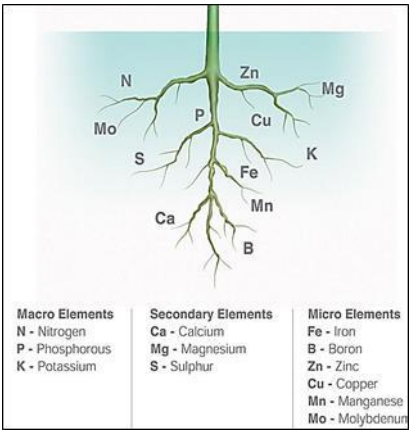
Abstract

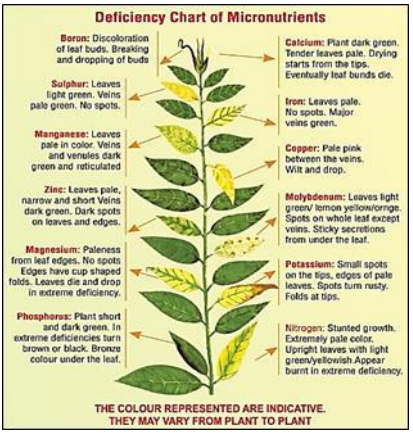
Plant species, age, plant part and the time of sampling can affect the values of mineral nutrients present in a given sample. Besides this, the option used for the digestion of plant sample is also very important. So, for taking up the analysis of mineral nutrients in plant samples and getting the accurate and realistic results, proper and careful sampling and selection of digestion method are some of the very important considerations. Most of the essential elements are not equally distributed in the plant or within the plant parts. Although, it is possible to assay any plant part or even the whole plant but the biological significance of such an analysis always depends on the availability of interpretative data from the plant part(s) collected and used for the analysis. While conducting a plant analysis, primary objective should be to obtain the plant part for which assay results in actual comparison of plant samples coming from different types or stress treatments. Prior to elemental analysis, possible errors can occur at various stages such as; sample collection, initial handling, transportation, decontamination, drying, particle size reduction etc. Thus, possible sources of error must be known to the investigator for the accuracy of the whole procedure and the analysis. The sampling procedures and precautions required at various stages of sample preparation for the analysis of mineral nutrients are described here in this article. The sampling result was affected by canopy height, leaf age and time of sampling. Therefore, the three factors were studied. The results illustrated that the stability in level of nutrient concentrations was in 3-6 month-old leaves from the central part of the canopy. The most stable period was from April to May for leaf sampling. It was recommended that the stable intracanopy and stable period of nutrient concentrations could be used as the standards of leaf sampling technique. Based on the leaf sampling technique, the standard of leaf nutrient concentrations was summarized, and could be used as the standard of nutrient suitability evaluation.

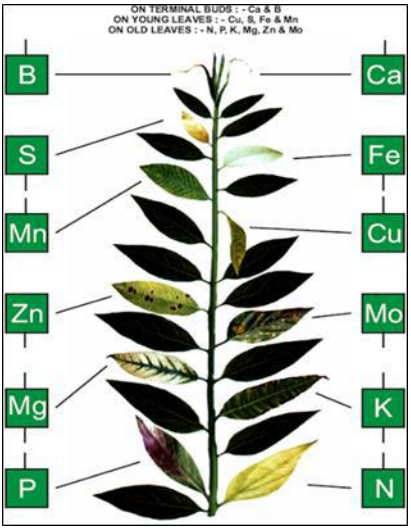
Keywords: Fruit crops, sample preparation, recent sampling techniques, macronutrient

Introduction

- Plants require adequate amount of nutrients to fulfil their functions in the plant metabolism throughout their life cycle.
- Thus it becomes very necessary to know whether the plant is well nourished or not because the adequate nutritional state is a determining factor on the production of any crop.
- The search for an effective method to determine plant nutritional status has been the target of many researches in plant nutrition.







- Current methods include both soil and tissues analysis.
- The soil analysis method is based on the assumption that the chemical extractants simulate the root system acquisition of soil nutrients in a comparable manner.
- However, it does not take into account factors such as soil temperature and aeration, and even the higher or lower absorption due to the own plant nutritional needs.

Steps of soil sampling in orchard

1. Soil assessment before planting

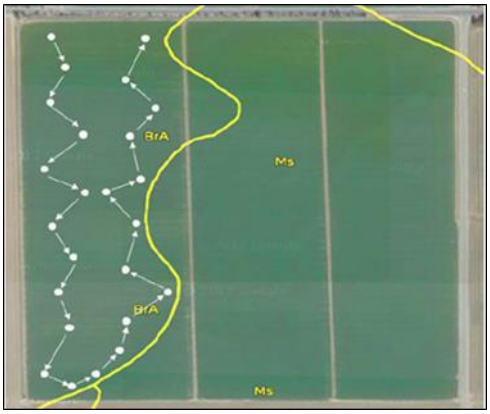
Soil sampling before planting serves three major purposes

- Determine soil properties such as pH, texture, nutrient availability, or salinity.
- Identify unsuitable areas due to physical barriers to root growth or drainage.
- Assess the variability in soil properties within the field to develop nutrient management plans.

Sampling procedure

- Divide each field into blocks based on soil survey data, slope, or cropping history. Soil survey data provide an overview of soil properties.
- Even when a field appears to be uniform, it is worth dividing it into several blocks which are sampled and analyzed separately.
- Ideally, a field is divided into blocks of 2-5 acres and a composite sample of five cores from each block is taken. When larger blocks are samples, 15 to 30 cores should be taken from each block for a composite sample.
- When soil survey data suggest possible physical limitations, a series of backhoe pits should be dug to identify textural changes and layers that restrict root growth and drainage. This information helps to determine the best method of soil modification and whether the site is suitable at all for orchards. As an alternative to backhoe pits, undisturbed soil cores may be taken with a soil probe.
- Soil sample cores are taken from the entire field or management area in a W-shaped sampling pattern or by walking a zigzag course around or through the area as shown in Figure 1 for the Brentwood soil.

- Mix the cores thoroughly; remove large stones, pieces or roots and other foreign material.
- Sample by foot increments to a depth of 2 to 4 feet or deeper if restrictive layers may be encountered in the subsoil.



- Map the field based on soil survey data and the results from the soil analyses of the different blocks. This map will help to determine the blocks for soil and tissue samples in the following years.

2. Sampling in established orchard

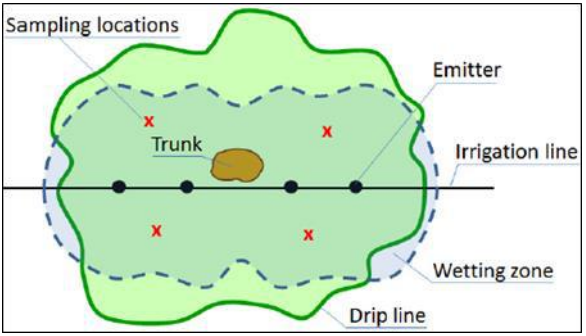
2.1 Time of sampling

- Taking soil samples every 3-5 years is usually adequate. In recently planted orchards, annual sampling may be done until the soil fertility program is established.
- To monitor available nutrients over the years, samples should always be taken during the same season, but preferably in fall.
- Samples need to be taken before fertilizer is applied.

2.2 Sampling procedure

- Divide each field into blocks based on soil survey data, slope, cropping history, variety, rootstock, age, growth pattern, or irrigation system
- Plant residue from the sample spot is removed. Samples are best taken with a soil probe or auger.
- The sample is taken halfway between the trunk and the drip line and within the wetting zone of the sprinkler/emitter.

- Cores are taken from the entire area of the field or management area in a W-shaped sampling pattern or by walking a zigzag course around or through the area as shown in Figure 1 for the Brentwood soil.



- Mix the cores thoroughly; remove large stones, pieces or roots and other foreign material.
- Sample by foot increments to a depth of 2 feet. When diagnosing a problem, deeper cores may be recommended.
- To obtain an accurate estimate of the nutrient availability, between 15 and 20 cores should be taken from each block for a composite sample.
- One sample per tree is generally taken. Within each block, make sure to sample different orientations relative to the trunk. Collect the samples in a clean plastic bucket.

Sample handling

- When all the cores for an area are taken, mix them thoroughly. Very wet samples should be air-dried before packaging. Do not dry the samples in an oven or at abnormally high temperature
- Put about one quart of soil in a clean bag and label it clearly. Follow the instructions of the laboratory that will do the analysis.
- To receive accurate fertilizer recommendations, the sample information sheet needs to be filled out carefully.
- Tissue/Plant analysis is considered a more direct method of plant nutritional status evaluation than soil analysis, but that method must necessarily involve a well-defined plant part analysis (Hallmark & Beverly, 1991).

- Among the several tissues to be considered for nutritional diagnosis purposes, leaves constitute the main plant sampling material (Chapman & Brown, 1950).
- Plant analysis has been considered a very practical approach for diagnosing nutritional disorders and formulating fertilizer recommendations.


Leaf/Tissue analysis

- Leaf is the principal site of plant metabolism; therefore, changes in nutrient supply are reflected in the composition of the leaf/petiole.
- Because of the dynamic nature of the leaf tissue composition, strongly influenced by:
  - Leaf age
  - Leaf maturation stage
  - Interactions involving nutrient absorption and translocation

The leaf analysis can be useful in one of the following ways

- To develop a nutrient guide for recommendation of manures and fertilizers for economic optimum yield.
- To correct defective manure and fertilizer application used by the growers which often lead to soil, water and environmental problems.
- To determine whether or not the supply of one or more nutrients is inadequate, satisfactory or unnecessarily high.
- To show that the lack of response to applied nutrients result from their failure to make into the leaves, thereby preventing a wrong conclusion from being drawn and directing attention to the cause of the lack of absorption.

Steps of leaf analysis

- Collection of sample
- Sample handling
- Decontamination
- Sample drying
- Grinding & storage
- Laboratory analysis

A. Leaf sampling technique

Components for plant sample collection

- Stage of plant growth at which sample is collected
- Number of plants selected per sampling
- Specific time of sampling
- Number of parts taken per plant
- Location on the plant for sample collection
- Plant part to be taken as sample for nutrient analysis
- Plants to be avoided for sample collection
- Damaged mechanically or by insects & diseases.
- Covered with dust or soil,
- Covered with foliar applied spray materials
- Border row plants

- Shaded leaves within the plant canopy.
- Sample containing a part or portion of dead plant tissue.

B. Sample handling

- The general steps to be followed in sample handling are:
- Field sampling should be carried out early in the morning.
- Scientist carrying out sampling should wash hands before sampling or use dispensable gloves.
- The collected material should be immediately shifted to cool containers.
- After transport from the field the samples can be stored in refrigerator at 5 °C till these are decontaminated.
- Samples covered with soil particles may be cleaned using deionized water.
- For micronutrient determination, avoid contact between the plant material and metals.
- Wash the sample at sampling site and partially dried before mailing to the laboratory.

C. Decontamination

- Fresh samples on receipt must be divided into 4 sub-samples for effective washing in minimum time.
- One sub-sample may be pre-washed with deionized water, if it is soiled.
- If sprayed chemical deposits are visible, the deposits can be removed by cotton soaked in 0.2% detergent solution.
- The sample should be passed through four solutions. viz. 0.2% detergent solution, N/10 HCl solution, distilled water and finally in deionized water or double distilled water.
- Samples for boron estimation should be washed in distilled water from stainless steel.

D. Sample drying

Initial drying

- It is designed to de-activate rapidly all plant enzymes thereby minimizing weight loss and biochemical changes, and to remove all water from the tissues so that the sample reaches an oven dry state when its weight remains unchanged with repeated drying.

- The samples may be dried at 65 °C to 70 °C in stainless steel lined hot air ovens which allow adequate circulation of air between samples.

Final drying

- Initially dried samples are sometimes weighed, ground and stored for a period to the analysis when these may absorb atmospheric moisture.
- This necessitates second drying at 70 °C for 12 hours immediately before analysis.

E. Grinding and Storage

- Dried samples are ground to reduce field samples to manageable size and facilitate the preparation of homogeneous sub-samples for chemical analysis.
- During grinding, care must be taken to ensure that it does not segregate into labeled, airtight glass or poly-carbonate containers which can withstand a second drying cycle.
- This should prevent samples from being infected by insects during storage.

F. Laboratory analysis

Standard and reproducible techniques should be used. With the advent of computer linked report generating instruments improvements in the efficiency and speed of quantitative plant analysis have become possible.

Sampling technique in fruit crops

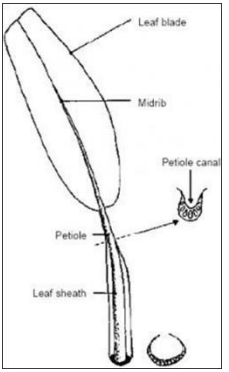
Mango (*Mangifera indica*)

- **Index tissue:** Leaf + Petiole.
- **Stage of sample collection:** 4 to 7 month old leaves (30-40 leaves).

- **Specific location in plant:** The leaves selected should come from end of the branch, the fourth and fifth leaf from the terminal bud. About 12 terminals should be sampled at even spaced points around the tree.


Banana

AAA group (*Musa cavendish* sub-group)

Index tissue: Petiole or Midrib.

Stage of sample collection: Bud differentiation.

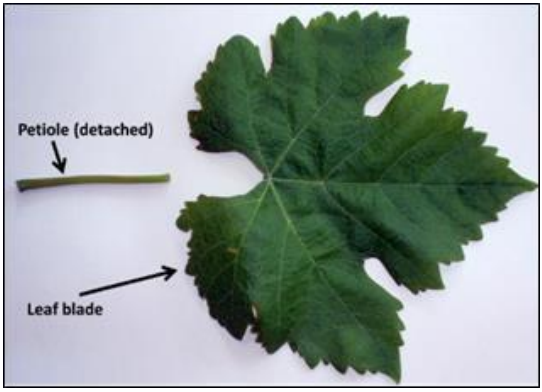
Specific location in plant: Petiole of 3rd open leaf from apex.

Grape (*Vitis vinifera* L)

1. **For yield forecast**

- **Index tissue:** Petiole.
- **Stage of sample collection:** Bud differentiation.
- **Specific location in plant:** 5th petiole from base.

2. **For quality**



- **Index tissue:** Petiole.
- **Stage of sample collection:** Bloom.
- **Specific location in plant:** Petiole opposite to bloom.

3. **Table/Wine/Juice**

- **Sampling procedure:** Collect 100 petioles from most-recent mature leaves next to fruit clusters.
- **Stage of sampling:** Collect petioles in mid to late summer before veraison (when the fruit begins to change colour).

4. **Muscadine grape**

- **Sampling procedure:** Collect 50 to 100 leaves from most-recent mature leaves next to fruit clusters.
- **Stage of sampling:** Collect leaves mid to late summer before final swell of the fruit.

Papaya (*Carica papaya* L)

- **Index tissue:** Petiole.
- **Stage of sample collection:** 6 months after planting.
- **Specific location in plant:** 6th petiole from apex.

Pomegranate (*Punica granatum* Linn)


- **Index tissue:** Leaf bud.
- **Stage of sample collection:** Differentiation stage.
- **Specific location in plant:** 8th leaf from apex.

Custard Apple (*Annona squamosa* L)


- **Index tissue:** Leaf.
- **Stage of sample collection:** 2 months after new growth.
- **Specific location in plant:** 5th leaf from apex.

Acid Lime (*Citrus aurantifolia*)

- **Index tissue:** Leaf.
- **Stage of sample collection:** June.
- **Specific location in plant:** 3 to 5 month old leaf from new flush.

Ber (*Ziziphus mauritiana* Lamk.)


- **Index tissue:** Leaf.
- **Stage of sample collection:** 2 month after pruning.
- **Specific location in plant:** 6th leaf from apex from secondary or tertiary shoots.

Apple (*Malus x domestica*)

Sampling procedure


- Select 60 to 100 leaves per sample from the middle of current-season terminal shoots.
- Select 1 or 2 leaves per shoot from several shoots on each of several trees exposed to light.
- Shoots to be sampled should be 5 to 7 feet above ground level in larger-sized trees and 3 to 6 feet above ground level in smaller-sized trees (young trees, trellised or slender spindle plantings).
- Sample 5 to 10 trees per acre.

Stage of Sampling: Eight to ten weeks after full bloom

Pear (*Pyrus communis*)

Sampling procedure

- Select 60 to 100 leaves from the middle of current-season terminal shoots.
- Select 1 or 2 leaves per shoot from several.
- Shoots on each of several trees exposed to light.
- **Stage of Sampling:** Eight to ten weeks after full bloom.

Peach (*Prunus persica*)

- Sampling Procedure:
- Collect 50 to 100 mature leaves from the mid-portion or near base of current-season terminal growth.
- Sample 5to 10 trees per acre.
- **Stage of Sampling:** Mid-season.

Blackberry (*Rubus fruticosus*)

Sampling procedure

- Collect 50 to 100 mature leaves from primocanes in the section 6 to 10 nodes from the terminal.
- **Stage of sampling:** Mid to late July.

Blueberry (*Vaccinium corymbosum*)

Sampling procedure

- Take 50 to 100 mature leaves from mid-portion of fruiting cane from across the field.
- **Stage of sampling:** Collect leaves during the first two weeks after harvest.

Pecannut (*Carya illinoinensis*)

Sampling procedure

- Sample the middle pair of leaflets from the
- Mid-portion of terminal growth 56 to 84 days after catkin fall.
- **Stage of Sampling:** Early July to early August
- **Leaf nutrient level categories:** For an ideal leaf nutrient guide, 5 range for each element can be used for classification of the nutritional status of fruit trees, namely, deficient, low, optimum, high and excessive/toxic based on yield and fruit quality responds.
- **Leaf nutrient guide:** The leaf nutrient guide must be calibrated through long-term well-designed experiments under normal field conditions and these may take as long as 20 to 25 years to obtain reliable results. For this reason, there are still fewer leaf analysis guides available than are necessary to establish accurate norms.

Leaf nutrient guide for mango cv. Dashehari

Nutrient	Unit	Critical limit
Nitrogen	%	1.23
Phosphorus	%	0.06
Potassium	%	0.54
Calcium	%	0.71
Magnesium	%	0.91
Sulphur	%	0.12
Iron	ppm	171
Manganese	ppm	66
Zinc	ppm	25
Copper	ppm	12
Leaf of 5-6 month age from center of shoot Samra and Thakur (1978)		

Leaf nutrient guide for guava (*Psidium guajava*)

Nutrient	Confidence Limit (%)
Nitrogen	1.63-1.96
Phosphorus	0.18-0.24
Potassium	1.31-1.71
Calcium	0.67-0.83
Magnesium	0.52-0.65
Khandhuja's and Garg (1980)	

Leaf nutrient guide for ber (*Ziziphus mauritiana* Lam K)

Nutrient	Unit	Confidence Limit
Nitrogen	%	2.77 - 3.32
Phosphorus	%	0.18 - 0.32
Potassium	%	1.69 - 1.96
Calcium	%	1.07 - 1.36

Leaf nutrient guide for Coorg mandarin (*Citrus reticulata*)

Nutrient	Unit	Vegetative Shoots	Floral Shoots
Nitrogen	%	2.08 - 2.79	1.85 - 2.48
Phosphorus	%	0.09 - 0.18	0.09 - 0.15
Potassium	%	0.78 - 2.08	0.76 - 1.89
Calcium	%	2.88 - 4.60	3.18 - 5.25
Magnesium	%	0.25 - 0.47	0.28 - 0.51
Iron	ppm	103 - 160	77 - 165
Manganese	ppm	54 - 220	50 - 124
Zinc	ppm	31 - 82	31 - 84

Leaf nutrient guide for mango cv. Totapuri

Nutrient	Unit	Status			
		Deficient	Low	Optimum	High
Nitrogen	%	< 0.47	0.47 - 0.83	0.84 - 1.53	1.54 - 1.88
Phosphorus	%	< 26	26 - 63	64 - 147	148 - 186
Potassium	%	< 0.22	0.22 - 0.51	0.52 - 1.10	1.20 - 1.40
Calcium	%	< 1.33	1.33 - 1.96	1.97 - 3.20	3.30 - 3.80
Magnesium	%	< 0.21	0.21 - 0.39	0.40 - 0.65	0.66 - 0.87
Sulphur	%	< 112	112 - 146	147 - 215	216 - 249
Iron	ppm	< 28	28 - 47	48 - 86	87 - 105
Manganese	ppm	< 20	20 - 57	57 - 174	175 - 232
Zinc	ppm	< 5	5 - 24	25 - 53	34 - 43
Copper	ppm	< 0.47	0.47 - 3.00	3.10 - 8.00	8.10 - 10.60
Yield	t/ha	< 9.20	9.20 - 10.40	10.50 - 13.70	12.80 - 14.00

Limitations of leaf analysis

- There are some limitations in its use, some of which are attributed to lack of required knowledge to get the full benefit from leaf analysis.
- One of the recognized limitations of legal analysis is that it is not a complete diagnostic device.

- The leaf analysis data do not indicate the doses of plant nutrients required for economically optimum yield.
- As a whole it reflects rather poorly the changes in soil reserves, soil acidity, alkalinity or salinity or other factors that affect growth and productivity.
- Thus, leaf analysis may give little or no information of encroaching root toxicities of such elements as Na, Cl, Se, Pb and Cu.

S. No.	Nutrient	Fertilizer
1.	Nitrogen	Urea
2.	Phosphorus	Mono ammonium phosphate (MAP) Mono potassium phosphate (MKP)
3.	Potassium	Potassium nitrate & Potassium sulfate
4.	Sulphur	Ammonium sulphate
5.	Calcium	Calcium nitrate & Calcium chloride
6.	Magnesium	Magnesium sulphate
7.	Boron	Boric acid & Solubor
8.	Copper	Copper Sulphate Pentahydrate
9.	Iron	Iron Sulphate Heptahydrate
10.	Manganese	Manganese Sulphate Monohydrate
11.	Zinc	Zinc Sulphate Heptahydrate
12.	Molybdenum	Sodium molybdate Ammonium molybdate

S. No.	Fertilizer	Rate (kg/ha per spray)	Typical Spray Volume (L/ha) & Spray Concentration (kg/100 L) 1 500 L/ha for trees
1.	Urea	10	0.5
2.	Mono ammonium phosphate (MAP) Mono potassium phosphate (MKP)	2.5-5	0.25-0.5
3.	Potassium nitrate	5-10	0.5-1
4.	Calcium nitrate	5	0.5
5.	Magnesium sulphate	2-5	0.25-0.5
6.	Solubor	0.5-2.5	0.1-0.25
7.	Copper Sulphate	0.5-1	0.05-0.1
8.	Iron Sulphate	1	0.05-0.1
9.	Manganese Sulphate	1-2	0.1-0.2
10.	Zinc Sulphate	1	0.1
11.	Sodium molybdate	50 g	

Conclusion

- Plant analysis is an authoritative tool for evaluating nutrient deficiencies, toxicities and imbalances, identifying hidden hunger, deciding fertilization plans, studying nutrient interactions, and determining the availability of elements for which reliable soil tests have not been developed.
- However, the results can be confusing if initial plant sampling, handling, and analysis of the sample are inaccurate.
- For that, cropping history, sampling techniques, soil test data, environmental influences and a knowledge of nutrient concentrations all need to be considered in the final diagnosis.
- Sampling techniques should be properly followed specifically for different fruit crops to get better result.
- The efficient and accurate plant analysis results in more efficient nutrient management and sustainable crop production.

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Chapter - 9
Prevalence, Characterization and Antibiogram Profiling of Fresh Leafy, Salad Vegetables and Rotten Fruits from Different Areas of Dhaka City, Bangladesh

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Chapter - 9

Prevalence, Characterization and Antibiogram Profiling of Fresh Leafy, Salad Vegetables and Rotten Fruits from Different Areas of Dhaka City, Bangladesh

Maruf Abony, Avijit Banik and Zakaria Ahmed

Abstract

This study was conducted to determine the microbiological quality of fresh raw and unwashed leafy and salad vegetables (red amaranth, spinach, carrot, radish, tomato, and cucumber), different fruits like-Sofeda, Pineapple, Grape, Banana, Apple, Orange, Guava, papaya, Jujube and Star fruit in Dhaka, Bangladesh, where *Salmonella* spp., *Acinetobacter* spp., *Klebsiella* spp., *Vibrio cholerae*, *V. parahaemolyticus*, *Staphylococcus aureus* and *E. coli* were bacteria and two strains of pathogenic fungi-*Aspergillus niger* and *A. flavus* were isolated. In betel leaves, both isolated fungi were showed their highest percentage of frequency (*A. niger*-66.66% and *A. flavus*-46.66%) where *A. niger* showed more pathogenic than *A. flavus*. The result of the pathogenicity test indicates that all the isolated fungi were pathogenic to their respective samples, except orange. The two species of *Aspergillus* spp. were found to be associated as the predominant fungi with the rotten fruits, vegetables and betel leaves. In case of fresh leafy and salad vegetables, the total microbial load ranged from $8x10^7$to $1.70x10^8$ having 7 different organisms where the most predominant organism was *Vibrio* sp. (23%) followed by *Klebsiella* sp. (20%), *Acinetobacter* sp. (19%), *Pseudomonas* sp. (19%), *Salmonella* sp. (8%), *Moraxella* sp. (8%) and *Escherichia coli* (3%) and 11% of the *Vibrio* sp. isolates were *V. cholerae*, found from 4 samples, but no presence of *V. cholerae* was observed in the tomato samples. *E. coli* was observed only in Carrot sample. Commercial antibiotic discs were used for antibiogram by Kirby-Bauer disc diffusion method on Mueller-Hinton agar medium. In case of antibiogram profiling of rotten fruits, majority exhibited resistance against Erythromycin, Vancomycin and Amoxycillin and showing sensitivity against Ciprofloxacin and Ceftriaxone. It was observed in the current study that 100% isolates were resistant against Erythromycin, followed by Amoxycillin 90.63% and Vancomycin 86.25%,

where only 35.27% isolates were resistant against Ciprofloxacin. In case of sensitivity 64.73% isolates were sensitive against Ciprofloxacin followed by Ceftriaxone 66.25%. on the contrary, in case of fresh leafy and salad vegetables, the isolated organisms were tested against antibiotics among which Imipenem showed the highest sensitivity (86%), followed by Ceftriaxone (100%), Nitrofurantoin (94%), Erythromycin (89%) and Amoxicillin (83%) had the highest resistance against the isolated organisms; however most of the isolates showed a multi-drug resistance pattern and they were resistant to at least four drugs.

Keywords: Fruits, vegetables, microbial load, antibiotic resistance

Introduction

Fruits are highly nutritious, sources of vitamins, minerals, fibers etc. and these are part of our daily diet. However, during cultivation, harvesting, transportation, handling fruits get contaminated with pathogenic microorganisms which leads to severe problems to community. Different types of vegetables are consumed raw worldwide for its highly nutritional value. Usually salad vegetables are often unwashed before consumption. Vegetables are rich in carbohydrates, anti-oxidants, minerals, vitamins and fibers and often consumed uncooked (Slavin and Lloyd 2012). In recent years many countries have undertaken various initiatives to encourage consumers to eat more vegetables as they are an essential ingredient of a healthy diet (Dias 2012). In the daily diet, vegetables have been strongly associated with improvement of gastrointestinal health, good vision, and reduced risk of heart disease, stroke, chronic diseases such as diabetes, and some forms of cancer. Some phyto-chemicals of vegetables are strong antioxidants and are thought to reduce the risk of chronic disease by protecting against free radical damage, by modifying metabolic activation and detoxification of carcinogens, or even by influencing processes that alter the course of tumor cells (Zhang *et al*. 2015). In Bangladesh total vegetable consumption reached 4,049 kt in 2013 in Bangladesh, according to FAO statistics. This is 0.226% more than in the previous year (FAO 2014). Hence consumption of raw vegetables for diet as salad has become more common among health-conscious people in recent years. Recently vegetables have been identified and confirmed as a significant source of pathogens due to the phyto-nutrients present in vegetables that act as effective media for the transmission of pathogens (Dias 2012). Consumption of raw vegetables contaminated with harmful microorganisms may result in food poisoning due to the fact that there is no killing step such as heating during preparation that would inactivate the harmful microorganisms (Younus *et al*. 2020).

Microorganisms capable of causing human disease may be found in raw produce. Sometimes they are part of the fruit or vegetable microflora as incidental contaminants from the soil and surroundings. In other instances, they are introduced into or on food by poor handling practices in agricultural production or post-harvest processes. The primary sources of microbial contamination of fresh vegetables include human and animal fecal matter, contaminated water, soil, dust, surroundings and handling equipment and poor sanitary practices throughout the production chain (FAO 2002). Contaminations may also occur at post-harvest stage through dirty wash water, cross-contamination, and consumption of raw or uncooked vegetables. Based previous investigations, pathogenic (disease-causing) strains of (STEC), *Salmonella*, *Norovirus* and *Listeria monocytogenes* are responsible for causing food borne illness via fruit (Bintsis 2017). Multiple investigations involved *E. coli* O157:H7 illnesses linked to leafy greens (FAO 2019). Bangladeshi people are more prone to microbial outbreak due to the relatively dense population with unsanitary condition. It is estimated 30 million people in Bangladesh suffer from foodborne illnesses annually (Noor and Feroz 2016). Children are more susceptible to unsafe food in comparison to adults which contributes to child mortality (Ali 2013). Often the vegetables are not properly washed before consumption due to the unawareness of the people living in Bangladesh about the hazards associated with it. To prevent the occurrence of food borne disease and its spread from raw vegetables it is necessary to minimize the contamination with microbes. Previously many researchers had also conducted similar studies in both developed and developing countries as well as in Bangladesh (Kabir *et al*. 2015, Eni *et al*. 2010, Bohaychuk *et al*. 2010). Many of them evaluated the presence of microbes after washing with tap water and/or other disinfectants to assess the proper cleansing method of vegetables. The significant source of bacterial and fungal transmission is food, fruits, vegetables and water. Food spoilage refers to several changes which make the food to be toxic and less palatable to consume and these could be associated with alterations in appearance, texture, taste and smell (Akinmusire 2011). But raw fruits and some categories of raw vegetables provide various nutrients and possess many health benefits (Ofuase 2014). In the human nutrition supplying, fruits play a vital role in the essential growth factors like as vitamins and essential minerals to the regular diet, which is necessary for the good and healthy consumption of fruits and vegetables products has dramatically increased by more than 30% during the past few decades (Barth *et al*. 2009). Vegetables also provide important roles to combat against cardiovascular and cancerous diseases (Akter *et al*. 2015). Fresh fruits and vegetables are a major source of

macro and micro nutrients such as a fiber, minerals and vitamin, thiamin, riboflavin, B_6 niacin, foliate, Vitamin A and E (Rickman *et al*. 2007). Fruits and vegetables are well known for their antioxidants compounds that protect against oxidative damage caused by free radicals, and they have been shown to be effective in helping to prevent retinal disease such as muscular degeneration (Wada and Ou 2002). In Bangladesh, 23.6%-43.5% loss postharvest of fruits and vegetables and over 30% loss of fruits are caused by fungal disease in transit and storage (Bashar *et al*. 2012, Hassan *et al*. 2010). Traditional varieties of fruits like apple, pineapple, grape, orange and tomato are affected by a wide array of microorganisms. The common postharvest and storage fungi of fruits infection are *Aspergillus flavus, A. fumigatus, A. niger, Alternaria alternata, Fusarium oxysporum, Geotrichum* sp. *Rhizopus nigricans, R. stolonifer* and *Penicillium* spp. (Bhale 2011). Garlic is most herbal medical crops grown all over the world and it's consumed in various forms like as it's known as to reduce blood sugar and cholesterol levels, medicinal pills, powder use in herbal medicine etc. But numerous fungi attack in garlic during storage result in decay causing considerable losses and decreasing the quality. Various fungi were reported to be associated with storage of garlic bulbs such as *A. niger, A. flavus* (Ghangaonkar 2013, Prasad *et al*. 1986, Mathur and Mathur 1958). Aspergillosis is one of the oldest genera of fungi. It's also one of the most common fungi in agricultural pathogens. *Aspergillus* spp. had become one of the best known and most studied in mould group. *A. niger* can produce a variety of fungal metabolites, termed mycotoxins depending upon growth condition and the strain of the organisms. *A. niger* capable of producing several mycotoxin show ever, mycotoxin production appears to be controlled by the conditions of fermentation. Another *A. flavus* is a saprophytic and pathogenic fungus. In globally, *A. flavus* is found as a saprophytic in soils and root of a common host of the pathogen on many important agricultural harvests. *A. flavus* has the potential to infect seedlings by sporulation on injured seeds generally excessive moisture conditions and high temp of storage grains and legumes increase the occurrence of *A. flavus* aflatoxin production (Barwant and Lavhate 2020). Among plant microbial pathogens, fungi are the most important and prevalent pathogens, infecting wild range of host plants, economical losses of crops in the field and harvesting during storage and transportation time. During consumption, Plant based fungal pathogens are unsafe (Riddhi and Yogesh 2013). In betel vine plants are occur some fungal infection like leaf rot disease, foot rot disease and leaf spot disease etc. (San and Naing 2016). Lots of research work has been done of fruits and vegetables rot in fungal infection in others country but insufficient

information available in Bangladesh. Therefore, our study focused on the presence of Gram-negative bacteria particularly, on the fresh and unwashed vegetables and to identify the selective fungal infection with rotten fruits, vegetables and leaves in Bangladesh in order to increase public awareness of the importance of good domestic hygiene practice.

Current chapter involves the prevalence of Gram-negative pathogens in fresh raw leafy and salad vegetables, without wash from different local markets in the Dhaka city and thereby analyze the quality of the products. Rotten fruits were analyzed for drug resistant microorganisms and fungal phytopathogens were detected in tomato, garlic and piper betel leaf. By analyzing the quality of these products, an estimated condition of the produce sold at random local markets in Dhaka city can be comprehended.

Methodology

Sample Collection, Isolation and Identification of bacterial isolates in case of fresh leafy and salad vegetables, samples of different kinds (red amaranth, spinach, tomato, cucumber, carrot and radish) were collected from different local markets in Dhaka city (Banani, Mirpur, Mohammadpur and Abdullahpur) and incubated at 120rpm and 37 ^{o}C in a reciprocal shaker for overnight (Motlagh and Kaviani 2008). In case of fruits, vegetables and betel leaves, samples of *Piper betle* (Betel leaf), *Allium sativum* (Garlic), *Solanum lycopersicum* (Tomato), *Malus pumila* (Apple), *Citrus sinensis* (Orange), *Vitis vinifera* (Grape) and *Ananas comosus* (Pineapple) were collected from two region southwestern zone (Jessore, Khulna and Satkhira) and central Dhaka in Bangladesh. All betel leaf samples were culled in directly betel vine garden (borojes) from South-western part and garlic, tomato, apple, orange, grape, pineapple collected from local market and superstore both central part in Bangladesh. In case of rotten fruits, unwashed and unprocessed rotten fruits were collected from different areas around Dhaka city (Savar, Mirpur, Uttara, Mohakhali and Banani) where ten different types of fruits were collected from each location with a total of 50 in number (Table 1) Fruits sold by street vendors often do not include rotten fruits as they are periodically removed from display, which is a major cause for lack of available rotten fruit samples.

Table 1: Rotten fruits collected from Dhaka city areas

Location	Fruit samples
Savar	Sapodilla (Sofada)
Mirpur	Pineapple, Orange
Uttara	Guava, Papaya, Jujube

Mohakhali	Star fruit, Grape, Apple
Banani	Banana

In case of rotten fruits, for enrichment, 25g of rotten fruits was added to 225ml of buffered peptone broth. Then samples were homogenized with 120rpm at 37 ºC for 35-48 h. After being homogenized, 1ml of each sample were transferred to different selective enrichment media such as 1ml of sample transferred in 9ml Tetrathionate broth, Phenol Red lactose broth and Alkaline peptone water (APW). They were incubated overnight at 37 ºC. After incubation period, enriched samples were transferred from TT broth by the four-way streak method in *Salmonella-Shigella* agar (SS agar) and Bismuth Sulphate Agar (BSA) for detection of *Shigella* spp. and *Salmonella* spp. Samples from Phenol red lactose broth were transferred to Eosin Methylene Blue (EMB) agar and MacConkey agar for detection of *E. coli* and other enterobacteriaceae and APW samples were transferred to Thiosulfate-Citrate-Bile salt-Sucrose (TCBS) Agar which was used for detection of *Vibrio* spp. An enriched sample from buffered peptone water was transferred to Manitol Salt agar and Cetrimide agar by streak plate method (Fig. 1). All selective Media were incubated 24±2h at 37 ºC. After incubation, single colonies were picked and were further sub cultured again in the selective media to get isolated pure colony (Giridhar and Ready 1997).

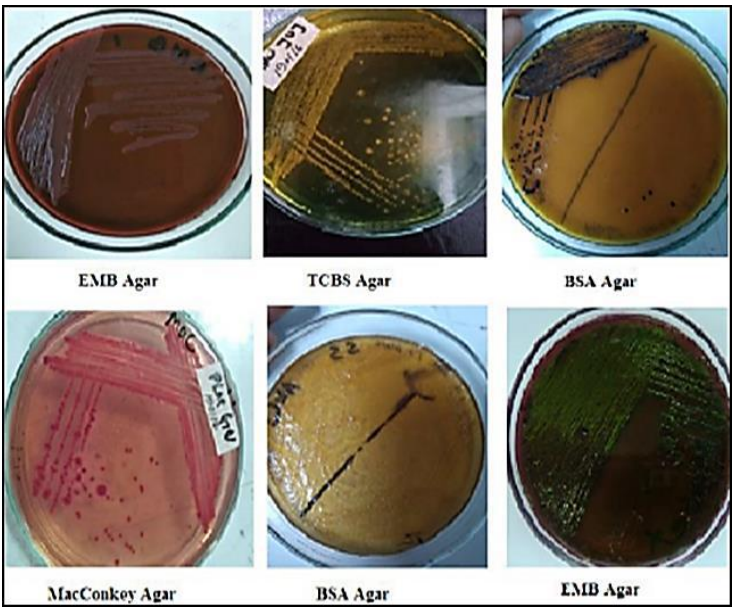


Fig 1: Streaking plate in different selective media (BSA Agar, EMB Agar, MacConkey Agar and TCBS Agar)

Spots, blights, anthracnose, rots etc. infections are visible in various parts such as stems, leaves, bulbs and fruits in fungi infections. The infected samples were transported in air-tight sterile polyethylene bags and stored at room temperature. Selected samples were examined morphologically, determined their mycoflora and also tested their pathogenicity against selective fugal. Different Gram-negative bacteria were isolated by culturing on different selective (e.g. Cetrimide agar, SS agar, etc.) and differential media (e.g. MacConkey Agar) and identified initially by the morphological characteristics of the colonies (Motlagh and Kaviani 2008). The presumptive organisms were further tested for biochemical, carbohydrate profiling and antibiotic susceptibility test in order to obtain a more precise identification (Riddhi and Yogesh 2013, San and Naing 2016).

Bacterial count was determined using pour plate technique. The plates were then incubated at 37 ^{o}C and the counts were taken after a day (Motlagh 2010). Identification of the isolated strains was done by conducted microscopy and biochemical characterization. The biochemical characteristics of a bacterium provide many traits that are useful for classification and identification. Triple Sugar Iron (TSI), Motility, Indole Test, MR-VP Test, Citrate Utilization, Nitrate Reduction, Catalase, Oxidase, Urease Test, Gelatin Hydrolysis, Starch Hydrolysis (San and Naing 2016) was conducted along with Carbohydrate Fermentation of mannitol, sorbitol, inositol, rhamnose, raffinose (Barnett and Hunter 1998). The bacterial isolates were identified based on their morphological characteristics, Gram stain of pure culture and biochemical tests according to the Bergey's Manual of systematic Bacteriology and database for different isolates (Martins *et al*. 2001, Bello *et al*. 2016).

Isolation and Identification of fungal pathogens

Collected infected parts of betel leaves, garlic, tomato, apple, orange, pineapple and grape were washed in tap water for 2-3 min and then the infected parts were cut into about 1cm small pieces, again washed with sterile distilled water in 2-3 times, then were exploitation and dried on the sterilized filter paper (Whatman No: 01) for 10-15 minutes. Then, cutting transmitted portions were placed on the Potato Dextrose Agar (PDA) medium for around two hours to observe proper deposition of fungal spores from the infected parts. Using sterile tweezers, removed the parts from plates and infectious spores were inoculated for 3-7 days in the room temperature (San and Naing 2016). Developing sporulation depends on type of fungi were sub-cultured on fresh PDA medium (with sulfate Streptomycin antibiotics solution) 4-6 times to get pure fungal spore where sulfate

Streptomycin antibiotics solution on prevented bacterial contamination in media (Barnett and Hunter 1998). Moreover, pure spore of fungus were also placed onto the sterilized filter paper than cut the filter positions were persevered in sterilized vials at freezer on -20 °C (Motlagh 2010). After the incubation period, the diameter of each inoculum in plates were measured and noted their morphological characteristics and the pure spores identified fungi were persevered as stock culture on half-strength PDA slants in the test tubes. The most significant morphological characteristics of fungus carried out for identification was spore-bearing structure and some fungus body extent characters. The isolated fungi species were identified on the basis of micro and macro morphological structure, identification level of genus using proper media, shape, size, color and arrangement of spore (Barnett and Hunter 1998). Isolated pure culture, freshly inoculated to proper media for species identification using macro-morphological and microscopic characteristics were observed (Barwant and Lavhate 2020). All the isolated fungi were grown in triplicates for the complete isolation and purification of colony and spore morphology. The frequency of different fungal species was assessed by calculation of the frequency percentage (Giridhar and Ready 1997) where the values were obtained according to:

$$\text{Frequency of percentage} = \frac{\text{No. of observation in which a species appeared}}{\text{Total number of observations}} \times 100$$

Pathogenicity test of isolated strains based on Koch's Postulate

In Koch's postulates method, leaf, stem and body surface tissues cross binding with healthy part to infected of fungus where the isolated fungi were placed into new proper growth media and after inoculation of pure culture replaced into covered moist cotton to promote sporulation of the fungi. Each specimen of isolated fungus were then wrapped to the infected moist cotton covering by healthy parts of selective samples and cotton was kept by adding required water for moist. After 1-7 days inoculated plant were keenly observed, recorded of typical symptoms produced by the pathogen and re-isolation and identification of fungi were done (Jahan *et al*. 2016).

Antibiotic susceptibility test

Antibiogram of identified isolates were conducted for *In vitro* antibiotic resistance profiling using Kirby-Bauer method against commercially available antibiotics were used (Bello *et al*. 2016, Giridhar and Ready 1997). The action of the antibiotics allowed determining the inhibition of the pathogen to the degree proportional to the diameter of the zone of inhibition where it shows that the clear zone resulted from diffusion of the

antimicrobial substance that surrounds the disc onto the agar medium. Using 5ml of Muller-Hinton broth all pure culture of specific isolates were inoculated and then incubated overnight at 37 ºC. The turbidity of the broth culture was adjusted to 0.5 McFarland standards and the standard turbid broth was spread evenly on complete surface of the plates of Muller-Hinton agar in order to measure the susceptibility of antibiotics (El-Marzoky and Shaban 2014). The zone diameter for individual antimicrobial agents was then interpreted into categories of susceptible, intermediate and resistant according to the guidelines from National Committee for Clinical and Laboratory Standards (NCCLS) (Khatoon *et al*. 2017).

In case of bacterial isolates from rotten fruits

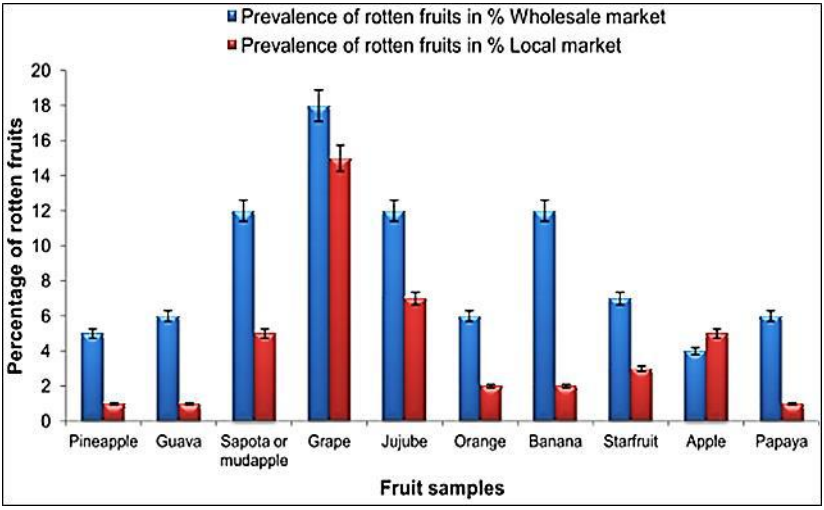
Fifty Fruit samples were examined of which 35 fruit samples showed the presence of bacteria and 15 samples showed other infections of fungi. Higher numbers of rotten fruits were present in wholesale markets where local markets showed lesser presence of rotten fruits due to removal of such fruits from display by the vendors (Fig. 2a). In daily food intake habits, most of the fruits are consumed in their raw conditions and may lead to the onset of human diseases that is a serious threat for overall public health, as they may cause food borne illness due to transmission during post-harvest (Daily Star 2019). During the storage period and transport, some fruits can be contaminated by other infected fruits and/or their roots (Rolle 2006). Fruits that are damaged post-harvest can be reservoir of microorganisms, as well as potential pathogens. Due to lack of proper handling microorganisms from rotten fruits may transmit on surface of healthy fruit, and pathogenic organisms present due to cross contamination may persist of surface of fruit. As a result when consumed raw due improper washing and cleaning food borne disease may occur (Daily Star 2019, Abadias *et al*. 2008). Fruits are easily contaminated with bacterial pathogens due to cross contamination during and post-harvest, which can be the major cause of bacterial infections caused by fruits. In this present study, 10 different bacterial spices were presumptive which were isolated from various rotten fruits and enumerated (Table 2).

Table 2: Morphological characteristics of bacteria isolated from ten types of rotten fruits

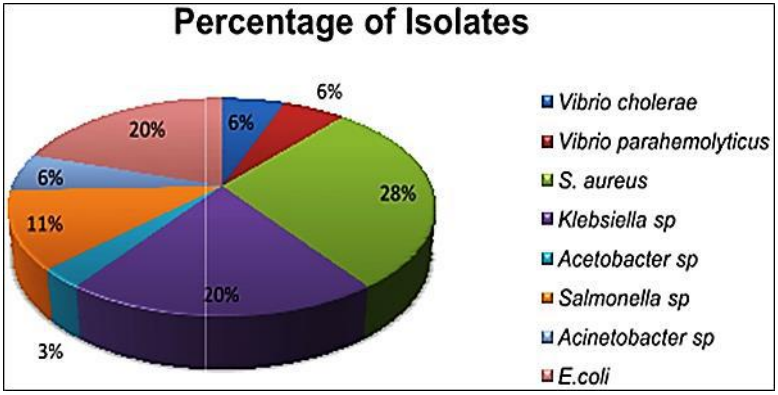
Using media plate	Colonial morphology				Gram reaction	Cell morphology
	Pigment	Consistency	Margin	Elevation		
SS agar	Colorless	Plaster	Circular	Flat	-	Cocci, Bacilli
SS agar	Pink	Plaster	Circular	Raised	-	Bacilli

BSA agar	Black	Plaster	Circular	Raised	-	Bacilli
BSA agar	Brown	Plaster	Irregular	Raised	-	Bacilli
EMB agar	Green Metallic Sheen	Plaster	Circular	Raised	-	Bacilli
EMB agar	Purplish	Mucoid	Irregular	Raised	-	Bacilli
Mac agar	Pink	Mucoid	Irregular	Flat	-	Bacilli
TCBS agar	Blue Green Center	Mucoid	Circular	Raised	-	Curved, Bacilli
TCBS agar	Yellow	Plaster	Circular	Raised	-	Comma shaped
MSA agar	Golden Yellow	Plaster	Circular	Raised	+	Cocci

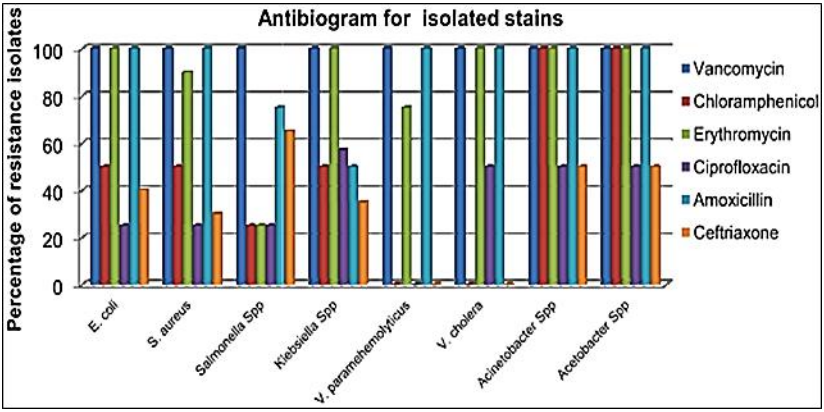
After biochemica tests, these were identified as *Salmonella* spp., *Acinetobacter* spp., *Acetobacter* spp., *Klebsiella* spp., *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Staphylococcus aureus* and *E. coli*. Among these microorganisms the most predominant species was *Klebsiella* spp., (20%), followed by *E. coli* (20%), *Acinetobacter* spp. (6%), *Acetobacter* spp. (3%), *Vibrio parahaemolyticus* (6%), *Vibrio cholerae* (6%), *Salmonella* spp. (11%) and *S. aureus* (28%) as shown in Fig. 2(b). The biochemical tests exhibit the biochemical profiles of respective isolated genera as presented in Bergey's Manual of Determinative Bacteriology (Clauss and Berkeley 1986). Six different Antibiotics were used against isolated microorganisms. The antibiotics showed the highest degree of resistance against Vancomycin, Erythromycin, Amoxicillin, Chloramphenicol, Ceftriaxone and Ciprofloxacin (Fig. 2c).



(a)



(b)



(c)

Fig 2: (a) Prevalence of rotten fruit in wholesale and local markets, (b) Percentage of Isolated organisms from rotten fruits, (c) Occurrence of drug-resistant isolates from rotten fruits

Most of the organisms showed resistance to almost every antibiotic. Some organisms showed resistance to one or two antibiotics, such as *E. coli* and *S. aureus* showed 100% resistance to Vancomycin, Erythromycin, Amoxicillin; *Acetobacter* spp. and *Acinetobacter* spp. both showed resistance against Chloramphenicol. More sensitivity was observed against Ceftriaxone by the isolates-*E. coli* (40%), *Salmonella* spp. (65%), *S. aureus* (30%) and *Klebsiella* spp. (35%). These results were more similar compared with the previous data found of Ceftriaxone (Hemalata *et al*. 2016). Identified *Vibrio* spp. isolates showed 100% sensitivity against Chloramphenicol and Ceftriaxone. Our antibiotics resistance pattern was found similar to that of previous reports on antibiogram of infected routine

fruit samples (Mamun *et al*. 2016). Although rotten fruits are not consumed directly, but in many industry and preparation of beverage items derived from fruits, such as street vended fruit juice. This in turn shows presence of various microorganisms in prepared food product from fruit (Kamal *et al*. 2014). In current study, it was observed that many bacterial strains isolated from rotten fruits are similar to isolates found in street vended fruit juices.

In case of bacterial isolates from fresh vegetables and betel leaves

All of the samples used in the present study were found to be contaminated with more or less organisms (Table 3). The microbial load ranged from $8x10^7$ to $1.70x10^8$ CFU/ml with the lowest in carrot and the highest in cucumber (Fig. 3). A total 35 isolates were identified, most predominant organism was *Vibrio* sp. (23%) followed by *Klebsiella* sp. (20%), *Acinetobacter* sp. (19%), *Pseudomonas* sp. (19%), *Salmonella* sp. (8%), *Moraxella* sp. (8%) and *E. coli* (3%). Among the *Vibrio* sp. isolates, 3 different species were identified which include *V. cholerae*, *V. parahaemolyticus* and *V. alginolyticus* (Fig. 3, 4). Pseudomonas spp. is part of the natural flora and is among the most common vegetable spoilage bacteria. *Moraxella* sp. was found just in Spinach and Radish samples, while *Salmonella* sp. was detected from Red Amaranth, Cucumber and Carrot. Presence of *V. cholerae*, *Salmonella* sp. and *E. coli* found from the present study are concerning for public health.

Table 3: Number of each organism isolated per sample

Sample	Number of organisms found per sample									Total number of organisms per sample
	V. cholerae	***V. parahaemolyticus***	***V. alginolyticus***	***Acinetobacter* sp.**	***Klebsiella* sp.**	***Pseudomonas* sp.**	***Moraxella* sp.**	***Salmonella* sp.**	***E. coli***	
Red Amaranth	1	-	-	1	2	1	-	1	-	6
Spinach	1	-	-	1	1	1	2	-	-	6
Tomato	-	-	1	1	1	-	-	-	-	3
Cucumber	1	1	-	1	1	1	-	1	-	6
Carrot	-	1	1	2	1	2	-	1	1	9
Radish	1	-	-	1	1	2	1	-	-	6
Total	4	2	2	7	7	7	3	3	1	36
Total Percentage of the Organisms found in the Sample	11	6	6	19	20	19	8	8	3	-

One of the isolated *Salmonella* sp. was resistant to 6 of the drugs (86%). Six of *Klebsiella* sp., two of *Pseudomonas* sp. and *Acinetobacter* sp. and one of *Moraxella* sp. were resistant to 5 of the drugs. All of the *Vibrio* sp. isolates was resistant up to 4 drugs (Fig. 5-7). All of the isolates were also tested for their susceptibility to a number of different antibiotics. In the present study, all of the isolates were resistant to Amoxicillin, except *Pseudomonas* sp. Imipenem showed the highest sensitivity (86%) among the tested antibiotics against all the organisms, followed by Ciprofloxacin (65%) and Chloramphenicol (42%) (Fig. 6).

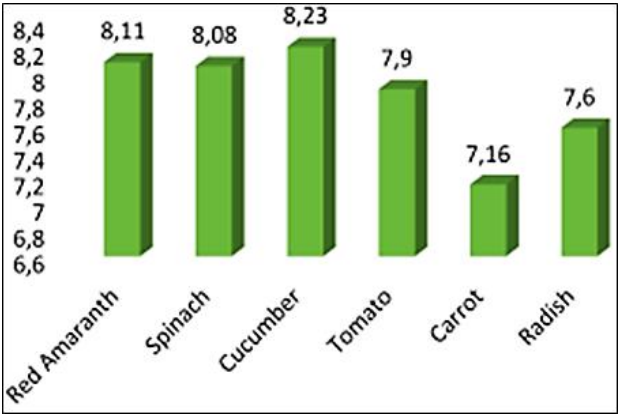


Fig 3: Total Viable Bacterial Count (log scale)

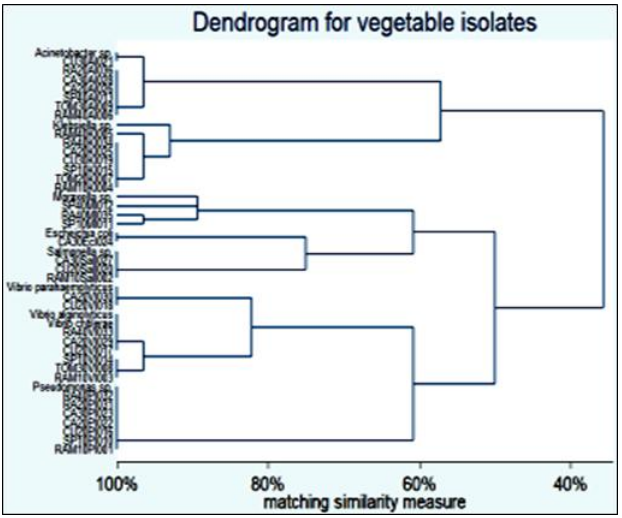


Fig 4: Dendrogram of similarity between isolates. Different Antibiotics in Percentage (%)

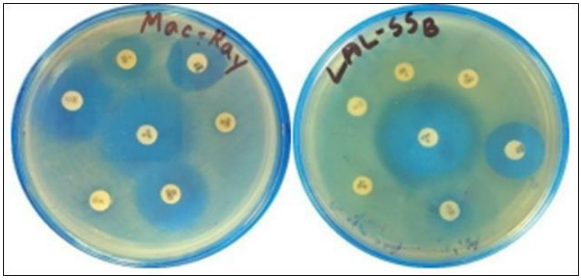


Fig 5: Antibiotic Susceptibility Test on Mueller-Hinton Agar

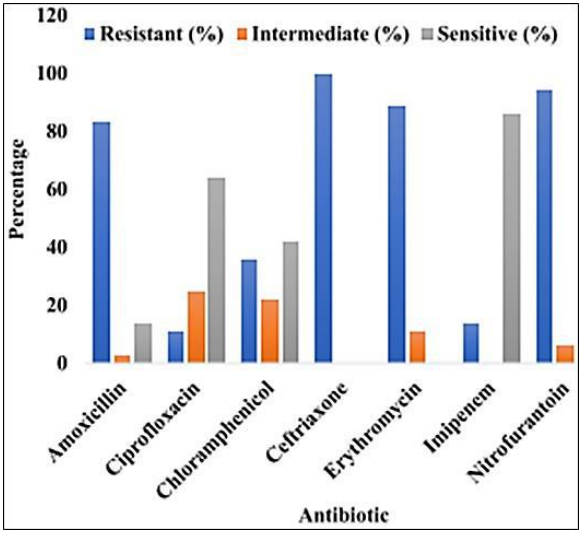


Fig 6: Frequency (%) of Antibiotic Susceptibility of the Isolates

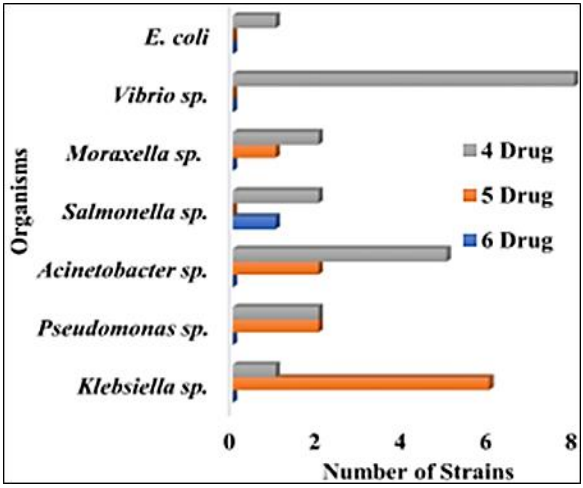


Fig 7: Multi-Drug Resistance Pattern of the Isolates

The increasing consciousness in people regarding the nutrition has resulted in the increased consumption of raw fresh unprocessed vegetables and fruits. These foods carry indigenous microflora besides pathogenic microorganisms. A number of diseases outbreaks due to consumption of these produce have been reported previously (Kabir *et al*. 2015, Francis 2002). The present study was focused on the detection of Gram- negative pathogenic bacteria from unwashed raw vegetables and thus determining the quality of those products. The total bacterial count showed higher bacterial load in Cucumber (1.70×10^8) than other samples which is similar to the findings of study conducted in Dhanbad, India (Mrittunjay and Kumar 2017). Among 35 isolated organisms *Acinetobacter* sp. and *Klebsiella* sp. were present in all of the samples. However, *Pseudomonas* sp. was absent only in Tomato samples. According to current study, *V. cholerae* was isolated from 4 samples out of 12 (11%), including red amaranth, spinach, cucumber and radish. Consumption of vegetables and fruits from fields where raw sewage was used for irrigation showed association with a cholera outbreak in Peru (Swerdlow 1992). In current study *Salmonella* sp. was isolated from 3 vegetables out of 12 (8%), which is higher than the findings of Mrittunjay and Kumar (2017) but close to findings (7.8%) of Kumar (2012). It also reported that pond water used for irrigation, cleansing and sprinkling of vegetables by vegetable farmers and vendors might be the primary source of contamination of by *Salmonella* sp. The presence of *E. coli* was observed only (6%) in the Carrot sample in current study, while it was the predominant (38.3%) bacterial pathogen from 50 different salad vegetables sold in Amravati City, India (Mundhada 2006). Another study reported 8.1% presence of *E. coli* in carrots, lettuce, green onions, and spinach samples (Bogomolny *et al*. 2013). According to our study, most of the isolates were resistant to at least four drugs. Ceftriaxone showed complete resistance to all of the isolates, which is a matter of great concern. Consumption of these multi-drug resistant (MDR) pathogen contaminated vegetables can result in serious illness to the consumers. Following Ceftriaxone (100%), Nitrofurantoin (94%), Erythromycin (89%) and Amoxicillin (83%) had the highest resistance against the isolated organisms (Fig. 7).

In case of Fungal Isolates (fruits, vegetables and betel leaves)

Pure cultures of the isolated fungi were identified according to the cultural properties, morphological, and microscopic characteristics of fungi. A total of 45 samples (leaves, fruits and vegetables) spoilage molds were Isolation from two specific fungi, *A. niger* (Barnett and Hunter 1998) and *A.*

flavus (San and Naing 2016), were deteriorating betel leaves, garlic, tomato, apple, pineapple and grape from collected samples but orange did not find target fungal activity (Table 4, Fig 8).

Table 4: Isolated and identification of fungi from various sources

Name of Samples	Number of sample	*Aspergillus niger*	*Aspergillus flavus*
Betel Leaves (paan)	15	10	7
Tomato	5	0	2
Garlic	5	3	0
Apple	5	3	2
Orange	5	0	0
Garpe	5	2	2
Pineapple	5	0	2

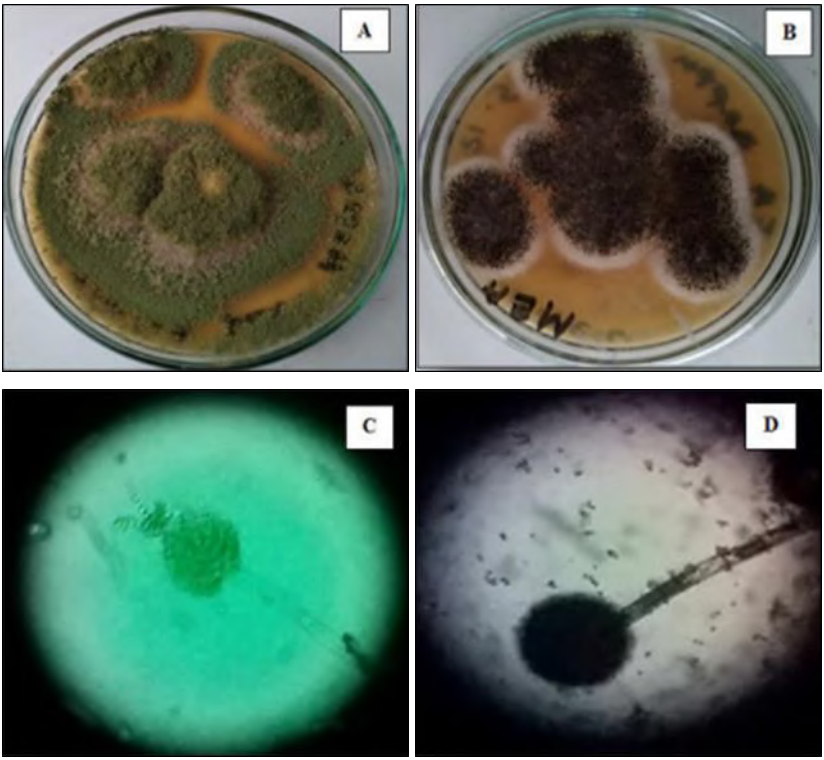


Fig 8: Isolated fungi on PDA plates (A: *A. flavus*, B: *A. niger*) and Microscopic structure of isolated fungi and spore's appearance (C: *A. flavus*; D: *A. niger*)

In betel leaves, both fungi, *A. flavus* and *A. niger*, were isolated and their percentage of frequency 66.66% and 46.66%, respectively. *A. flavus* were

identified in tomato, apple, grape and pineapple and three of them showed the similar percentage of frequency 40% against *A. flavus* and garlic (60%), apple (60%) and grape (40%) of frequency showed against *A. niger* (Fig 9 and 10). Identified *A. niger* and *A. flavus* from selective samples was subjected to pathogenicity test by Koch's Postulates.

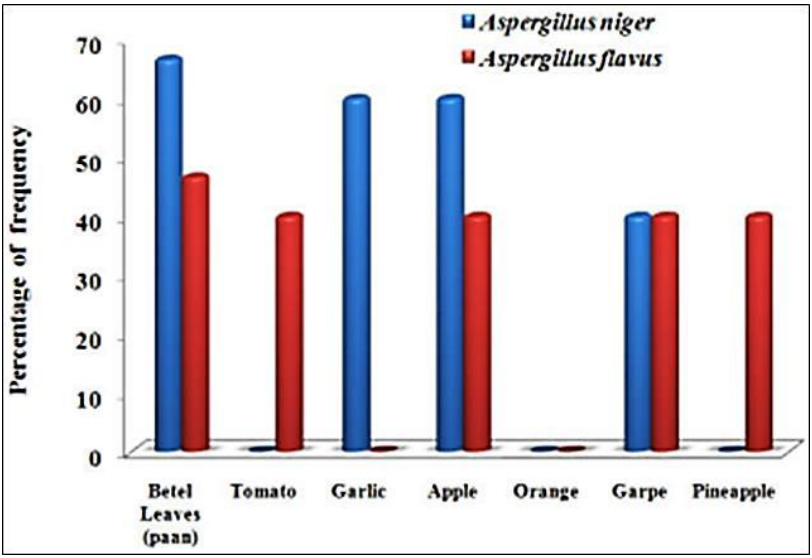


Fig 9: Frequency (%) test of both identified fungus

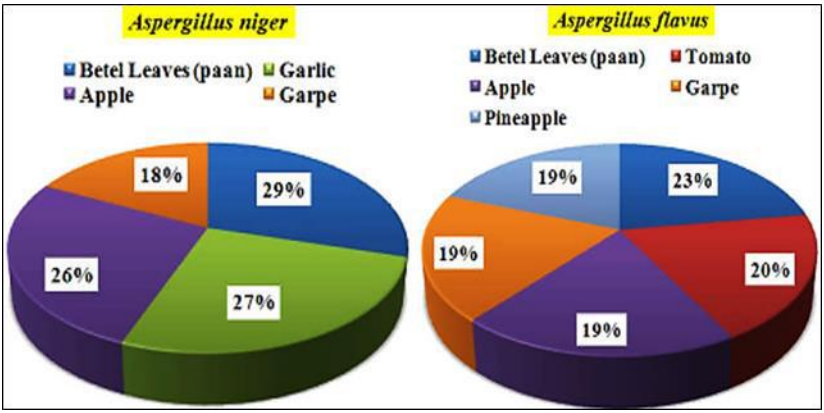


Fig 10: Total percentage of identified fungus against selected samples

Take on each healthy sample to inoculate the isolated fungus to exhibit to typical symptoms. After 2-4 days selected samples showed mycelium formation growth on their skin, bulk and their plant base. On the 3-6 days of inoculation, spread of infections rapidly towards the isolated place of the

inoculation and started to germinate producing. Finally, the artificially inoculated samples developed by characteristics symptoms resulting in healthy selective samples (Table 5).

Table 5: Pathogenicity test by Koch's postulation

Isolates	Observation						Mortality (%)	
	Mycelium formation (days)		Wilting (days)		Killing (days)			
	A. niger	***A. flavus***	***A. niger***	***A. flavus***	***A. niger***	***A. flavus***	***A. niger***	***A. flavus***
Betel leaves	2	2	3	3	4	4	100	100
Garlic	3	2	4	3	5	5	100	100
Tomato	2	2	3	3	4	4	100	100
Apple	3	4	5	6	6	7	100	100
Graph	2	2	3	3	4	4	100	100
Pineapple	4	4	6	6	8	9	100	100

All samples have done twice the pathogenicity test. On re-isolation from the artificially inoculated fungus from selective samples were found that the pathogen represented same characteristics on PDA culture as found earlier on isolation from naturally infected selective samples caused by *A. niger* and *A. flavus*. Most of the selected samples in these studies showed *Aspergillus* infection except orange could not show infection. Betel leaves, apple and grape samples identified both *Aspergillus* species (*niger* and *flavus*). In the previous study resulted as a similar result in the present researcher. Bashar *et al*. (2012), rotten fruits infection (apple and grape) in Dhaka city showed *A. niger* was the highest frequency of 44% in apple and 11.33% *A. flavus* in the grape as similar as our study also showed *A. niger* 60% in apple and *A. flavus* 40% in grape (Bashar *et al*. 2012). *A. flavus* was responsible of pineapple infection; in pervious study also showed similar result in pineapple infection (Onyemata and Ibrahim 2018). *A. niger* is most known as the Black mould. *A. flavus* and *A. niger* also parasitizes man and animals and also cause a number of disease grouped under the name Aspergillosis. Without getting sick, most of the people breathe *Aspergillus* spores in their daily life. But, *Aspergillus* infection due to a higher risk of developing health problems that have weakened immune systems or lung diseases infection and allergic reaction.

This *Aspergillus* infection may also be seen in the human ear and is called Otomycosis. *A. flavus* is reported to produce the mycotoxin known as Aflatoxin which is a significant potent carcinogenic, toxigenic, mutagenic,

teratogenic and has been directly correlated with adverse health effects, such as liver cancer and other organs disease in many animal species (Martins *et al*. 2001). In the present study; tomato showed *A. flavus* (20%) infection that was most similar result to show in previously in Nigeria (Bello *et al*. 2016). *A. niger* was found in black mold garlic in our study that was most common in other research. Most of the research showed infection of garlic bulbs decay yielding the associated of one or more fungi but *Fusarium solani*, *Botrytis allii* and *A. niger* were the most common fungi associated with rotted garlic (El-Marzoky and Shaban 2014, Khatoon *et al*. 2017). In our study *A. niger* showed in 27% in garlic isolation in total percentage of *A. niger*. In this study, Betel leaves showed the highest percentage of infection in *A. niger* (29%) and *A. flavus* (23%). In previous study, showed betel leaves infected in *Aspergillus* spp. (5.43%) in highest fungal from that research (Nahar *et al*. 2018). Another research reported that the stem root and yellow leaf in betel leaves disease were caused by *Aspergillus* spp. (San and Naing 2016).

Over the last 50 years, various studies have shown that the excessive use of antimicrobial drugs to treat human and animal infections has resulted in selective pressure, leading to antibiotic resistance. Recent studies have demonstrated that reservoirs of genes coding for antibiotic resistance in humans, as well as in animals, plants and the environment and that these genes can be transferred to human by direct contact or indirectly by ingestion of contaminated foods (Amorim and Nascimento 2017). Outbreaks of human infection associated with the consumption of raw fruits and vegetables often occur in developing countries and have become more frequent in developed counties over the past decade. Results obtained from this study showed the presence of various pathogenic bacteria on the surface of unwashed fresh raw vegetables from different local markets in Dhaka city. Moreover, most of the pathogens were multi-drug resistance. It indicates the necessity of proper hygiene practice during and post-harvesting stage of the vegetables. For raw consumption further processing of these products in compulsory to ensure their quality and safety of the user. Proper washing with water and/or pretreatment with different antimicrobial agents can reduce the chance of such contamination. The presence of MDR bacteria in the raw leafy and salad vegetables indicates the poor sanitation during and post-harvesting stage, even during transportation from the fields to the market and then to the consumers. This study showed that consumption of raw vegetables without wash can cause serious illness. To reduce the risk of getting infected by these MDR bacteria the raw vegetables must be wahed properly before the comsumption.

Betel leaves are chewed directly human; so there is need to evaluate the fungicide reduce in leaves before the human consumption. It's difficult to gain control of diseases at the same time where both fungal and bacterial pathogens are associated. But chemical fungicide application is effective method of plant disease control is indisputably. However, indiscriminate use fungicides could produce environmental and health hazards especially direct consume fruits, vegetables and leaves (Betel leaves). *Aspergillus* spp. is one of the most common fungi sources in agricultural different plant parts (seed, root, leaf, and stem) occur infection is caused or do the harmful effect such reduction in crop yields, loss in germination percentage, development of plant disease discoloration and shriveling, biochemical change in a seed. For that reason, the present finding in this study focused on *A. niger* and *A. flavus* were isolated from Betel leaves, vegetables and fruits in the southern and central part of Bangladesh. The high prevalence of fungi isolates in the selected fruits and vegetables showed that fungi are the major cause of the spoilage. Deterioration of most fruits, vegetables and leaves is caused by fungi infection. The isolated fungi are economic and public health importance. Isolated *A. niger* have been reported to produce mycotoxins that can be harmful to human and animals and also harmful aflatoxin produce by *A. flavus*. For those reasons, we recommend using fungicides spraying timely of the harvest to reduce the damaging activities of the fungal pathogens and contamination with mycotoxins and related fungal metabolites infection that might be hazardous to human health.

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Chapter - 10
Recent Advances in Gene Transfer Biotechnology of Fruit Crops

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Chapter - 10

Recent Advances in Gene Transfer Biotechnology of Fruit Crops

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Abstract

Plant genetic engineering has created new pathways for crop modification as well as novel solutions to specific problems. Significant progress has been achieved in the development of novel and efficient plant transformation methods during the previous decade. Despite the availability of a multitude of DNA delivery mechanisms, Agrobacterium and biolistic-mediated transformation remain the two most often used ways. Now in the agricultural post-genome age, with increasing genome sequence information, BAC clones, ESTs, and full-length cDNAs are accessible opened a new pathway of marker-free transformation methods. Under these conditions, significant attempts have been made to create alternative marker-free technologies in fruit species. Multigene transfer technology would be highly desirable for commercial fruit species cultivars to obtain multiple new traits at the same time without the need for several rounds of introgressive backcrossing. Considerable progress has been made in the development of novel and efficient plant transformation methods during the previous decade. In this literature, we describe several methods of genetic transformation, as well as alternative transformation without markers and multigene transformation of fruit crops using genotype-independent protocols, accuracy molecular tools to drive T-DNA insertion and expression, and efficiency and high-yield selection and regeneration *in vitro* culture methodology to address public concerns and improve market acceptance of transgenic crops.

Keywords: Transformation, agrobacterium, fruit crops, marker, technology

Introduction

Rapid increase of human population together with global climate variability resulted in increased demand of plan-based food and energy sources (Varshney *et al*., 2011). Fruits and nuts have essential role to enhance quality of humankind life since a diet based on cereal grains, root

and tuber crops, and legumes is generally lacked a wide range of products such as fiber, vitamin, provitamins or other micronutrients and compounds exist in fruit and nut species (Heslop-Harrison, 2005). About 654 million tonnes fruits were produced in the world in 2014 (Anonymous, 2015). Because an increase demand exists in global food production, many economically important fruit crops production need to be improved. During the past, some progress was made through classical breeding with regard to these problems.

However, conventional fruit breeding has had little success in improving fruit plants and is constrained

1) Due to long juvenile period, breeding programs for such plants can involve the professional lifetimes of several generations of scientists.
2) Erosion of naturally occurring genetic variability.
3) Transfer of undesirable genes along with desirable traits.
4) Reproductive obstacles that limit the transfer of favourable alleles from diverse genetic resources (Gomez-Lim and Litz, 2004; Varshney *et al*., 2011).

Thus, there is an urgent need for the biotechnology-assisted crop improvement, which ultimately aimed to obtain novel plant traits (Petri and Burgos, 2005). Tissue culture based technologies including somatic hybridization (Grosser *et al*., 2010), *in vitro* selection (Rai *et al*., 2011), haploid and double haploid production (Germana, 2011), encapsulation technology (Rai *et al*., 2009) have also been applied to some fruit plants for crop improvement.

Plant genetic engineering has opened new avenues to modify crops, and provided new solutions to solve specific needs (Rao *et al*., 2009). Contrary to conventional plant breeding, this technology can integrate foreign DNA into different plant cells to produce transgenic plants with new desirable traits (Chilton *et al*., 1977; Newell, 2000). These biotechnological approaches are a great option to improve fruit genotypes with significant commercial properties such as increased biotic (resistance to disease of virus, fungi, pests and bacteria) (Ghorbel *et al*., 2001; Fagoaga *et al*., 2001; Fagoaga *et al*., 2006; Fagoaga *et al*., 2007) or abiotic (temperature, salinity, light, drought) stress tolerances (Fu *et al*., 2011); nutrition; yield and quality (delayed fruit ripening and longer shelf life) and to use as bioreactor to produce proteins, edible vaccines and biodegradable plastics (Khandelwal *et al*., 2011).

Currently, public concerns and reduced market acceptance of transgenic crops have promoted the development of alternative marker free system technology as a research priority, to avoid the use of genes without any purpose after the transformation protocol as selectable and reporter marker genes. Typically, it is employed for the selection strategy that confers resistance to antibiotics and to herbicides (Miki and McHugh, 2004; Manimaran *et al*., 2011). A large proportion of European consumers considered genetically modified crops as highly potential risks for human health and the environment. European laws are restrictive and do not allow the deliberate release of plant modified organism (Directive 2001/18/EEC of the European Parliament and the Council of the European Union). Under these premises, great efforts have also been realized to develop alternative marker free technologies in fruit species. Over the last decade, significant progress has been made in the development of new and efficient transformation methods in plants. Despite a variety of available DNA delivery methods, *Agrobacterium* and biolistic-mediated transformation remain the two predominantly employed approaches. Efficient regeneration systems for the generation of transgenic tissues still appear as an important bottleneck for most of the species and cultivars.

Plant transformation methods

Recent advances in genetic transformation have made it possible to transfer genes of both academic and agronomic importance into various crop species. A prerequisite for successful transformation system is an efficient regeneration protocol when tissue culture-based transformation process is employed. The very basis of plant regeneration relies on the realization that plant somatic cells are totipotent and can be stimulated to regenerate into whole plants. However, this insight is limited because, in reality, only a limited number of plant species and certain types of explant tissues have been found to be capable of regenerating whole plants under appropriate culture conditions. Therefore, much effort has been aimed at establishing and improving plant regeneration systems. Yet, efficient regeneration alone does not necessarily lead to efficient transformation. There is a need to develop advanced transformation methods that would not only incorporate the required characteristics (stable and desirable transgene integration and expression) into plants but also enable generation of transgenic events in a high-throughput manner. These requirements are particularly relevant now in the crop post-genome era in which ever-increasing amounts of genome sequence information, BAC clones, ESTs and full-length cDNAs are available. This situation presents both new challenges and opportunities for

plant transformation research. At present, *Agrobacterium* and microprojectile are the commonly used methods for this purpose; other methods, such as electroporation and microinjection, are still used only rarely.

1. *Agrobacterium* mediated transformation

In this method, *A. tumefaciens* or *A. rhizogenes* is employed to introduce foreign genes into plant cells. *A. tumefaciensis* a soil borne gram-negative bacterium that causes crown-gall, a plant tumor. The tumor-inducing capability of this bacterium is due to the presence of a large Ti (tumor-inducing) plasmid in its virulent strains. Similarly, Ri (root-inducing) megaplasmids are found in virulent strains of *A. rhizogenes*, the causative agent of "hairy root" disease. Both Ti and Ri-plamids contain a form of "T-DNA" (transferred DNA). The T-DNA contains two types of genes: oncogenic genes, encoding enzymes involved in the synthesis of auxins and cytokinins (causing tumor formation) and genes involved in opine production. The T-DNA element is flanked by two 25-bp direct repeats called the left border (LB) and right border (RB), respectively, which act as a cis element signal for the T-DNA transfer. Both oncogenic and opine catabolism genes are located inside the T-DNA of the Ti plasmid whereas the virulence (*vir*) genes are situated outside the T-DNA on the Ti plasmid and bacterial chromosome. These *vir* genes are organized into several operons (*vir*A, *vir*B, *vir*C, *vir*D, *vir*E, *vir*F, *vir*G, and *vir*H) on the Ti-plasmid and other operons (*chv*A, chvB, and *chv*F) that are chromosomal and are essential for T-DNA transfer. The mechanism of gene transfer from *A. tumefaciens* to plant cells involves several steps, which include bacterial colonization, induction of the bacterial virulence system, generation of the T-DNA transfer complex, T-DNA transfer, and integration of the T-DNA into the plant genome. The process of T-DNA transfer is initiated upon receipt of specific signals (e.g., phenolic compounds) received from host cells. Previous observations suggested that wounding or vigorous cell division also promotes T-DNA transfer, presumably due to induction by phenolic compounds produced during cell repair or during the formation of new cells. In response, a signal received by *vir*A activates a cascade of other *vir* protein machinery genes. However, very little is known about the nature and function of the factors that *Agrobacterium* utilizes, for instance, specific receptors on the host cell surface and/or cell wall. Subsequently, *vir*D1 and *vir*D2 proteins nick both the left and right borders on the bottom strand of the T-DNA. The resulting single-stranded T-DNA molecule (T-strand), together with several *vir* proteins, is then exported into the host cell

cytoplasm through a channel formed by the *Agrobacterium Vir*D4 and *Vir*B protein complex. Before its entry into the host cell cytoplasm, the *Vir*D2–T-strand conjugate is most likely coated by *Vir*E2, forming the T-complex. *Vir*E2 is a single-stranded DNA-binding *Agrobacterium* protein that is transported into the plant cell, where it presumably functions to protect the T-DNA from degradation. The *Agrobacterium* T-complex is likely transported through the host cell cytoplasm by a cellular-motor-assisted mechanism. The T-complex then enters the cell nucleus by an active mechanism mediated by the nuclear import machinery of the host cell. This facilitates integration of the T-strand into the host genome at random positions by a process of non-homologous, or more precisely, illegitimate recombination.

Table 1: Some recent reports on genetic transformation of fruit crops via *A. tumefaciens* or *A. rhizogenes*

Species	Aim	Plasmid	Transgenes	References
Apple	Stability of scab resistance	pMOG402.hth.gus.intron	*npt*II, *gusA*	Krens *et al*., 2011
Almond	Method optimisation	pBI121mgfp-5-ER pNOV2819	*npt*II, *pmi*	Ramesh *et al*., 2006
Avocado	Method optimisation	pMON9749, pTiT37-SE	*nptII*, *gusA*	Hernandez *et al*., 1998
Banana	Method optimisation (Agro + SAAT+ Vacuum infiltration)	pCAMBIA1301	*hptII*, *gusA*	Subramanyam *et al*., 2011
Blueberry	Method optimisation	pBISN1	*nptII*, *gusA*	Song and Sink, 2004
Grapevine	Method optimisation	Nr	*gusA*, *nptII*	Gago *et al*., 2011
Grapefruit	Resistance to Citrus tristeza virus	pGA482GG CP	*gusA*, *nptII*	Febres *et al*., 2008
Kiwifruit	Manipulation of plant architecture	pBI121	*İpt*	Honda *et al*., 2011
Mango	Rooting enhancement	Nr	*rol B*	Chavarri *et al*., 2010
Trifoliate Orange	Enhanced salt tolerance	pBin438	*nptII*, *AhBADH*	Fu *et al*., 2011
Papaya	Resistance to PRSV	pRPTW PRSV	*replicase gene*, *neo*	Chen *et al*., 2001
Pear	Method optimisation	PBISPG	*nptII*, *gusA*	Sun *et al*., 2011
Plum	New selection system with	pC1381, pC1301, pC2301	*hpt*, *nptII*	Tian *et al*., 2009

	hygromycin			
Pomegranate	Method optimisation	pBIN19-sgfp	*nptII, gfp*	Terakami *et al*., 2007
Strawberry	Method optimisation	pBI121	*nptII, gusA*	Barcelo *et al*., 1998
White mulberry	Method optimisation	pBI121	*nptII, gusA*	Agarwal and Kanwar, 2007

2. Microprojectile (Particle) bombardment transformation

Microprojectile bombardment is one of the direct gene transfer method for development of transgenics. This method was developed in 1980s to genetically engineer plants that were recalcitrant to transformation with *Agrobacterium*. Subsequently, the technique has been widely used to produce transgenic plants in a wide range of plant species. The first particle delivery method was developed by Sanford and co-workers. The Sanford device was extensively modified to produce the PDS-1000/He machine, which was licensed to DuPont. The technique involves coating microcarriers (gold or tungsten particles approx. 0.6-1.0 mm in diameter) with the DNA of interest and then accelerating them at high velocities, to penetrate into the cell of essentially any organism. Briefly, the microcarriers are spread evenly on circular plastic film (macrocarrier). The entire unit is then placed below the rupture disk in the main vacuum chamber of the biolistic device. A variety of rupture disks are available that burst at pressures ranging from 450 to 2,200 psi. Below the macrocarrier is a stopping screen, in which a wire-mesh is designed to retain the macrocarrier, while allowing the microcarriers to pass through. The target tissue is placed below the launch assembly unit. Under a partial vacuum, the microprojectile is fired, and helium is then allowed to fill the gas-acceleration tube. The helium pressure builds up behind a rupture disk, which bursts at a specific pressure, thus releasing a shock wave of helium that forces the macrocarriers down onto the stopping screen. The microcarriers leave the circular plastic film and continue flying down the chamber to hit and penetrate the target tissue, thus delivering the DNA. Several factors must be considered for successful gene transfer using particle bombardment technology. These factors include the design of a suitable vector with a small size and high copy number, as well as the quantity and quality of the delivered DNA. The entire process must be performed under sterile conditions to prevent contaminations of target tissue during subsequent tissue culture. The types and sizes of microcarriers are important choices because they affect the depth of penetration of the accelerated microcarrier as well as the degree of damage to the target cells. Gold particles ranging from 0.6 to 3.0 mm in diameter are commercially

available. The degree of penetration required will depend on the thickness of the cell wall, the type of tissue being transformed, and the depth of the target cell layers. Variation in the helium pressures, the level of vacuum generated, the size of the particles, and the position of target tissues will dictate the momentum and penetrating power at which the micro projectiles strike the tissue. All of these parameters are under the experimenter's control and must be optimized for a given target tissue. Treatment of the target tissues prior to and after particle bombardment has a significant effect on the frequency of recoverable transgenic cell lines and plants. An attractive feature of particle bombardment is its ability to transfer foreign DNA into any cell or tissue type whose cell wall and plasma membrane can be penetrated. Embryogenic and meristematic tissues are the most commonly employed target tissues for the production of genetically transformed plants. Particle bombardment of embryogenic tissues has been successfully exploited to produce transgenic plants in a wide range of agronomically important plants. One of the advantages of particle bombardment is the possible expression of multiple transgenes in the target tissue, which can be achieved by fusion of genes within the same plasmid that is then bombarded into the target tissues. However, this technology is limited due to several drawbacks, such as the integration of multiple copies of the desired transgene, in addition to superfluous DNA sequences that are associated with the plasmid vector. Multicopy integrations and superfluous DNA can lead to silencing of the gene of interest in the transformed plant. This problem was overcome by transferring the desired coding region only with its control elements into the target cells of plant genome.

Table 2: Some recent reports on genetic transformation of fruit crops via direct gene transfer

Species	Aim	Transfer system	Plasmid	Transgenes	References
Apple	Method optimisation	PEG-mediated	pKR10	*Gfp*	Maddumage *et al*., 2002
Banana	Resistance to virus	Particle bombardment	pAB6, pAHC17,pH1	*gusA, bar, ubi, BBTV-G-cp*	Ismail *et al*., 2011
	Tolerance to Sigatoka leaf spot	Particle bombardment	pYC39 ThEn-42	*StSy, Cu, ZnSOD*	Vishnevetsky *et al*., 2011
Cranberry	Herbicide résistance	Particle bombardment	pUC19 bar,	*aphII*	Zeldin *et al*., 2002
Grape	Method optimisation	Particle bombardment	pGA482GG	*nptII, gusA TomRSV-CP*	Scorza *et a*l., 1996

Papaya	Use of PMI/Man	Particle bombardment	pNOV3610	*Pmi*	Zhu *et al*., 2005
Kiwifruit	Method optimisation	PEG 4000	p35SGUS	*gusA*	Raquel and Oliveira, 1996

3. **Electroporation mediated transformation**

Electroporation-mediated transformation requires the application of strong electric field pulses to cells and tissues and is known to cause some type of structural rearrangement of the cell membrane. *In vitro* introduction of DNA into cells is now the most common application of electroporation. The technique was originally developed for protoplast transformation but has subsequently been shown to work with intact plant cells as well. A voltage of 25 mV and an amperage of 0.5 mA for 15 min are the most often used parameters. However, factors such as surface concentration of DNA and tolerance of cells to membrane permeation may affect electroporation efficiency. Using the electroporation method, successful transformation has been achieved with protoplasts of both monocot and dicot plants. However, using protoplasts as explants for regeneration of transformants limits the use of electroporation for stable transformation because the protoplast to-plant regeneration system has not been developed in most plant species. The electroporation of plant cells and tissues is very similar in its principles to the electroporation of protoplasts. Compared to biolistics, it is inexpensive and simple, but the technique has only been successful in a few plant species. The thick cell walls of intact tissues represent key physical barriers to electroporation.

4. PEG/Liposome mediated transformation

Polyethylene glycol (PEG)-mediated transformation is a method used to deliver DNA using protoplasts as explants. The method is similar to electroporation in that the DNA to be introduced is simply mixed with the protoplast, and uptake of DNA is then stimulated by the addition of PEG, rather than an electrical pulse. PEG-mediated transformation has several advantages because it is easy to handle and no specialized equipment is required. However, the technique is rarely used due to the low frequency of transformation and because many species cannot be regenerated into whole plants from protoplasts. In addition, fertility may be a concern because of the soma clonal variation of the transgenic plants derived from protoplast cultures. Related to PEG-mediated transformation is the liposome mediated transformation technique. In this method, DNA enters protoplasts via endocytosis of liposomes. Generally, this process involves three steps: adhesion of liposomes to the protoplast surface, fusion of liposomes at the

site of adhesion, and release of the plasmid inside the cell. Liposomes are microscopic spherical vesicles that form when phospholipids are hydrated. Liposomes being positively charged tend to attract negatively charged DNA and cell membrane. In this process, the engulfed DNA is free to integrate into the host genome. However, there have been very few successful reports on the application of this technique in plant species because the technique is very laborious and is associated with low efficiency. In tobacco, intact YACs were transformed via lipofection-PEG technique.

5. Silicon Carbide Mediated Transformation (SCMT)

Kaeppier *et al*. first reported the use of silicon-mediated transformation, which is one of the least complicated methods. In this method, small needle-type silicon carbide whiskers are mixed with plant cells and the gene of interest, and the mixture is then vortexed. In the process, the whiskers pierce the cells, permitting DNA entry into the cells. The fibers most often used in this procedure have an elongated shape, a length of 10-80 mm and a diameter of 0.6 mm and show high resistance to expandability. The method is simple, inexpensive, and effective on a variety of cell types. The efficiency of SCMT depends on fiber size, vortexing, the shape of vortexing vessel, as well as the plant material used for transformation. Furthermore, silicon carbide fibers have been found to improve the efficiency of *Agrobacterium*-mediated transformation. The main disadvantages of SCMT include low transformation efficiency and damage to cells, thereby negatively affecting their regeneration capacity. Furthermore, this method imposes health hazards due to fiber inhalation if not performed properly.

6. Microinjection

Microinjection is the direct mechanical introduction of DNA into the nucleus or cytoplasm using a glass microcapillary injection pipette. Using a microscope, cells (protoplasts) are immobilized in low-melting-point agar with a holding pipette and gentle suction; DNA is then injected into the cytoplasm or nucleus. The microinjection technique requires relatively expensive technical equipment for the micro manipulation of single cells under a microscope and involves precise injection of small amounts of DNA solution; the procedure is also very time-consuming. The injected cells or clumps of cells are subsequently cultured in tissue culture systems and regenerated into plants. However, microinjection has achieved only limited success in plant transformation due to the thick cell walls of plants and, more challengingly, to a lack of availability of a single-cell-to-plant regeneration system in most plant species.

7. Chloroplast mediated transformation

In genetically modified plants, the gene of interest usually integrates into the nucleus; however, it is also possible to transfer the gene into the plastid. The chloroplast genome is highly conserved among plant species and typically consists of double-stranded DNA of 120-220 kb, arranged in monomeric circles or in linear molecules. In most higher plant species, the chloroplast genome has two similar inverted repeat (IRA and IRB) regions of 20-30 kb, that separate a large single copy (LSC) region and small single copy (SSC) region. Both the microprojectile and protoplast mediated transformation methods are capable of delivering DNA to plastids, but to achieve successful transformation, chloroplast-specific vectors are required. The basic plastid transformation vector is comprised of chloroplast-specific expression cassettes and target-specific flanking sequences. Integration of the transgene into the chloroplast occurs via homologous recombination of the flanking sequences used in the chloroplast vectors. The first successful chloroplast transformation was reported in Chlamydomonas. In recent years, several crop chloroplast genomes have been transformed through organogenesis, and maternal inheritance has been observed. Advancement in chloroplast engineering has made it possible to use chloroplasts as bioreactors for the production of recombinant proteins and biopharmaceuticals. Because plastid genes are maternally inherited, transgenes inserted into these plastids are not disseminated by pollen. Additional advantages of this transformation system include the ability to express several genes as a polycistronic unit, thereby potentially eliminating position effects and gene silencing in chloroplast genetic engineering.

8. Alternative transformation systems: Transgenics without marker genes

A highly desirable approach to promote public acceptation for future commercialization of transgenic plants and products is focused on the elimination of marker genes from transformed plants or the direct production of marker-free transgenics (Kraus, 2010). These newly and promising approaches are highly dependent on previously established highly efficient regeneration protocols that may be based on organogenesis or embryogenesis (Petri *et al*., 2011). There are various technologies such as homologous recombination, cotransformation, site-specific recombination (Cre/loxP site specific recombination system, R/RS system, FLP/FRT system etc.) or marker elimination by transposons to remove selective marker genes (Hao *et al*., 2011; Manimaran *et al*., 2011). However, there are still few marker-free fruit species transformation protocols. Strawberry leaf

explants were transformed with site-specific recombinase for the precise elimination of undesired DNA sequences and a bifunctional selectable marker gene used for the initial positive selection of transgenic tissue and subsequent negative selection for fully marker-free plants (Schaart *et al*., 2004).

MAT (multi-auto-transformation) (Ebinuma *et al*., 1997) combined with the *Agrobacterium* oncogene *ipt* gene, for positive selection with the recombinase system R/RS for removal of marker genes acting as "molecular scissors" after transformation were used as alternative approach in citrus plants (Ballester *et al*., 2007; 2008). Also, in apricot (López-Noguera *et al*., 2009) a similar strategy was used. Regeneration of apricot transgenic shoots was significantly improved to non-transformed plants (regenerated in non-selective media).

Moreover, it was significantly higher in comparison with previous published data using resistance to kanamycin mediated by *nptII* gene. The lack of *ipt* differential phenotype promoted difficulties to assess the excision of the marker genes that require periodic assays. Complete excision of marker genes ranged from 5 to 12 months, however, only 41% of the regenerated transgenic shoots R-mediated recombination occurs correctly. In Citrus sp., it was also reported that anomalous excision of marker genes promoting failures in the expression of the reporter genes (Ballester *et al*., 2007, 2008). Apple (Malnoy *et al*., 2010) and pineapple sweet orange (Ballester *et al*., 2010) transformation using ''clean'' binary vector including only the transgene of interest were carried out to create marker-free transformants. In plum (*Prunus domestica*), transformation was carried out without reporter or selectable marker genes using a high-throughput transformation system (Petri *et al*., 2011).

9. Cisgenesis, the P-DNA technology and multigene transformation

Other relevant advance in fruit species transformation was the proposal made by Schouten *et al*. (2006), the "cisgenesis". This term means the use of recombinant DNA technology to introduce genes from crossable donors plants, isolated from within the existing genome or sexually compatible relative species for centuries therefore, unlikely to alter the gene pool of the recipient species. Cisgenesis includes all the genetic events of the T-DNA as introns, flanking regions, promoters, and terminators (Vanblaere *et al*., 2011). This methodology proposes to transfer the own plant DNAs, the P-DNAs. The use of this technology requires the construction of whole plant derived vector from the target species. Within the target species genome, it

must be a DNA fragment with two T-DNA border-like sequences oriented as direct repeats ideally about 1-2 kb apart with suitable restriction sites for cloning of a desirable gene. In the last years, different works were considered to step towards introducing regulatory elements and genes of interest from crossable donor plants, however with some foreign elements as marker genes in species as melon and apples (Szankowski *et al*., 2009). Up to 2011 there is no any report of real "cisgenesis" plantlets, in agreement with Schouten *et al*. (2006) definition of the topic. In 2011, Vanblaere *et al*. developed apple cv. Gala cisgenic plants by expressing the apple scab resistance gene *HcrVf2* encoding resistance to apple scab. Marker-free system was employed for the development of three cisgenic lines containing one insert of the P-DNA after removing by recombination with using chemical induction. These lines were not observed different from non-transformed cv. Gala plants. Cisgenic plants are essentially the same as the traditionally bred varieties, and they might be easier to commercialise than the "problematic" transgenic plants (Schouten *et al*., 2006; Rommens *et al*., 2007). Critical opinions to these proposals also were clearly exposed, the uncontrolled P-DNA integration into the plant target genome can cause mutations or affect to the expression of other native genes, altering the behaviour of that cisgenic plants in an unpredictable manner (Schubert and Willims, 2006; Akhond and Machray, 2009). Recently, interesting approaches are being proposed for genome editing using ZFNs (Zinc finger nucleases) that can promote induction of double-strand breaks at specific genomic sites and promote the replacement of native DNA with foreign T-DNA (Weinthal *et al*., 2010).

The multigene transfer (MGT) methodology consist in introducing more than one gene at once. Commonly, most of the transgenic plants are generated by introducing just one single gene of interest, but now MGT are being developed to obtain more ambitious phenotypes as the complete import of metabolic pathways, whole protein complex and the development of transgenic fruit species with various new traits simultaneously transferred (Naqvi *et al*., 2009). In this sense, this technology would be highly desirable for commercial fruit species cultivars to obtain new traits related with large fruit size, high coloration of the fruit epidermis, flesh firmness and virus resistance (Petri *et al*., 2011) at the same time without the need of several rounds of introgressive backcrossing.

Future perspectives and concluding remarks

The future of fruit genetic transformation is required of genotype-independent protocols, accuracy molecular tools to drive the T-DNA

insertion and its expression, and efficiency and highly-yield selection and regeneration *in vitro* culture methodology. But *Agrobacterium* mediated transformation procedure is a high nonlinear complex biological process, and its complexity can be understood with the composition of many different and interacting elements governed by non-deterministic rules and influenced by external factors. In this sense, the emergent technology dedicated to meta-analysis can be really useful to increase our understanding of fruit genetic transformation, making possible to identify relationships among several factors and extracting use full information generating understable and reusable knowledge (Gago *et al*., 2011; Gallego *et al*., 2011; Perez-Pineiro *et al*., 2012) Under these perspectives, modeling any fruit transformation procedure (*Agrobacterium-mediated*, biolistics, electroporation etc.) including the genetic engineering, *in vitro* plant tissue culture and regeneration stages will be improved for the next years. Genetic modification of fruit plants via cisgenesis or intragenesis may be other potential useful strategies. The acceptance of science-based approaches like cisgenesis or intragenesis or use of selection marker free transgenic will encourage confidence, and bring the benefits of GM products to consumers. In addition, there is a great need for global coordination of regulations to remove artificial trade barriers, promoting technology transfer, and protecting developing countries from exploitation (Harfouche *et al*., 2011; Gambino and Gribaudo, 2012).

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