

Biodiversity of Temperate Fruits



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Introduction

Many of the world's well-known fruits (apple, pear, peach, plum, grape, and strawberry) that grow in particular environments found in regions with Mediterranean climate are known as temperate fruits. These areas include California (USA), North Africa, Turkey, the Middle East, southern Europe, Greece, Central Chile, Australia, and some parts of Asia. These fruits can adapt well in two different climatic conditions, where they undergo a dormancy period to complete their cycle. This dormancy period helps the fruit to adapt well in tropical climates, and they have various degrees of winter hardiness, which helps the fruits to adapt cold conditions (Encyclopedia of Food and Culture 2003; Retamales 2011). Fruits are considered a valuable food commodity with many potential health benefits due to the presence of natural antioxidant components, which can contribute to the prevention of cardiovascular and other chronic diseases, such as heart disease, cancer, diabetes, and Alzheimer's disease. It has been revealed that carotenoids and polyphenols, such as anthocyanins, flavonoids, phenolics, and phenylpropanoids, present in fruits might act as antioxidants and therapeutic agents contributing to such action (Manzoor et al. 2012; Khomdram et al. 2014).

The major temperate fruit crops, viz., apple, peach, cherry, plum, apricot, and pear, belong to the Rosaceae family. Most of these woody perennials have a long intergeneration period because of their large plant sizes and juvenile phase, which make them poorly suited for classical genetic analysis. On the other hand, fruit trees also possess some advantageous features, such as long life, possibility of producing interspecific crosses, existence of efficient methods of vegetative reproduction, and a smaller genome size. The breeding methods used in these temperate fruits have undergone minor changes over the last 50 years. The incorporation of specific

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alleles from wild or exotic materials into elite breeding lines has rarely produced new commercial cultivars. The knowledge provided by advances in molecular genetics, notably molecular markers, provides faster and more efficient approaches of cultivar improvement (Dirlewanger et al. 2004).

Geographical Location, Cultivation, and Production of Temperate Fruit Crops

Based on the climate conditions, temperate fruits can be classified into two categories. The first category includes fruits such as apple, pear, plum, cherry, etc. which can grow well in cold conditions, while the second category includes fruits such as peach, plum, apricot, etc. which can also grow in warmer climates. The proper development of these fruits depends on soil conditions also. Different fruits require different soil conditions, like type, pH, fertilizers, etc. Here, in this section, a brief about the cultivation practices of a few of the temperate fruits is given. The geographical distribution, production, and major common diseases of temperate fruits are presented in Table 1.

Factors Affecting the Growth of Temperate Fruit Crops and Their Production

For successful growing of temperate fruit crops, various components of climate, such as temperature, humidity, light, rainfall, hail, and frost, should be carefully studied. Man cannot control these environmental factors. It is not possible to make any changes to them. However, the effect of these factors can be altered. For these, we can take certain steps to increase or decrease the effects, i.e., the effects of high or low temperatures can be altered, additional moisture can be provided, high wind velocity can be reduced by growing a wind break around the orchard. The climate of a particular region is mainly influenced by different factors, viz., latitude, altitude, topography, position related to continents and oceans, and large-scale atmospheric circulation patterns.

Temperature

Temperature is one of the most important components of climate. It plays a vital role in the production of temperate fruit crops. There are various different activities of plants, like growth and development, respiration, photosynthesis, transpiration, uptake of nutrients, water and reproduction (such as pollen viability, blossom fertilization fruit set, etc.), carbohydrate and growth regulators balance, rate of maturation, senescence, quality, yield, and shelf life of the edible products.

Table 1 Geographical distribution, production, and common diseases of temperate fruits

S. no.	Fruit	Geographical distribution (top five countries)	Worldwide production (in MT) (FAO 2014)	Disease
1	Apricots	Armenia, Afghanistan, Iran, Italy, Turkey	3.36	Brown rot of blossom/fruit and twig blight, shot hole disease, jacket rot, bacterial canker and <i>Eutypa</i> dieback disease, <i>Armillaria</i> root rot, <i>Phytophthora</i> root and crown disease, and crown gall disease
2	Figs	Turkey, Egypt, Algeria, Morocco, Iran	1.14	Surface mold or <i>Alternaria</i> rot, fig endosepsis, brown rot, internal rot, eye-end rot, pink rot, or soft rot Fig mosaic virus, <i>Aspergillus</i> rot, limb blight or dieback, smut, sour rot
3	Pears	China, Argentina, United States, Italy, Turkey	25.79	Fire blight, pear scab, <i>Pseudomonas</i> blossom blast and canker, crown gall, powdery mildew, black rot, black Spot
4	Peaches and nectarines	China, Spain, Italy, United States, Greece	22.79	Bacterial canker, bacterial spot, crown gall, phony disease, <i>Alternaria</i> rot, brown rot, <i>Phytophthora</i> crown and root rot
5	Apples	China, United States, Turkey, Poland, India	84.63	Fire blight, <i>Alternaria</i> blotch, apple blotch, apple canker, apple scab, cedar apple rust, crown rot, powdery mildew, woolly apple aphid, rosy apple aphid, and rosy curling aphid
6	Quinces	Turkey, China, Uzbekistan, Morocco, Iran	0.64	Quince leaf blight, cedar-quince rust, fire blight
7	Plums	China, Serbia, Romania, Chile, Turkey	11.28	Plum leaf spot, plum pox virus, plum pockets, crown gall, brown rot, black knot of plum
8	Raspberries	Russia, Poland, United States, Serbia, Mexico	0.61	Spur blight, cane blight and anthracnose, raspberry leaf spot, <i>Botrytis</i> fruit rot
9	Gooseberries	Germany, Russia, Poland, Ukraine, United Kingdom	0.17	Anthracnose, powdery mildew, leaf spot, cane blight or wilt, <i>Botrytis</i> dieback and gray mold berry rot, white pine blister rust
10	Strawberries	United States, Mexico, Turkey, Spain, Egypt	8.11	Angular leaf spot, bacterial wilt, cauliflower disease, <i>Alternaria</i> fruit rot, anthracnose and anthracnose fruit rot and black spot, black root rot, gray mold, leather rot, hard rot, leak, leaf blotch
11	Blueberries	United States, Canada, Poland, Germany, Mexico	0.53	Mummy berry, <i>Phomopsis</i> twig blight, <i>Phomopsis</i> canker, <i>Phomopsis</i> leaf spot and fruit rot, <i>Botryosphaeria</i> stem blight, bacterial blight/canker, gray mold
12	Persimmons	China, Korea, Spain, Japan, Brazil	5.19	<i>Armillaria</i> root rot, gray mold, leaf spots and blights, root and crown rot, wood decay or heart rots
13	Kiwifruit	China, Italy, New Zealand, Chile, Greece	3.44	Bacterial blossom blight, oak root fungus, <i>Phytophthora</i> , bleeding canker, gray mold

Top five countries data taken from www.mapsofworld.com, Production data taken from FAO (2014)

These functions of the plant should be ideal when the temperature is in the optimum range. During high temperatures, plants do not perform proper functions of growth, while at low temperatures, the physiological activities of plants are stopped. According to the different temperature ranges in the tropics, specific trees are grown in different locations, e.g., apple, pear, peach, and almond are successfully grown in regions of low temperature, known as temperate fruits. In warm winter areas, due to insufficient chilling temperature, fruit trees fail to complete their physiological rest period or meet their chilling requirements. As a consequence, buds remain dormant, and leaves and blossoms do not appear on the trees in the following spring. For this reason, temperate fruits like apple, apricot, pear, and plums are not considered suitable for tropical or subtropical regions.

The activities of the plant are affected by very high or very low temperatures. The temperature ranges are given below:

Minimum: 4.5–6.5 °C (40–43 °F)

Optimum: 24–27 °C (75–85 °F)

Maximum: 29.5–45.4 °C (85–114 °F)

Effect of Low Temperatures

Low temperatures have adverse influences on fruit trees. There are many effects of low temperatures, i.e.:

- **Desiccation:** Imbalance between absorption rate and transpiration rate
- **Chilling injury:** There is a disturbance in the metabolic and physiological process
- **Freezing injury:** Termed as undercooling protoplasm coagulation

Chilling Requirements

Chilling is needed for fruit crops that fall dormant in the winter in order to avoid frost injury and they do not resume their growth until a certain amount of chill has accumulated for fulfilling their chilling requirements. Climate change is likely to affect the chilling requirements of temperate fruit crops significantly and, therefore, the opportunity to meet this requirement will be reduced as the climate becomes warmer. The results of these climate changes are clearly apparent in the shifting of apple cultivation from lower elevations to higher altitudes in India. Insufficient chilling greatly influences flower initiation and fruit coloration, along with deterioration in fruit texture and taste. High temperatures and moisture stress increase sunburn and cracking in apples, apricots, and cherries in the higher altitudes. Insufficient chilling reduces pollination, fruit set, and, ultimately, the yield in walnuts, pistachios, and peaches. Low temperatures in the winter are important for fulfilling their chilling requirements to ensure uniform flowering, fruit set, and generate economically sufficient yields. In order to escape certain damages of tissues from low temperatures, fruit trees of temperate or cold climates have evolved

the mechanism of dormancy. After a certain duration of cold temperature (chilling), endodormancy is inhibited and the tree is ready to resume its growth cycle in the following spring (Luedeling et al. 2011).

Humidity and Frost

The atmospheric humidity plays a vital role in deciding the amount of moisture needed to produce a fruit crop. In hot and dry weather, an enormous amount of water is lost through transpiration. If the atmosphere is humid, even though hot, the amount is much smaller and, thus, a site in a humid belt needs less irrigation. High humidity combined with high temperature also promotes rapid growth and higher yield, but increases the incidence of pests and diseases. The water requirements of a plant also depends on humidity, but, generally, the requirements for water differ as per different plant species, e.g., to produce 1 kg of apples requires 250 L of water. The plant gets water from soil, but there are many factors affecting this process, i.e., (a) amount of water in the soil, (b) the availability of water also depends on the texture and structure of the soil, (c) water absorbing area of the tree. Water is lost from the plant through transpiration by leaves. Transpiration depends on humidity, temperature, wind, light, etc., and is necessary to maintain the health of the plant by maintaining the balance between the uptake and loss of water.

Light

Light is an electromagnetic radiation which is a form of kinetic energy (Fig. 1). It comes from the sun to the Earth as discrete particles called quanta or photons. Light is one of the most important factors affecting plant life. It is an integral part of the

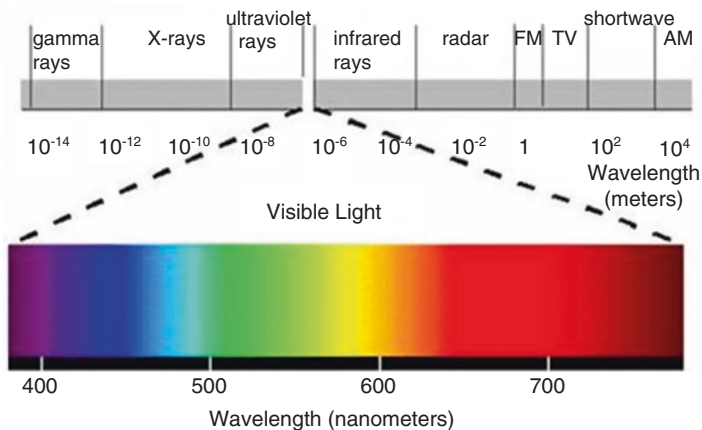
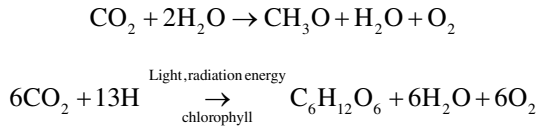


Fig. 1 Visible light spectrum showing different colors corresponding to different wavelengths of visible light

photosynthetic reaction, in which it provides the energy for the combination of carbon dioxide (CO₂) and water (H₂O) in the green cells having chlorophyll for the formation of carbohydrates with the release of oxygen. The following equation illustrates the oxidation of water during photosynthesis:



The crop growth performance of plants is mainly influenced by (a) the quality of light and (b) the intensity of light:

(a) **Light intensity**

Light intensity refers to the number of photons falling on a given area or to the total amount of light which plants receive; the intensity of light varies with day, season, distance from the equator, dust particles and water vapor in the atmosphere, slope of the land, and elevation. Symptoms associated with low light intensity are decrease in the rate of photosynthesis with normal rates of respiration, decrease in supplies of carbohydrates for growth and yield, leaf tips become discolored, leaves and buds drop, leaves and flowers become light in color. With high light intensity, the plant wilts and light-colored leaves may become gray in color due to the reduction in chlorophyll, and the rate of photosynthesis is lowered while respiration continues. All the above reasons can cause low yields.

(b) **Quality of light**

This refers to the length of waves. The visible part of the spectrum of electromagnetic radiation ranges from 390 to 730 nm in wavelength. It is also called photosynthetically active radiation.

In general, red and blue light produce a greater dry weight, whereas green light inhibits plant growth. Red light promotes seed germination, growth, and flower bud formation in long day/short night plants. Photosynthesis is greater in the red region. In apple, the blue-violet region is more important for the development of red pigments and color.

Rainfall

Rainfall is a very important factor for temperate fruit crops if an orchard is to be established in a new area. It is essential that the pattern of rainfall in the prospective region be studied before any decision is made concerning the types of crop to be cultivated. Well-distributed and consistent rainfall is always desirable for an ideal orchard site. Rain at the time of flowering is not suitable, because most fruit crops are sensitive to rain.

Biodiversity of Temperate Fruit Crops

Diversity is an essential raw material for evolution, which enables populations of the crop species to survive, adapt to new circumstances, and evolve to produce new genetic variants (Gerrano 2010). Genetic diversity arises due to differences between individuals that can be visualized as variations in the DNA sequence, biochemical properties (isoenzyme structure or properties), physiological characteristics (abiotic stress resistance), and morphology (Goncalves et al. 2009). Diversity estimates also provide conservation techniques and enable the careful selection of plant material for breeding programs. Diversity within a given plant population is said to be a product of biotic factors, the physical environment, artificial selection and plant characters such as size, mating system, mutation, migration, dispersal, and the influence of man through domestication and selection (Frankel et al. 1995).

Morphological Diversity

Temperate fruit crops vary from stone to berry fruits, with wide variation within the fruit crops. The variation in a particular fruit crop can be studied in terms of phenotypically, morphologically, biochemically, and genotypically. To identify different varieties with the same name, it is important to perform primary characterization of varieties for the proper management of plant genetic resources (Hend et al. 2009; Rao et al. 2010). Morphological characterization like color, size, and shape of the fruit allows the scientist to study variation in fruits by using visual attributes and have been widely used to discriminate between varieties of the same species (Cantini et al. 1999; Barranco and Rallo 2000). Morphological traits are affected by environmental conditions such as rainfall or solar radiation (Rotondi et al. 2003). Therefore, these traits are less reliable for studying the diversity in plants. However, on one hand, plant breeders are more interested in studying the diversity of a particular crop at the molecular level, but on the other hand, farmers believe that morphological and agronomic variation is best for attaining sustainable farming (Dempsey 1996). The utilization of morphological markers is the first and simplest method of evaluating crop diversity, which should be done before molecular characterization (Hoogendijk and Williams 2001). Chalak et al. (2006) characterized peach cultivars of Lebanon at the molecular and morphological levels for the first time. The results of this study revealed that flowering dates, maturity dates, fruit type, and flesh color had significant contributions to the total variation. Principal component analysis was used to study the morphological characters, viz., flowering time, time of harvesting, size and shape of fruit, percentage of russetting, and firmness of the flesh, to identify the main origins of pear cultivar variability collected from Spain (Pereira-Lorenzo et al. 2012). Pérez-Romero et al. (2015) studied the morphological and molecular characters of apples grown in Spain for diversity analysis and observed wide variation among morphological traits, such as diameter,

primary fruit height, and shape. Wide morphological variation has been observed in cherry based on the height of cherry rootstock (Ganji Moghadam et al. 2006). In the case of plum fruit, tree growth and pomological traits are important for studying morphological variation (Hend et al. 2009; Aran et al. 2012). Morphological characterization revealed a large diversity among almond accessions of Lebanon (Chalak et al. 2007). Thus, it can be concluded that the high diversity of the cultivars analyzed suggests a diverse origin of tropical fruit grown worldwide and have an important role in preserving endangered plant material for future use in breeding programs (Pérez-Romero et al. 2015).

Biochemical Diversity

Over the last few decades, people have become very conscious of their health and diet. In such a time, the importance of fruits for nutrition and their health benefits cannot be ignored. Fruits are the oldest food of mankind and abound with vitamins A, B, and C, and minerals like calcium, magnesium, iron, and potassium, as well as being a rich source of energy. These vitamins and minerals function as antioxidants, phytoestrogens, and anti-inflammatory agents (Slavin 2012). The antioxidant compounds, such as tocopherol, ascorbic acid, glutathione, and carotenoids, provide protection against oxidative damage from reactive oxygen species (Blokina et al. 2003). The antioxidants work by scavenging reactive oxygen species, inhibiting their formation, and preventing the formation of hydroxyl radicals and decomposition of lipid hydroperoxides (Niwa et al. 2001). Nutritionists advise us to consume at least 115 g of fruit every day for a balanced diet. In many countries, people eat fruits as their staple food. For example, people in the South American countries eat bananas as the main course of their meal (Kazi et al. 2015). Fruits can also increase our digestive power. An intake of fruit every day keeps us hale and hearty. Phosphorus and amino acids are abundant in apple, almond, etc. Citrus fruits and aonla are rich in vitamin C, while the richest source of vitamin C is Barbados cherry. Dry apricot is the richest source of calcium, phosphorus, and niacin. Additionally, fruits supply dietary fiber, and fiber intake is linked to a lower incidence of cardiovascular disease and obesity. It was also reported that a high consumption of fruits can help in preventing several non-communicable diseases, such as cardiovascular diseases, type 2 diabetes, and cancer (Ganry 2006).

Rapid progress has been made in plant molecular biology and biotechnology, which has opened up challenging possibilities in characterizing and evaluating biochemical diversity for estimating the nutrition profile of different temperate fruits for the benefit of mankind. The varied nutritional profile of different temperate fruits grown across the globe is shown in Table 2. The nutritional composition and content can also vary between the different genotypes and varieties of the same fruit. The biochemical parameters and antioxidant profiles of nine different genotypes of strawberry fruit were characterized and variation was observed in the total flavonoid, anthocyanin, vitamin C, and folate contents (Tulipani et al. 2008).

Table 2 Nutritional composition of different temperate fruits per 100 g

Fruit	Water (%)	Energy (Kcal)	Fiber (g)	Carbo hydrate (g)	Sugar (g)	Fat (g)	Protein (g)	Vitamin A (mg)	Vitamin B1 (mg)	Vitamin B2 (mg)	Vitamin B6 (mg)	Vitamin C (mg)	Vitamin E (mg)	Sodium (mg)	Potassium (mg)	Calcium (mg)	Iron (mg)
Apple	86.00	52.00	2.40	13.80	10.39	0.17	0.26	2.00	0.02	0.03	0.04	4.60	0.18	1.00	107.00	6.00	0.12
Apricot	86.00	48.00	2.00	11.00	9.00	0.40	1.40	420.00	0.03	0.04	0.05	10.00	0.89	1.00	259.00	13.00	0.40
Blueberry	84.00	57.00	2.40	14.50	9.96	0.33	0.74	0.00	0.04	0.04	0.05	9.70	0.57	1.00	77.00	6.00	0.28
Blackberry	85.00	43.00	5.30	9.61	4.88	0.49	1.39	30.00	0.02	0.03	0.03	21.00	1.17	1.00	162.00	29.00	0.62
Cranberry	87.00	46.00	4.60	12.20	4.00	0.13	0.39	0.00	0.01	0.02	0.06	13.30	1.20	2.00	85.00	8.00	0.25
Cherry	82.00	63.00	2.10	16.00	12.80	0.20	1.10	40.00	0.03	0.03	0.05	7.00	0.10	0.00	222.00	13.00	0.36
Elderberry	80.00	73.00	7.00	18.40	0.00	0.50	0.66	0.18	0.07	0.06	0.23	36.00	0.00	0.00	280.00	38.00	1.60
Fig	80.00	74.00	2.90	19.18	16.26	0.30	0.75	3.00	0.06	0.05	0.11	2.00	0.00	1.00	242.00	35.00	0.37
Gooseberry	88.00	44.00	4.30	10.18	0.00	0.58	0.88	0.00	0.04	0.03	0.08	27.70	0.37	1.00	198.00	25.00	0.31
Grape	83.00	69.00	0.90	18.10	15.50	0.16	0.72	0.00	0.07	0.07	0.09	3.20	0.19	2.00	191.00	10.00	0.36
Kiwifruit	84.00	61.00	3.00	14.66	9.00	0.52	1.10	5.00	0.03	0.02	0.06	92.70	1.46	3.00	312.00	34.00	0.31
Mulberry	88.00	43.00	1.70	9.80	8.10	0.39	1.44	0.00	0.03	0.10	0.05	36.40	0.87	10.00	194.00	39.00	1.85
Peach	89.00	39.00	1.50	9.54	8.39	0.25	0.91	15.00	0.02	0.03	0.02	6.60	0.73	0.00	190.00	6.00	0.25
Pear	84.00	57.00	3.10	15.23	9.75	0.14	0.36	0.00	0.01	0.03	0.03	4.30	0.12	1.00	116.00	9.00	0.18
Plum	87.00	46.00	1.40	11.42	9.90	0.28	0.70	18.00	0.03	0.03	0.03	9.50	0.26	0.00	157.00	6.00	0.17
Quince	84.00	57.00	1.90	15.30	0.00	0.10	0.40	0.00	0.02	0.03	0.04	15.00	0.00	4.00	197.00	11.00	0.70
Raspberry	86.00	53.00	6.50	11.94	4.42	0.65	1.20	0.00	0.03	0.04	0.05	26.20	0.87	0.00	151.00	25.00	0.69
Strawberry	91.00	33.00	2.00	7.68	4.89	0.30	0.67	10.00	0.02	0.02	0.05	58.80	0.29	1.00	153.00	16.00	0.41

Source: USDA Nutrient Database (USDA National Agricultural Statistics Service 2014), Kazi et al. (2015)

Belakud et al. (2015) reported the highest total sugar content (7.23%) in Belruby and the highest ascorbic acid content (50%) in the fruits of cv. Sweet Charlie.

Similar variations of biochemical contents between different apple cultivars was observed and it was reported that Golden Delicious had the minimum flavonoid content when compared to Red Delicious, Granny Smith, and Reinette apples (Escarpa and Gonzalez 1998). Hammerstone et al. (2000) analyzed the biochemical variation in different apple cultivars and found that Red Delicious and Granny Smith had the maximum procyanidin content, whereas the varieties Golden Delicious and McIntosh had the minimum contents. Jonagold apples were believed to contain the highest concentration of catechins, quercetin glycosides, and chlorogenic acid than Cox's Orange Pippin, Golden Delicious, and Elstar apples (Van der Sluis et al. 2001).

The variation in the nutritional composition of 16 plum genotypes growing at different locations of Pakistan was studied by Nisar et al. (2015). Their results suggested that the genotypes differed in their nutritional composition of fruits, anthocyanin, phenolic contents, and antioxidant activity of fruits. The vitamin C content in plum genotypes ranged between 52.51 and 137.6 mg/kg, the total sugar content from 67.17 to 105.07 g/kg, the anthocyanin content from 14.23 to 212.38 mg/100 g, and the total phenolic content ranged from 2.63 to 7.62 mg/g.

The findings of Kan et al. (2014) indicated that a higher content of phenolic compounds and vitamins was found in apricot fruits grown in irrigated conditions. The Cataloglu cultivar had the highest rutin contents and the Hacıhaliloglu cultivar contained the highest chlorogenic acid content in both irrigated and dry farming conditions. Vitamin C content was found to be higher than β -carotene, retinol, vitamin E, and lycopene contents in apricot fruits in both irrigated and dry farming conditions.

The biochemical variation of minerals, phenolics, and antioxidant activity in peel and pulp extracts of different genotypes of peach was studied by Manzoor et al. (2012). They observed that peach peel had significantly higher levels of minerals, antioxidant capacity, and phenolics than peach pulp, suggesting that the intake of unpeeled peach in the diet could act as a valuable source of high-value nutritional components and natural antioxidants for nutraceutical applications. Later, Liu et al. (2015a) studied the variation of nutrients in peach blossoms at different developmental stages and reported that total phenolics and flavonoids contents and antioxidant capacities were decreased during blossom development.

The highest content of organic acids, vitamin C, phenol, and flavonoids in indigenous pear cultivars were found in Karamut (0.44%), Gradišćanka (1.61 mg/100 g), Poljakinja (717.08 mg gallic acid equivalent/100 g of fresh fruit), and Mlolinjača cultivars (120.20 mg catechin equivalent/100 g of fresh fruit), respectively. The researchers observed the strongest antioxidant activity in cv. Karamut (Đurić et al. 2015).

Karlidag et al. (2009) studied six wild-growing sweet cherry genotypes with different fruit skin color, which were analyzed for their antioxidant activity, ascorbic acid contents, total anthocyanins, and total phenolic and total soluble solid contents. They found that the antioxidant activity was relatively higher in blackish skin colored

fruits than light ones. The total antioxidant activity ranged from 51.13 to 75.33%, whereas the total phenolic content was between 148 and 321 mg gallic acid equivalents (GAE)/100 g FW. Vitamin C was found to be highest in blackish colored fruits (21–27 mg/100 mL). The total soluble solid content varied between 19.35 and 23.98%.

Çağlarırnak (2003) carried out a beautiful study by investigating kernel properties and the composition of Turkish walnut genotypes. He found that Sebin Type-I and Güvenli showed good quality. These genotypes contained 6.9% palmitic acid, 7.5% stearic acid, 21.2% oleic acid, 43.4% linoleic acid, 6.3% linolenic acid, and 11.8% arachidic acid. The proximate components were protein (13.8%), ash (1.8%), moisture (3%), fat (62.8%), and carbohydrates (18.7%). The mineral components were phosphorus (316 mg/100 g), potassium (270 mg/100 g), calcium (85 mg/100 g), magnesium (90 mg/100 g), and iron (2.9 mg/100 g).

The study regarding the biochemical nutritional variability of temperate fruits will increase our knowledge for a better understanding of broadening the gene pool available for plant breeding programs to produce new varieties with superior biochemical and pomological characteristics. The selection of high quality fruit genotypes could help us to reduce fruit-related malnutrition issues in the human diet.

Genetic Diversity

Genetic diversity refers to the variability in heritable characters observed among individuals of a population (Kremer et al. 1998). The ultimate source of genetic diversity is gene mutations causing permanent change in the DNA sequence, molded and shaped by selection, recombination, gene flow, genetic drift, and migration in heterogeneous environments in space and time (Hartl and Clark 1997). Given the threat of abiotic and biotic stresses leading to crop loss, it is, thus, important to understand the genetic diversity in plant genetic resources and conserve it efficiently (Zhang et al. 2000). Further genetic diversity is vital in plant breeding programs for developing high yielding varieties and protecting the productivity of such varieties by integrating genes for disease and pest resistance or tolerance to abiotic stresses (Allard 1999). Research in plant genetics is focused on determining the amount of genetic variation in natural and domestic populations and developing methods for maintaining such variability to tackle the changing global environment (Gerrano 2010).

In a country with highly varied agroecological and diverse growing conditions, the availability of genetic diversity is significantly important for the maintenance, conservation, and enhancement of productivity in fruit crops. Such diversity has been providing security for farmers against biotic and abiotic stresses. Genetic diversity and the advent of molecular marker technologies offer great potential to add to the genetic improvement in temperate fruit breeding programs. Estimates of genetic diversity using new molecular tools, especially molecular markers, have proven to be useful in delineating heterotic groups already existing or to identify new ones (Casa et al. 2002).

DNA markers are commonly used for estimating genetic diversity in temperate fruit crops. RAPD, RFLP, and AFLP clearly distinguished the different stone fruit cultivars present in the international fruit market. RFLP markers developed for European and North American apricots produce unique profiles for most cultivars. Spanish cultivars cluster together, distinct from the remaining European and North American apricots (Hurtado et al. 2001; Romero et al. 2003). Recently, microsatellite or single sequence repeat (SSR) markers have been developed for peach (Cipriani et al. 1999), apricot (Messina et al. 2004), and applied for the characterization of cultivars and confirmation of geographic origin.

Warburton and Bliss (1996) analyzed 136 peach cultivars with RAPDs from different geographical origins. The genotypes were clustered into 12 main groups and nine of these clusters comprised Asian cultivars, while the European and American cultivars were grouped into three clusters, revealing less genetic diversity. Initially, for the analysis and classification of the major apricot, cultivars a group of 190 accessions were analyzed with ten newly developed microsatellite loci (Messina et al. 2004). The genetic distance was reflected in the grouping of cultivars in agreement with their geographic origin and pedigree.

Pinar et al. (2013) determined the genetic diversity in 57 Turkish apricot genotypes using 19 sequence-related amplified polymorphism (SRAP) primers and reported 87 amplified fragments with 64% polymorphism. Cluster analysis classified the 57 genotypes into three major groups with similarity ratios among genotypes between 0.73 and 0.94. Their study revealed that the SRAP marker system can be useful for genetic diversity analysis and identification of wild-grown apricots.

Yamamoto et al. (2001) identified 36 pear accessions that included Japanese pears, Chinese pears, and European pears. Liu et al. (2015b) studied the genetic diversity in 385 pear genotypes by using 134 SSR markers. A total of 690 alleles were produced at an average of 5.45. The clustering relationship divided the genotypes into three groups, with the primary division between occidental and oriental pears, revealing separate evolution processes. Population structure analysis with K values of 2–8 reflected a clear genetic composition within different genotypes, supporting *Pyrus sinkiangensis* as a hybrid of oriental and occidental pears, and *P. pyrifolia* and *P. bretschneideri* sharing a common ancestor. The comprehensive evaluation of a wide range of pear cultivars by SSR markers demonstrated their excellent application for the study of their genetic diversity and genetic relationships.

Hokanson et al. (2001) studied 142 *Malus* accessions with eight SSR primers and observed a high level of variation. Gross et al. (2014) studied the genetic diversity in apple (*Malus × domestica*) and found 96% genetic diversity by using SSR markers. There was no significant difference reported in heterozygosity (He) for *M. × domestica* compared with *M. sieversii* and *M. orientalis*. The improvement of genetic linkage maps using transferable markers, microsatellites, and RFLPs has provided a base for fruit genetics and breeding. Marker-assisted selection and comparative mapping was done in fruit crops of the Rosaceae family by Dirlwanger et al. (2004). About 13 maps were constructed from 562 markers that helped in comparing genomes among seven species of *Prunus* that included peach, cherry, apricot, almond, *P. davidiana*, *P. ferganensis*, and *P. cerasifera*.

Khoramdel et al. (2013) screened 40 quince genotypes that originated from six distinct geographic areas using 15 SSR markers. They reported 5.36 alleles per locus, with a mean PIC value of 0.76. An unweighted pair group method with arithmetic mean (UPGMA) and principal coordinate analysis divided the quince genotypes into five major clusters. They found that 83% of individuals in the clusters were positioned in their place of origin and concluded that geographic isolation leads to considerable genetic differentiation among Iranian quince collections.

Sharma et al. (2015) analyzed 24 sweet and wild cherry genotypes collected from the Czech Republic to determine genetic variation using 16 SSR primers. All the SSRs were found to be polymorphic and they generated a total number of 70 alleles with a mean of 4.4 alleles per primer combination. The allele frequency varied from 2.1 to 87.5% and the observed heterozygosity ranged from 0.25 to 0.96, with an average of 0.72, while the expected heterozygosity values varied from 0.22 to 0.75, with an average of 0.59. The PIC value ranged from 0.21 to 0.71, with a mean value of 0.523. Cluster analysis separated the cherry genotypes into two groups. The high level of genetic diversity obtained in the collection proved to be genetically diverse and, therefore, these genotypes would be useful to breeders for the development of new cultivars.

Ahmed et al. (2012) analyzed 82 walnut genotypes collected from the North Western Himalayan region of Jammu and Kashmir, India by a combination of 13 SSR and 20 RAPD primers. They observed a high level of genetic diversity within populations, and the number of alleles per locus ranged from 1 to 5 in the case of SSR and 2 to 6 in the case of RAPD primers. The clustering pattern of the dendrogram showed that all the accessions divided into four main clusters with various degree of subclustering within the clusters. These results have very good implications for walnut breeding and conservation programs.

Biotechnological Aspects of Temperate Fruits

Plant biotechnology is an interdisciplinary science that provides solutions to many agricultural challenges by the rapid selection and propagation of elite cultivars, conservation and maintenance of valuable germplasm, molecular-assisted selection, genetic improvement, and safeguarding human health. Such a type of biotechnology allows researchers to detect and map genes, discover their functions, select specific genes in breeding, and transfer genes for specific traits into plants for the development of agriculture and the purpose of improving food quality and nutritional value (Laimer et al. 2005). The implication of plant biotechnology in temperate fruit trees is very helpful for agriculture, health, and mankind by taking part in many areas of research that include:

- Maintaining postharvest biology and technology of fruits
- Identification and introduction of useful traits and genes that can contribute to national and global goals for agriculture

Maintaining Postharvest Biology and Technology of Fruits

The primary goal of biotechnology on postharvest biology and technology of fresh fruits is to reduce their losses in quantity and quality from harvest to consumption. Thus, biotechnology can be a useful tool for addressing some of the issues related to quality attributes and biological deterioration. Biotechnology can be used to improve color uniformity and intensity and to minimize undesirable colors, such as browning. Efforts have been directed to produce genotypes with low browning potential by lowering phenolic content and activities of phenylalanine ammonia-lyase and polyphenol oxidase. Fruit softening was reduced to maintain firmness by altering the cell wall metabolism in fruits and ethylene biosynthesis. Transgenic tomatoes with blocked ethylene biosynthesis have been developed and tested on a commercial scale. The flavor and nutritional quality of fruits was maintained and improved by the manipulation of multiple genes. Isolation of the polygalacturonase gene, antisense construction, and gene transfer has provided useful insights into the role of polygalacturonase in tomato fruit softening (Giovannoni et al. 1989). Gene expression during peach fruit development and softening is being studied with the objective of manipulating softening through gene transfer (Callahan et al. 1991). Genotypic differences in susceptibility to chilling injury have been shown in most chilling-sensitive commodities. Thus, it is possible to produce cultivars with lower chilling sensitivity to allow their handling at lower temperatures, especially in the winter season, for extending their postharvest life (Kader 2000).

Identification and Introduction of Useful Traits and Genes

Genetic transformation is a technique of altering the phenotype of fruit trees by adding single horticultural traits in existing cultivars without changing their commercial characteristics. Genetic improvement of fruit trees is essential for increasing fruit production. For most of these species, the desired new varieties contemplate the presence of agronomic and horticultural traits related to propagation, yield, appearance, quality, disease, pest control, abiotic stress, and shelf life. Incorporation of these traits into the genetic backgrounds of species by conventional breeding needs overcome some major disadvantages, such as long generation time, complex reproductive biology, high levels of heterozygosity, limited genetic sources, and linkage drag of undesirable traits from wild relatives. In addition, breeding by controlled crosses is hampered due to factors specifically related to complex characteristics, such as delayed flowering, unsuccessful fruit setting due to abortive embryos, massive fruit drop, and self-incompatibility barriers. Although the use of new technologies based on high-throughput platforms for sequencing and genotyping has deeply contributed to accelerating the association of molecular markers and major genes to their relevant traits, the feasibility of genetic modification relies on adequate technical systems which allow for results to be obtained in a reasonable time

frame. Regardless of the transformed events, highly regenerative systems for explant production and whole-plant regeneration are key steps in fruit tree genetic transformation (Prieto 2011).

Different traits have been modified in transgenic fruit trees, which comprise: (a) altered processing and storage qualities, (b) modified nutritional properties, i.e., the effect of desirable and undesirable components, (c) modified growth habit and vigor, (d) resistance to abiotic stresses, e.g., drought, low temperature, soil factors, and (e) resistance to biotic stresses (Laimer 2003). The breeding and cultivation of virus-resistant plants is a major contribution for the control of viral diseases. Work on pathogen-mediated resistance focusing on virus resistance breeding in fruit trees started in 1988 at the Institute of Applied Microbiology (IAM). For explaining the pathogen-mediated protection approach, the coat protein gene of the stone fruit pathogen plum pox potyvirus (PPV) was isolated and transformed in different explants, i.e., cotyledons, leaf disks, and embryogenic callus cultures, of different *Prunus* species (Baulcombe 1996; Waterhouse et al. 2001). Several transgenic lines were then regenerated and subjected to genetic characterization and evaluation of pathogen protection. Additionally, sequences of the PPV genome involving structural and non structural genes were introduced in different plasmids in both sense and antisense orientations for transformation and showed good levels of resistance in herbaceous model plants (Korte et al. 1995).

Embryogenic transformation lines of 14 different grapevine cultivars and rootstocks have been established that reported enhanced resistance to nematodes (Gölles et al. 2000). Currently at the IAM, many different fruit trees have been transformed, such as apricot, plum, cherry, and grapevine lines, with different sequences of the PPV genome, the *Prunus* necrotic ringspot virus genome, the genome of different grape viruses, e.g., grapevine fanleaf virus (GFLV), arabis mosaic virus (ArMV), grapevine virus A (GVA), and grapevine virus B (GVB), together with different marker genes, e.g., GUS, GFP, or NPTII (Korte et al. 1995; Gribaudo et al. 2003). These plants represent valuable tools to improve our understanding of host–pathogen interactions and may possibly allow the development of alternative defense strategies for crop plants (Laimer 2005). Efforts are required to create public understanding and acceptance for these crop plants. To build public confidence, many projects were initiated to demonstrate the step-by-step principle of working with genetically modified organisms (GMOs) in the case of transgenic fruit trees.

The damage and losses caused by fruit pathogens are unacceptable to orchardists, marketers, and consumers. For the last 20 years, genetic transformation of fruit crops has focused on enhancing disease resistance (viruses, fungi, and bacteria), increasing tolerance of abiotic stresses (drought, frost, and salt), and many agronomic and horticulturally important traits, such as improved fruit quality, long shelf storage life of fruit, and fruit softening and ripening (Litz and Padilla 2012). However, the development of genetically modified fruit plants and their commercialization are hindered by many regulatory and social hurdles. From the biosafety and consumer points of view, the presence of selectable marker genes, which are essential for the initial selection of transgenic plants, is undesirable in the latter stages (Tuteja et al. 2012). Therefore, the production of ‘clean’ marker-free

transgenic fruit plants is now an essential requisite for their commercial exploitation (Gambino and Gribaudo 2012; Rai and Shekhawat 2013). Genome-wide genetic maps became important for the identification and isolation of genes and study of their structure, expression, and function. These maps allowed the mapping of genes associated with many agronomical characteristics of interest, such as dwarfing, fruit acidity, apomixes, male sterility, resistance to salinity, and disease resistance. The integration of transcriptomics and metabolomics could also generate more accurate information about the biochemistry and physiology of fruit plants, such as transcription, translation, environmental effects, and metabolite accumulation (Machado et al. 2011).

Conclusion

Temperature, light, rainfall, humidity, frost, fertilizers, and soil requirements affect the growth and production of temperate crops. The high biodiversity in morphological, biochemical, and genetic terms revealed a diverse origin of temperate fruits that have an imperative role in preserving germplasm for future use in breeding programs. The increase of desirable genes in the breeding stock is a step-by-step process in which the choice of parents is only one step. Since genetic uniformity is a threat to the improvement of breeding families, a wide genetic base is necessary to avoid an increase in inbreeding levels. The implication of plant biotechnology in temperate fruit trees allows researchers to detect and map genes, identify their functions, and transfer specific genes into plants for the development of agriculture, health, and mankind.

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