Christoph-Martin Geilfus

Controlled Environment Horticulture

Improving Quality of Vegetables and Medicinal Plants

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Dedicated by C.-M. Geilfus: To my family

Preface

Plant-based food not only is important for caloric supply, but also acts as a crucial source of human health-promoting metabolites. These metabolites belong to groups including glycosides, polyphenols, alkaloids or terpenes, the members of which can act as anticarcinogens or in an analgesic, antihyperglycemic, antibacterial, antiarrhythmic or even antimalarial manner. Many of these compounds are produced by plants in response to stresses from the environment and allow plants to withstand unfavourable conditions.

The horticulturist can utilize such stress responses to enrich vegetables or medicinal plants with beneficial compounds. For instance, after the induction of a mild stress event in a controlled manner (e.g. lack of water, high temperature, nutrient shortage), a plant can initiate defence reactions, viz. the production of these metabolites; however, the stress intensity is controlled in order that biomass formation is not affected.

The fine-tuned control of production factors such as water, temperature or nutrient availability is only possible during protected cropping. In horticulture, this refers to production within, under or sheltered by structures such as covers, artificial shading, plastic tunnels or greenhouses.

This textbook entitled *Controlled Environment Horticulture: Improving Quality of Vegetables and Medicinal Plants* describes in detail ways in which the horticulturist can control abiotic and biotic production factors in order to adjust the metabolism of the crop to the production of metabolites that are beneficial when ingested as part of a plant-based diet or as plant-based pharmaceuticals. Before guidance in this regard is given, a theoretical background is provided that allows the reader to apply their acquired knowledge to other situations.

The unique character of the book is that undergraduate and graduate students have written many parts of this textbook. In this way, complex plant physiological concepts are presented in a simple and understandable manner, making this book the perfect format for undergraduate students.

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Contents

III Exercises

Author's Biography

Christoph-Martin Geilfus

is a horticulturist and agronomist, born in 1983, who studied Agriculture and Agrobiotechnology in Giessen, Germany. He received a doctorate and habilitated in Plant Nutrition in Kiel, Germany. After working as a Visiting Professor in Leuven, Belgium, he was appointed as Professor for Controlled Environment Horticulture at Berlin, Germany. His research interests include horticulture, the mineral nutrition of crops, apoplastic stress signalling and guard cell biology.

Abbreviations

ATP	Adenosine triphosphate
CO_2	Carbon dioxide
CRI	Colour rendering index
DLI	Daily light integral
DNA	Deoxyribonucleic acid
LED	Light-emitting diodes
nm	Nanometre
OH	Hydroxyl group
PAR	Photosynthetically active radiation
PPFD	Photosynthetic photon flux density
RNA	Ribonucleic acid
UV	Ultraviolet

Introduction

Contents

Introduction

Contributions by Roland Sier (rolandsier@gmail.com).

C.-M. Geilfus, *Controlled Environment Horticulture*,
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1

The development of horticultural production systems is thought to have had a major influence on human history. Von Hagen (1957) speculated that 'the transition of human communities from nomadic hunter-gatherers to sedentary or semi-sedentary horticultural communities' was partly determined by the start of crop cultivation on a small scale around human dwellings. Horticulture is a sector of agriculture in which plants are primarily grown to serve as food (fruits and vegetables), herbs or medicinal plants (cannabis, garlic) or for hedonistic pleasure (ornamental plants). Horticulture comes from the Latin words *hortus* (= garden) and *cultura* (= managing). Nowadays, horticultural production is often extremely intensive and highly concentrated in certain areas. Examples of these regions are California (USA), Andalusia (Spain), the Netherlands or the Shandong province in China. The horticultural and medicinally produced goods are mostly highly perishable. Reasons for this are, for example, the high surface-area-to-volume ratio of, for example, leafy greens (Watada et al. 1996). Processing and/or distribution should therefore take place as quickly as possible and may require extra cooling in the 0–10 °C range (Watada et al. 1996). This maintains freshness and preserves health-promoting characteristics of certain substances. Otherwise, the nutritive value and/or activity and amount of pharmaceutically active substances might decrease because of time delays, transport distance or postharvest temperature. Important attributes that determine the nutritive value (inner quality) of vegetables are the amount and nature of carbohydrates, proteins, lipids, fibres, vitamins, minerals or other secondary metabolites (Fig. 1.1). Secondary metabolites have several functions and important for the stress response of plants to environmental factors (see ▶ Chap. 3). As many of these secondary metabolites are phytochemicals, they can sometimes be used to modulate human health. This textbook focuses on horticultural strategies to enrich these compounds within vegetables and medicinal plants without decreasing their yield. The inner quality of crops can also be affected by a plant's uptake of drug residues or agrochemicals. Factors determining the outer quality of horticultural crops are size, shape and colour (Fig. 1.1). These outer quality features will not be discussed in this book.

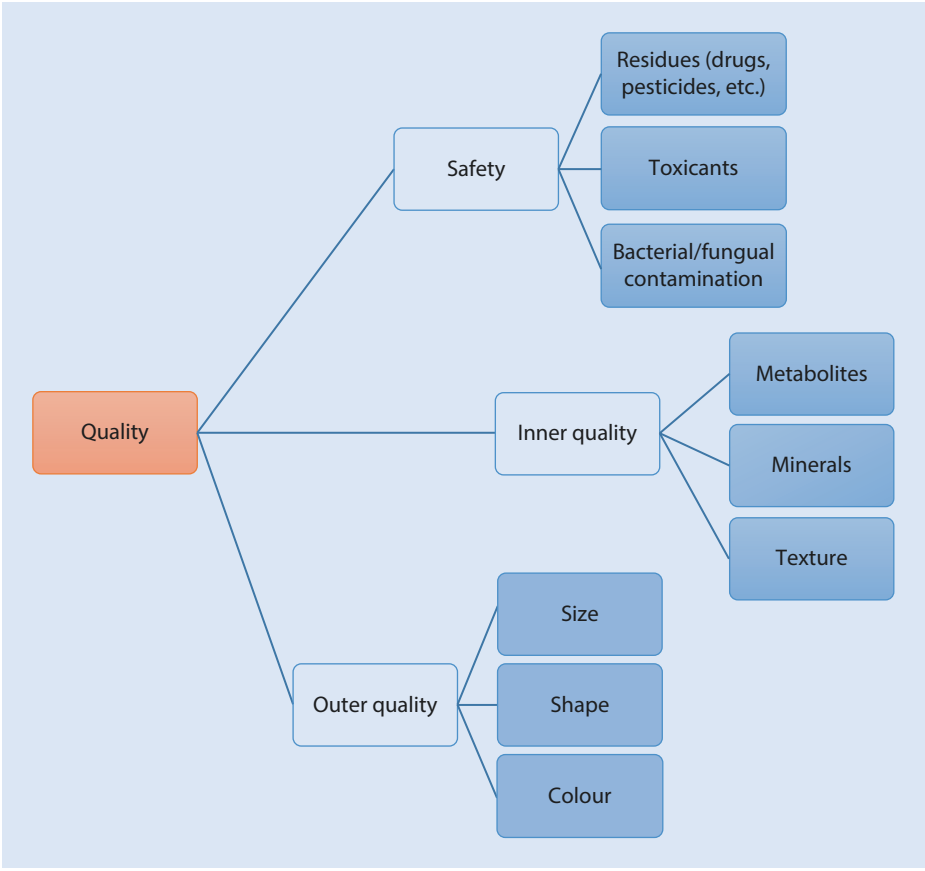

Fig. 1.1 Overview of various aspects of plant quality (simplified representation)

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Protected Cropping in Horticulture

Contributions by Roland Sier (rolandsier@gmail.com).

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2

The production of horticultural crops within, under or sheltered by structures such as covering material (e.g. mulch), shade cloth, plastic tunnels or greenhouses is called protected cropping. These structures or materials can help growers to modify conditions for plant growth, as they can protect plants against pests or adverse weather conditions. The degree of protection and control can range from a low-cost protection shelter (e.g. fabric/cloth) on a field to greenhouses and further to completely controlled environment horticulture (CEH) systems ('plant factory') (■ Table 2.1).

The highest level of protection and control is given by CEH by means of which the sophisticated integration of structures and technologies allows the attainment of an entirely controlled growth environment. The objective is to minimize or remove all constraints in a production system. Thus, most production factors such as temperature, humidity, nutrients and lighting are controlled. The controlled overdose or critical shortage of any of these production factors, e.g. light (▶ Chap. 5), can induce a stressful situation for plants and (if applied gently and controlled tightly) the plants accumulate

■ **Table 2.1** Direct comparison of cultivation practices in open fields, greenhouses and indoor systems

		Degree of protection			
		Open fields	Soil culture (in greenhouses)	Hydroponics (in greenhouses)	Indoor systems (in closed rooms or buildings)
Stability and controllability	Natural stability of aerial zone	Very low	Low	Low	Low
	Artificial controllability of aerial zone	Very low	Medium	Medium	Very high
	Given stability of root zone	High	High	Low	Low
	Artificial controllability of root zone	Low	Low	High	High
	Vulnerability of yield and quality	High	Medium	Relatively low	Low
	Initial investment per unit (land) area	Low	Medium	Relatively high	Extremely high
	Possible yield	Medium	Medium	Relatively high	Extremely high

Adapted from Kozai et al. (2016)
To achieve, for example, low yield and quality vulnerability in hydroponics and indoor systems, the manager's skill must be high to maintain the best growing conditions with all factors (Dicu and Badescu 2011)

metabolites that help them to endure these unfavourable conditions without decreasing yield. In some instances, these metabolites add human health-promoting features to the respective horticultural products/food. An example of the way in which plant metabolism can be changed by CEH is the foliar application of plant hormones. This measure has the potential to improve the quality of the crop and is discussed in detail in ▶ Chap. 15 and outlined in ▶ Chap. 21. For instance, plant hormones can be sprayed onto the leaves of growing plants with the aim of inducing a hormone-specific response in the plant and resulting in the synthesis and accumulation of human health-promoting metabolites. Of course, these newly synthesized metabolites help the plant to cope with the applied stress.

In the northern hemisphere, CEH allows year-round plant production, which would be impossible on an open field as it is too cold and dark in winter. Because of (1) the costly acquisition of production factors (ranging from nutrients to heating and to water) and (2) the high investment costs in buildings, structure and maintenance, CEH is viable only for high-value crops such as tomato, capsicum, lettuce/other leafy greens, cucumbers, eggplant, herbs, medicinal plants and some types of cut flowers (Hadley 2017).

An impressive example of a CEH facility in extreme conditions is the EDEN ISS project in Antarctica (◘ Fig. 2.1). This CEH test module, operated by the German Aerospace Centre, provides scientists with leafy greens, cucumbers, tomatoes and other produce. It can be seen as an experimental CEH to gain knowledge for its future possible implementation in the ISS space station. From mid-February to the beginning of September 2018, scientists produced around 77 kg leafy greens, 51 kg cucumbers and 29 kg tomatoes in a shipping-container-like module with a length of 6 m. In total (with herbs, radish and kohlrabi), 183 kg vegetables were produced in the Antarctica (DLR 2018).

Biotic and Abiotic Environmental Factors Plants, like all living beings, have special needs. For successful reproduction, insects or other pollinators might be mandatory. In order to grow and develop, plants need, for example, light, water and nutrients. If the supply of these growing factors exceeds or falls below a certain range, plants might experience stress. This can result in adaptive reactions resulting in the accumulation of certain metabolites that are important for the fitness/defence of the plants (Jahangir et al. 2009). If the stress is too severe, this may result in stunted plant growth or, in the worst case, the death of the plants. However, 'not every deviation from conditions that permit optimal growth is regarded as stress. (…). For plants, such constructive, conditioning stress includes, e.g., periodic lack of water, changes in temperature, and large fluctuations in irradiation, all having a "training" and "hardening" effect and are thus "constructive" stresses, even though they decrease the biomass production somewhat. These fluctuations in conditions and the demands they place on the plant are required for life and contrast with destructive stress' (Bresinsky et al. 2013). The dose and the intensity of the environmental factor are critical. If overdosed, any production factor can become a stress factor (◘ Table 2.2). For instance, light is necessary for growth. However, high light exposure can induce light stress (▶ Chap. 5). Water is vital for all plants, but, when the water content in a rooting medium is too high, this can cause root rot or anoxia, whereas a lack of water can result in wilting. Mild water scarcity, on the other hand, might lead to adaptation reactions that accumulate bioactive plant compounds (▶ Chap. 8).

Fig. 2.1 EDEN:ISS, a greenhouse in the Antarctic for the food supply of the future. The EDEN ISS greenhouse is located at the Neumayer III Antarctic station, which is operated by the Alfred Wegener Institute. German Aerospace Centre (DLR) scientists are using the EDEN ISS greenhouse in the inhospitable environment of the Antarctic to replicate as closely as possible the conditions of a long-term mission in space. *Top* EDEN:ISS in the Antarctic. *Middle* DLR scientist Paul Zabel grows vegetables in the eternal ice. *Bottom* Even penguins queue up to get some of the fresh food. EDEN:ISS is a project of the DLR. Pictures and text reprinted with permission of the DLR. (Source 1: ► https://www.dlr.de/dlr/en/desktopdefault.aspx/tabid-11008/. Source 2: ► https://www.flickr.com/photos/dlr_de/sets/72157683682719480)

Table 2.2 Abiotic factors that affect horticultural production in closed systems, possible control units and their problems/difficulties

Abiotic factor	Control unit	Problems/difficulties of management
Light	LEDs, special lamps, shading	Excess light can cause burns; deficiency causes stunted growth or death of the plant
Temperature, energy and heat	Heaters, air conditioning, shading	Excess high temperature can impair growth and photosynthesis (see ► Chap. 9) resulting in stunted growth; optimal temperatures are needed for optimal metabolic activity
Humidity	(De)humidifier, air conditioning, temperature	Excess humid air can favour fungi infections and minimize transpiration-driven nutrient uptake
(Soil) water	Irrigation, water management	Deficiency causes plants to wilt; excess water can cause root rot
Nutrients	Fertilization	Excess nutrients can cause toxicity, whereas too little causes deficiencies
Wind and air exchange	Openings, fans	Strong or permanent wind can harm plants; however, wind is important for the hardening and stability of plants
Soil/growing medium	Soil amendments, tillage, liming	If deficient or exhibiting an extreme pH, plant nutrients can be suboptimal
CO_2	CO_2 gas cylinders	Too much CO_2 can lead to growth retardation, whereas too little results in inefficient photosynthesis (see ► Chap. 14)

Stress can be grouped into abiotic and biotic factors. Abiotic means that the factor is non-living (e.g. water, wind, radiation). Biotic means that the factor is a living entity (e.g. bacteria, fungi, plants, insects such as aphids) or that it is a virus. Biotic factors can threaten the production of the whole crop. Aphids, for example, can additionally transfer viruses. In a well-managed CEH facility, certain precautions can help to avoid pest insects entering the facility. Moreover, the tight control of temperature, air exchange and humidity avoids the growth of fungal pathogens.

Not all of the production factors shown in Table 2.2 are controllable in each type of production system. Moreover, the control and/or adjustment of all of them is not always economical. Every production system has a tradeoff. In cooler regions, heating costs are higher and could increase further with poor insulation. However, the more efficient the insulation is, the more photosynthetic active radiation derived from the sun is excluded resulting in an increased need for artificial lightning, which consumes electricity (see ► Chap. 5 for further reading). Finding the right balance between input (energy and labour) and output (yield and quality) is the key for producing crops economically. The

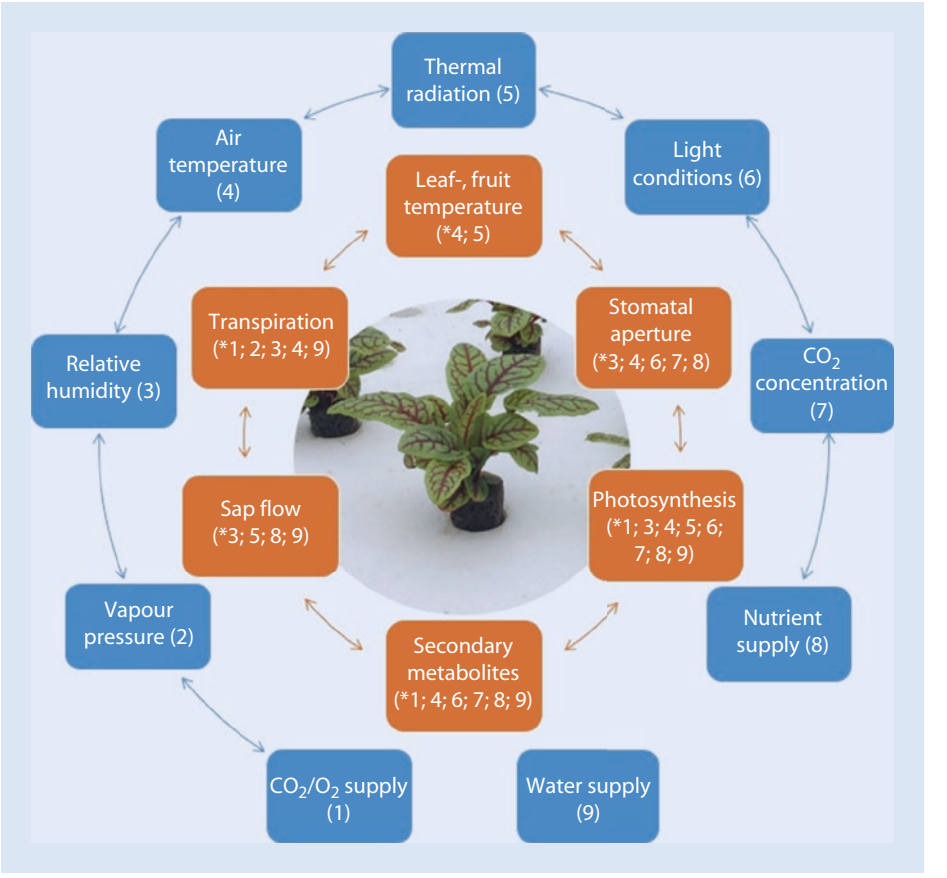

Fig. 2.2 Interaction between various abiotic factors (*blue boxes*) and effects on the plant (*orange boxes*). The *numbers* in the *blue boxes* represent the indicated abiotic factor. These shortcuts are shown in the *orange boxes* to link the stress factor with the plant response (∗ interaction with)

horticulturist must also be aware of the fact that most controllable production factors interact with each other. For instance, a change in air temperature also effects relative humidity. Both factors in turn influence the plant's internal processes involved in yield and crop quality, such as transpiration or photosynthesis (Fig. 2.2).

2.1 Horticultural Vegetables

Vegetables are extremely important for a healthy diet. A suitable daily consumption of around 400 g (excluding starchy tubers such as potatoes) is recommended by the World Health Organization (WHO) and the Food and Agriculture Organization of the

Table 2.3 Quantity of the most commonly produced vegetables worldwide in 2016

Crops	Produced mass (t)
Tomatoes	177,040,000
Onions, dry	93,170,000
Cucumbers and gherkins	80,620,000
Cabbages and other brassicas	71,260,000
Eggplants	5,129,000
Carrots and turnips	42,710,000

United Nations (FAO). They recommend this level because the eating of vegetables provides many essential compounds for the human body and is effective against many diseases including cardiovascular diseases and cancer (World Health Organization 2018). Horticultural vegetables can be grouped into:

- Fruit vegetables (e.g. tomatoes, chillis, cucumbers, watermelons)
- Root and tuber vegetables (e.g. radish, beets, carrots)
- Leaf vegetables (e.g. spinach, various salad types, kale)
- Stem vegetables (e.g. celery, asparagus, lemongrass)
- Flower vegetables (e.g. cauliflower, broccoli)
- Podded, bulb and corm vegetables

All of the above can be grown in soil systems. Root, tuber and bulb vegetables cannot be produced in hydroponic systems unless a culture substrate is available that allows tuber or bulb formation. Nevertheless, the cultivation of tuber-forming crops such as potato (*Solanum tuberosum* L.) will never be practicable in hydroponic systems, as the yield is not comparable to soil-based cultivation systems. Cucumbers and tomatoes are the main vegetable products from greenhouse horticulture in the Netherlands, one of the most important horticultural producers in Europe. In 2017, around 910,000 tons (t) tomatoes and 40,000 t cucumbers were harvested in the Netherlands (Statistics Netherlands, CBS 2018). Of all vegetables produced worldwide, tomatoes (*Solanum lycopersicum* L.) are top of the list (Podmirseg 2016). In the 1950s, the popularity of tomatoes, especially in Europe, increased until it became one of the most widely produced and demanded vegetables in the world (Podmirseg 2016). In 2011, around 158,019,580 t tomatoes were produced worldwide (Statistics Netherlands, CBS 2018), a status that further increased as shown in Table 2.3. The main producer of vegetables in general is China (mainland) with around 169,230,000 t (Statista, statistics portal 2018).

2.2 Medicinal Plants

Hardy et al. (2012) showed the first evidence for the use of medicinal plants by Neanderthal individuals. Back then, medicinal plants and herbs were the primary source of healthcare agents and are still used worldwide as a significant part of traditional and modern healthcare systems. In a recent report by the WHO, the percentage of the population that used plant-based medicine at least once amounts to 48% in Australia, 70% in Canada and 75% in France (WHO, Fifty-sixth World Health Assembly 2003). The distinction between medicinal and spice plants can be seamless. Garlic (*Allium sativum*), for example, is both a spicy and a medicinal plant. The active compound in garlic, namely, aniline, has health-promoting effects and is responsible for its desired sensory features such as taste and smell. This main compound of garlic shows multiple beneficial effects, such as antimicrobial, antithrombotic, antiarthritic and antitumour activity (Thomson and Ali 2003). China is the biggest producer of garlic with around 21,197,000 t in the year 2016 (FAO 2018). International trade in medicinal plants has become a major part of the global economy, and demand is increasing in both developing and industrialized nations (Kozai et al. 2016). However, the consumption of plant-based medicines has been accompanied by issues of quality and consistency, compromising its safety and efficacy and leading to serious health issues (Zobayed et al. 2005). Within different plant tissues, the concentration of wanted phytochemicals naturally varies strongly. Moreover, unwanted ingredients might be present in some parts of a medicinal plant. Therefore, only certain parts of a medicinal plant are harvested and used. ◘ Table 2.4 summarizes the various plant parts that can be harvested.

Important secondary metabolites for medicinal use are, for example, saponins, flavonoids, alkaloids and capsaicin (◘ Table 2.5; see also ► Chap. 3). Saponins act in an anti-inflammatory, immunostimulatory, hypocholesterolaemic, hypoglycaemic, antifungal and cytotoxic manner (Marrelli et al. 2016). Flavonoids neutralize free radicals, which possibly allows them to reduce risk of cancer (Hirschi 2009). Furthermore, they possess anti-inflammatory, anticancerogenic and antiviral properties (Lee et al. 2007). Alkaloids are often used in traditional medicine and have specific effects on the nervous systems of animals and humans. Capsaicin, which tastes spicy (hot), is found in chilli peppers and confers many health-promoting features. The most recent studies indicate that capsaicin-rich diets have favourable effects on atherosclerosis, metabolic syndrome, diabetes, obesity, non-alcoholic fatty liver, cardiac hypertrophy, hypertension and stroke risk (McCarty et al. 2015). It is also added to skin creams to fight fissures/cracking.

Recent research on medicinal plants shows that growing them in CEH with artificial lightning is suitable for guaranteeing the activity of the active ingredient and the safety of the medicinal plant products. Moreover, it ensures the year-round harvesting of products and maximizes biomass production and quality by optimizing nutrient uptake and environmental factors such as temperature and CO_2 concentration (Kozai et al. 2016). Experiments on sprouts performed by Mewis et al. (2012), for example, revealed that additional UV-B radiation was linked to increases in phenolic compounds and flavonoids such as kaempferol and quercetin, which accumulated in broccoli sprouts (*Brassica oleracea* var. *italica*) 24 h after UV-B treatment. Afreen

Table 2.4 Overview of parts from medicinal plants and their Latin nomenclature

Harvested part	Latin name in medicine
Blossom	Flos
Leaf	Folium
Herb	Herbe
Fruit	Fructus
Seed	Semen
Bulb	Bulbus
Bark	Cortex
Root	Radix
Rhizome	Rhizoma
Tuber	Tuber
Wood	Lignum
Stem	Stipes/caulis
Twig	Ramulus

The various parts of the medicinal plants are processed in different ways. Specific terms that name a certain kind of processing or postharvest treatment such as 'peeled' (mundatus) or 'ethanolic extract' (extractum spiritosum) are widespread. Such a specific treatment is critical for preserving health-promoting substances and their characteristics

Table 2.5 Examples of medicinal plants and their active ingredients

Plant species	Secondary metabolite (group)
Sisal (*Agave amanuensis*), Chinese liquorice (*Glycyrrhiza uralensis*), fenugreek (*Trigonella foenum-graecum*)	Saponins
Roman licorice (*Glycyrrhiza echinata*), water pepper (*Polygonum hydropiper* L.)	Flavonoids
Arabian coffee (*Coffea arabica* L.), tree of heaven (*Ailanthus altissima*), rauvolfia (*Rauwolfia sellowii*)	Alkaloids
Chilli pepper (*Capsicum annuum* L.)	Capsaicin

et al. (2005) treated Chinese liquorice (*Glycyrrhiza uralensis*) with UV-B radiation together with elevated CO_2 levels and found that the content of glycyrrhizin increased in root tissues. A study on St. John's wort (*Hypericum perforatum*) established that the enrichment of the CO_2 atmosphere in CEH induced the accumulation of hypericin and pseudohypericin (Mosaleeyanona et al. 2005) (see ▶ Chap. 14). Both compounds are known for their antidepressant efficacy.

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Plant Secondary Compounds

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In the plant cell, all the enzyme-catalysed transformation processes that allow and regulate growth, development and reproduction are summarized as metabolism. This process can be divided into anabolic and catabolic metabolism. In anabolism, smaller cell components are built up or assimilated to give more complex molecules. Important examples for anabolic processes are carbon fixation or fatty acid synthesis. During catabolism, organic material is broken down (dissimilation), e.g. during glycolysis, respiration or fatty acid beta-oxidation. Other examples of catabolism include the breakdown of polysaccharides to monosaccharides or the breakdown of nucleic acids to nucleotides. Plants also use catabolic processes to release previously stored energy, metabolic intermediates or nutrients for new anabolic reactions. Thus, metabolism comprises the energy exchange between energy-producing catabolic processes and energy-consuming anabolic processes (Bresinsky et al. 2013).

3.1 Primary Metabolites

Substances produced by the plant can be grouped into primary and secondary compounds, the so-called metabolites (Bresinsky et al. 2013). Before dealing with secondary metabolites, which are the focus of this textbook, primary compounds will be briefly highlighted in this chapter because an understanding of primary metabolism is essential for an understanding of secondary metabolism. *Primary compounds* are necessary for the growth and the propagation of plants, as these compounds are involved in overall cellular maintenance. These metabolites include carbohydrates, lipids and proteins and are found in relatively large quantities in almost all organisms and cells in which they fulfil important physiological functions (Yang et al. 2018). Most importantly, they ensure basic cellular homeostasis. Homeostasis can be understood as the capacity of a system in which parameters are steered in a way such that internal conditions remain stable and relatively constant (Torday 2015). The metabolic processes by which compounds (metabolites) are synthesized and broken down are called pathways. Remarkably, primary compounds and their metabolic pathways are relatively well conserved in all living organisms (McMurry and Begley 2016).

Among the primary metabolites, *carbohydrates* serve in plants for the storage and transport of energy (e.g. starch and sucrose) and as structural elements (e.g. cellulose). They are also part of molecules that carry genetic information (RNA and DNA) or are part of coenzymes (e.g. ATP). They consist of carbon, hydrogen (which is why we call them carbohydrates) and oxygen (Berg et al. 2015).

Proteins also have many functions. They serve, for example, in the replication of DNA, in the catalysis of metabolic processes, in cell signalling, in immune responses and in transport processes within the plant. The formation of proteins takes place in the cytoplasm, in the endoplasmic reticulum and in the Golgi, where they are synthesized from amino acids, their sequence being genetically determined. Biochemically, amino acids consist of amino group(s), carboxyl group(s) and a variable side chain. After being synthesized, enzymatically active proteins in plant cells are permanently degraded and replaced, and their amino acids are reutilized in order to adapt to the relevant development stages and any new environmental conditions (Berg et al. 2015).

Lipids are molecules consisting of hydrocarbon chains. They store energy, are involved in cell signalling and are components of cell and organelle membranes. The several cat-

egories of lipids include fatty acids and triglycerides. In most instances, the relevant metabolic pathways take place in the cytoplasm and endoplasmic reticulum in the case of triglycerides and in the plastids in the case of fatty acids (Bresinsky et al. 2013).

3.2 Secondary Metabolites

Secondary compounds (or *metabolites*) fulfil many ecophysiological functions. More than 200,000 secondary compounds have been identified to date (Wink 2016). However, they are less relevant for facilitating cellular homeostasis than primary metabolites. With regard to their chemical structure, secondary metabolites are highly diverse. The various plant species contrast in their metabolic composition, and, thus, species are characterized by a typical spectrum of chemical molecules. Whereas some secondary compounds are always present (constitutive), others depending on the individual species are elicited by certain biotic or abiotic environmental factors (► Chap. 3). Secondary compounds are usually found in small concentrations far below 1% of the dry weight of a plant (Akula and Ravishankar 2011). In principle, a tradeoff occurs between primary and secondary metabolism. First, primary metabolic activity is reduced when the production of secondary compounds is induced, e.g. by a stressful environment. This is not surprising given the fact that the production of secondary compounds requires substantial resources such as energy and metabolic precursors. Second, the enzymes that build up the metabolites have to be produced, and this alone consumes energy and precursors such as amino acids. Third, secondary metabolites have to be translocated from source (cell organelles of synthesis or storage) to sink (tissue of usage) organs (Wink 2010). Furthermore, specific storage organs for secondary metabolites have to be formed.

From a plant perspective, secondary compounds present a strategy to react fast and flexibly to various environmental cues (e.g. stresses). They may serve as attractants or repellents, inhibit herbivores, function as antimicrobial agents, provide a shield from excess light or act as inhibitors against other competing plant species (allelopathic function, Wink 2015a; please refer to ► Chap. 16). Secondary compounds are frequently relevant for plant fitness. Plants including hydrophytes have developed defence strategies in which secondary metabolites play a paramount role. After an infection, plants produce antibacterial or antifungal compounds and fortify their cell walls by lignin (Malinovsky et al. 2014). Often, secondary metabolites operate not only cumulatively but also synergistically, forming powerful chemical protection against pathogens. Therefore, it is very challenging for pathogens including viruses, bacteria, fungi and herbivores to become chemically resistant (Wink 2015a). Domesticated crops such as rapeseed are manipulated by breeding activities in such a way that they synthesize and accumulate less of a certain secondary compound, e.g. the bitter substance sinapine, because of its repellent taste or other properties that livestock does not appreciate (Bhinu et al. 2008). A disadvantage is that these crops lose their initial self-protective properties; this goes hand in hand with the necessity to use chemical pest management.

In general, and from a human perspective, plant secondary metabolites are extremely important. In agriculture, they are applied as biopesticides because of their antiviral, insecticide, fungicide and herbicide properties (Gutzeit and Ludwig-Müller 2014). In medicine, e.g. phytotherapy, they are appreciated for their anesthetic, antioxidant, anti-

inflammatory, antibacterial, antidepressive, antiviral, relaxing or digestive functions (Table 3.1; Wink 2015b). Furthermore, plant secondary compounds are used as flavouring agents, fragrances, colourants, artificial sweeteners and hallucinogens (Seigler 1998; Erdogan Orhan 2012). However, certain substances may harm human health, e.g. atropine is toxic, coumarin is carcinogenic, furanocoumarin is allergenic, and pyrrolizidine alkaloids are hepatotoxic (Table 3.2; Neuman et al. 2015). Throughout human

Table 3.1 Properties and bioactivity of selected secondary metabolites that are applied as isolated compounds in medicine

Plant species	Substance (class)	Properties/applications
Aconitum napellus	Aconitine (A)	Analgesic
Atropa belladonna	L-hyoscyamine (A)	Parasympathomimetic
Campthotheca acuminate	Camptothecin (A)	Tumour therapy
Cannabis sativa	Tetrahydrocannabinol (T)	Analgesic
Catharanthus roseus	Dimeric Vinca alkaloids (A)	Tumour therapy
Chondrodendron tomentosum	Tubocurarine (A)	Muscle relaxant
Cinchona pubescens	Quinidine (A)	Antiarrhythmic
Coffea arabica	Caffeine (A)	Stimulant
Colchicum autumnale	Colchicine (A)	Gout treatment
Crotalaria	Pyrrolizidine (A)	Hepatic veno-occlusive disease
Cytisus scoparius	Sparteine (A)	Antiarrhythmic
Digitalis lanata	Digitoxin, digoxin (A)	Heart insufficiency therapy
Erythroxylum coca	Cocaine (A)	Analgesic, stimulant
Galanthus woronowii	Galantamine (A)	Alzheimer's disease treatment
Lycopodium clavatum	Huperzine (A)	Alzheimer's disease treatment
Papaver somniferum	Morphine (A)	Analgesic, hallucinogen
Physostigma venenosum	Physostigmine (A)	Alzheimer's disease treatment
Pilocarpus jaborandi	Pilocarpine (A)	Glaucoma treatment
Rauvolfia serpentina	Reserpine (A)	Hypertonia treatment
Sanguinaria canadensis	Sanguinarine (A)	Antibacterial, antiviral
Strophanthus gratus	Ouabain (T)	Heart insufficiency therapy
Taxus brevifolia	Paclitaxel (taxol) (A)	Tumour therapy

Modified after Wink (2015b); Van Wyk and Wink (2015, 2017); Van Wyk et al. (2015); Wagner et al. (2007)
A alkaloid, *T* terpenoid

Table 3.2 Estimated number of described secondary metabolites and their main functions in plants

Class	Estimated numbers of structures	Toxic or repellent for herbivores	Antimicrobial activity	Attraction of pollinators or fruit dispersers
With nitrogen				
Alkaloids	27,000	++++	++	–
Nonprotein amino acids	700	++++	+++	–
Cyanogenic glucosides/ HCN	60	++++	+	–
Glucosinolates	150	++++	++++	–
Amines	100	+++	+	+++
Lectins, peptides, AMPs	2000	+++	+++	–
Without nitrogen				
Terpenes				
Monoterpenes (incl. iridoid glucosides)	3000	++	+++	+++
Sesquiterpenes	5000	+++	+++	++
Diterpenes	2500	+++	+++	–
Triterpenes, steroids, saponins	5000	+++	+++	–
Tetraterpenes	500	+	+	+++
Phenols				
Phenylpropanoids, coumarines, lignans	2000	+++	+++	++
Flavonoids, anthocyanins, tannins	4000	+++	+++	++
Polyketides (anthraquinones)	800	++++	+++	–
Others				
Polyacetylenes	1500	++++	++++	–
Carbohydrates, organic acids	600	+	++	–

Modified after Wink (2015b); Van Wyk and Wink (2017); Van Wyk et al. (2015)
Activity: – no or very few secondary metabolites (SM) active; + few SM active; ++ many SM active; +++ most SM active; ++++ all SM active

history, horticulturists have therefore tried to improve not only the quantity and quality of primary plant metabolites but also the properties of secondary compounds.

Another major function of secondary metabolites for flowering plants is to attract pollinators and other animals that disseminate their seeds. For this purpose, plants produce colourants in petals (e.g. anthocyanins, carotenoids) or volatile fragrances in blossoms (e.g. terpenes, amines). In order to prevent pollinators eating the entire flower, plants produce nectar, pollen or essential oils as a reward. For the promotion of the dispersal of seeds, plants produce fruits that contain secondary metabolites that confer certain colours, fragrances and flavours to attract animals that eat and thus disperse the seeds.

Another feature of importance is that plants synthesize secondary metabolites, e.g. antioxidant agent, in order to protect themselves against abiotic stress (e.g. heat, drought, UV radiation). Secondary metabolism is dynamic and flexible: upon stress, namely, attacks or infection, molecular cues (themselves being secondary metabolites) induce plants rapidly to increase the production of the necessary compounds. Production, storage and transport within the plant are energy-consuming and require considerable amounts of ATP or reduction equivalents, thus decreasing growth and development, explaining the tradeoff between primary and secondary metabolism as explained above (Züst and Agrawal 2017).

Many secondary compounds are synthesized in the cytoplasm of the cell. However, they can also be produced in chloroplasts (some terpenes and some alkaloids), in mitochondria (some amines, a few alkaloids) or in vesicles (Wink 2015a). Plants not only build up secondary compounds upon stimulation but also store them in considerable amounts in their vacuoles, in which they fulfil their function (e.g. vacuolic anthocyanins act as pigment to shield the cell from solar radiation) or where they can be released when they are needed (e.g. vacuolic glucosinolates are needed during herbivore attack). The site of storage of a compound also depends on its polarity. Hydrophilic compounds can be sequestered and stored in water-based compartments (e.g. the vacuole), whereas lyophilic compounds cannot. Lipophilic compounds such as terpenes are stored in specific cells, in small oil reservoirs or in the cuticle or in trichomes (Wink 2015a). Papaveraceae (poppy flowers) and many Euphorbiaceae (surge flowers) plants, which produce lactiferous compounds that include toxic alkaloids, sesquiterpenes or diterpenes, store them in particular tubes, called laticifers (Wink 2015a). Plants manufacture and store specific blends of secondary metabolites derived from various groups of compounds. Even within a single plant, one organ may contain a compound mix differing from that of another. The metabolite composition differs also according to the plant's developmental stage (e.g. germination or flowering stage) and among or within populations of the same species (Wink 2015a).

The most widespread groups of secondary metabolites are phenols, terpenoids and alkaloids:

- *Phenols* are characterized by several phenolic rings and phenolic OH groups (Wink 2015b). Several metabolic pathways lead to phenols, including the shikimate pathway, the acetate-malonate pathway and the terpenoid synthetic pathway (Fig. 3.1) (Wink 2010; Crozier et al. 2006). According to the number of carbon atoms, the most important subgroups of phenols are:
  - *Coumarines*: these phenols are fragrant with a sweet odour and naturally found in many plants, particularly in the Tonka bean (*Dipteryx odorata*), vanilla grass

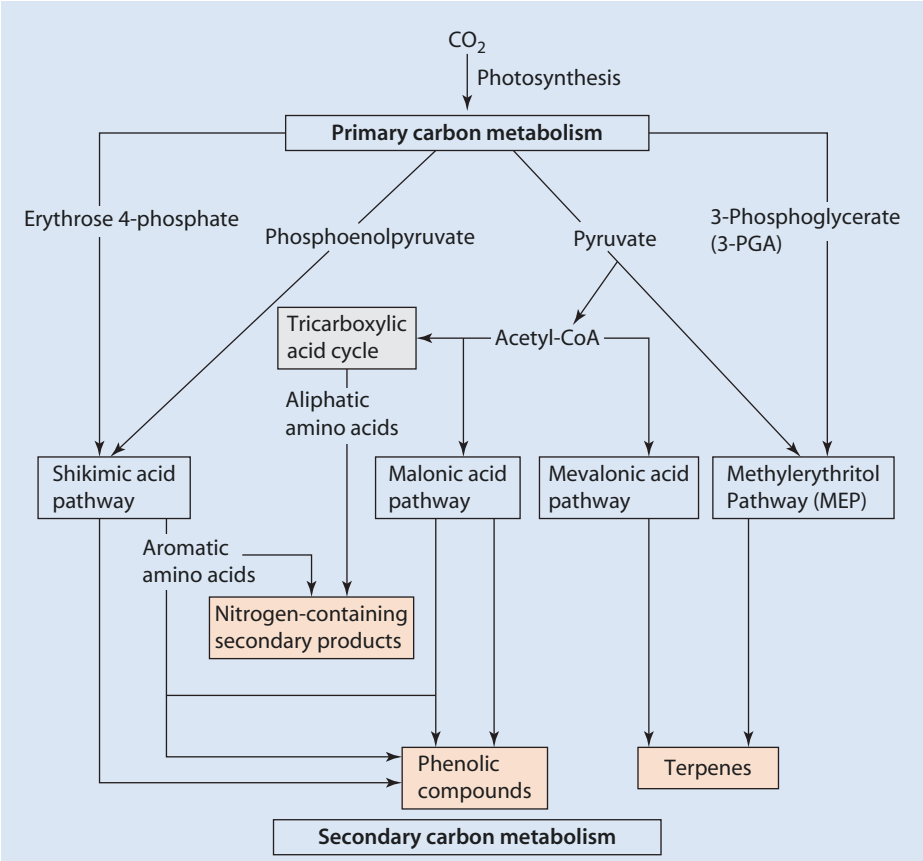

Fig. 3.1 A simplified general overview of the biosynthetic pathways involved in the biosynthesis of secondary metabolites showing a tight association with the product of primary/central metabolism. *Pink boxes* represent secondary metabolites, whereas primary metabolites are given without a frame. The pathways in *unshaded boxes* represent secondary metabolism and that shaded *grey* is part of primary metabolism (most not shown). (Figure taken from Ncube and van Staden (2015).)

(*Anthoxanthum odoratum*), sweet woodruff (*Galium odoratum*), sweet grass (*Hierochloe odorata*) and cassia cinnamon (*Cinnamomum cassia*). Furanocoumarins (e.g. celery, parsley) have to be activated by UV-A light (320–400 nm) in order to be toxic for plant pathogens (Hänsel and Sticher 2010; Petersen et al. 2010).

- *Flavonoids and anthocyanins*: flavonoids appear in many plants and fulfil important functions. Flavonoids are (or are precursors for) yellow, red and purple plant pigments for flower coloration and serve in the attraction of pollinators. They are also involved in UV filtration and symbiotic nitrogen

fixation and provide protection against oxidation. Anthocyanins (glycosides of anthocyanidin) are water-soluble vacuolar pigments in leaves (red cabbage), flowers (e.g. roses, delphiniums, corn cockles, begonias) and fruits (e.g. apple) and, sometimes, in roots (balsams). Among the best known flavonoids are quercetin and kaempferol (Hänsel and Sticher 2010).

- *Lignins*: they stabilize the tissues of vascular plants and algae and support wood formation. Upon infection by pathogens or wounding by feeding insects, additional lignification is induced in plants followed by a thickening of the cell walls through the accumulation of lignin (Hänsel and Sticher 2010; Lattanzio et al. 2006).
- *Polyphenols*: these occur in most plant families and are often concentrated in leaf tissue, the epidermis, bark layers, flowers and fruits. They may release and suppress growth hormones, deter herbivores and prevent microbial infections and may function as signalling molecules (Hänsel and Sticher 2010).

- *Terpenes* are derived from five-carbon isoprene units by biosynthesis from isopentenyl pyrophosphate and are modified in multiple ways. They are produced in the acetate-mevalonate pathway or the non-mevalonate pathway: MEP/DOXP (2-C-methyl-D-erythritol 4-phosphate/1-deoxy-D-xylulose 5-phosphate) (Hänsel and Sticher 2010). They form a large class of naturally occurring organic substances. Cytokinins, major plant hormones involved in cell growth and differentiation, belong to the terpenoids (please refer to ▶ Chap. 15). Isoprenes are thought to protect the photosynthetic membranes from heat damage (Hänsel and Sticher 2010; Ashour et al. 2010).
  - *Monoterpenes* are substantial components of ethereal oils, which attract or repel insects, and are characterized by their aromatic smell. They are synthesized in multiple plant species, e.g. in the Asteraceae (sunflower family), Apiaceae (celery family), Lamiaceae (mint family), Myristicaceae (nutmeg family) and Poaceae (grasses) and are present in the resins of conifers (Wink 2015b).
  - An important subgroup of terpenoids are the *saponins*. Because of their detergent properties, they are toxic, particularly to fish. Historically, humans used them as soaps. In plants, saponins are found in leaves, stems, bulbs, roots, blossoms and fruits. They are present in several monocot and dicot families including the Amaranthaceae (amaranth) and Sapindaceae (soapberry) families. In plants, they serve as antifeedants and protection against microorganisms by damaging their membranes (Hänsel and Sticher 2010).
  - *Tetraterpenes* are needed for the biosynthesis of carotenoids. Carotenoids are dominant pigments in flowers (e.g. violaxanthin in viola) and in fruits (the red pigment of tomato, lycopene) and appear in other organs (e.g. ß-carotene in the taproot of carrots). Carotenoids absorb light energy for photosynthesis, protect chlorophyll from radiation damage and serve as antioxidants (Hänsel and Sticher 2010). Other well-known terpenoids are the cannabinoids synthesized in cannabis plants (Kinghorn et al. 2017), the carotenes and xanthophylls and the essential oils synthesized, for example, by peppermint, chamomile and eucalyptus (Lüttge and Kluge 2012).
- *Alkaloids*: with 27,000 substances, this is the largest group of identified secondary plant compounds (Wink 2015b). Alkaloids are particularly frequent in the

Solanaceae (nightshades), Papaveraceae (poppies), Apocynaceae (dogbanes) and Ranunculaceae (buttercups or crowfeet) families. They are characterized by heterocyclically bound nitrogen and are synthesized from amino acids. Alkaloids mostly taste bitter. They are usually toxic, a feature that protects the plant against herbivory attacks. Upon attack by pathogens, plants might synthesize these antimicrobial compounds. Plants produce alkaloids mainly in their leaves, fruits, seeds, roots or bark, although different parts of the plant may contain different alkaloids.
  - The main alkaloid subgroups are the tropanes (e.g. atropine, cocaine), pyridines (e.g. nicotine), isoquinoline (e.g. morphine, codeine), purine (e.g. caffeine) and colchicine. Alkaloids include morphine, mescaline and cocaine, which provoke specific effects on the nervous systems of animals and humans (Roberts et al. 2010). Other alkaloids (e.g. caffeine, nicotine) show stimulating effects. Because of their pharmacological properties, they are used in traditional and modern medicine. Such properties include antimalarial, anticarcinogen, analgesic, antibacterial and antiarrhythmic effects (Hänsel and Sticher 2010).
- *Glycosides* are 'originally mixed acetals resulting from the attachment of a glycosyl group to a non-acyl group RO- (which itself may be derived from a saccharide and chalcogen replacements thereof (RS-, RSe-)' (IUPAC 2014). Their functions in plants are related to detoxification processes and protection against herbivory (Brito-Arias 2016). Because of their membrane-damaging property, glycosides may be toxic to bacteria and fungi. An important subgroup is the glucosinolates, which are composed of specific amino acids (Selmar 2010). They contain sulphur and nitrogen and are found particularly in the Brassicaceae (e.g. cabbage, horseradish, mustard), Capparidaceae (e.g. caper) and Tropaeolaceae (e.g. garden nasturtium). Typical is the spicy smell and taste of mustard oil. Increasing indications suggest that they protect people from colon cancer (Schneider et al. 2017).
- *Plant hormones* (also called phytohormones) are extremely important secondary metabolites. They serve as signal molecules, regulate gene expression, control cellular processes and determine the formation of major plant organs (Gray 2004; Depuydt and Hardtke 2011). Moreover, they are involved in stress responses. Plants usually produce them in low concentrations. The most important phytohormones include the abscisic acids (ABA), auxins, cytokinins, ethylene and gibberellins. However, Taiz et al. (2018) group phytohormones as primary metabolites, since all plants require them for growth and development. In general, phytohormones are derivatives of secondary metabolite pathways, except for the auxins and ethylene whose precursors are synthesized in primary metabolism: A more detailed review on the role of phytohormones in CEH is given in ▶ Chap. 15.

3.2.1 Improving Quality by Adjusting Metabolites Through the Regulation of Controlling Environmental Factors

Although the metabolome is predetermined by the genetic background of the plant, metabolism is a dynamic process and is not fixed. This is because during growth and development, plants are permanently adapting to their changing environment. Thus, the metabolomic composition is dynamic in terms of quality (this means in both the

pattern and biological properties of the metabolites) and quantity (Gorelick and Bernstein 2014). Environmental factors contribute highly to changes in the secondary metabolome (Gorelick and Bernstein 2017; Yang et al. 2018). These factors include light (see ► Chap. 5), nutrient deficiency (see ► Chap. 6), salinity (see ► Chap. 7), water availability (see ► Chap. 8), temperature (see ► Chap. 9) or wounding (see ► Chap. 10). Apart from these physical stressors, hormonal elicitors also exert their effects, such as jasmonic acids, salicylic acids, brassinosteroids, abscisic acids and auxins, and inorganic chemical elicitors in the form of heavy metals.

Definition of Metabolome

A metabolome is a set of small molecules within a biological organism (e.g. cell, organ, tissue). A metabolome can include primary and secondary metabolites and substances not necessarily produced naturally by organisms, e.g. toxins. Very small molecules, including metabolites, are part of the metabolome. Macromolecules, such as DNA and RNA, are not.

Moreover, also biotic stressors, e.g. pathogens, fungi or insects, elicit the production of specific secondary compounds in plants. After exposure of a plant to stressors, enzymatic pathways are induced that alter the content of bioactive secondary compounds, namely, alkaloids, terpenoids and phenylpropanoids. By regulating controllable stressors, growers can shift metabolism towards the accumulation of favourable compounds. However, an important point to note is that plants might reduce their production of primary metabolites when stress factors induce the production of secondary compounds. This might result in a reduction of the biomass, an effect that has to be avoided in horticultural production as crops need to be marketable. Stress exposure must be strong enough to adjust the metabolism towards compounds that are favourable in the human diet but, at the same time, must be so mild that biomass and yield formation is not reduced. This is the challenge for the horticulturist.

Since the production of secondary metabolites often depends on the physiological and developmental stage of the plant and on environmental conditions, the time of harvest is of great importance, as is the postharvest treatment (e.g. drying technology and storage conditions) (Ncube et al. 2012). In order to avoid yield losses, it makes sense to start the controlled stress treatment shortly before harvest, by which time yield has been set (Schreiner et al. 2003; Pareek 2017). Usually, the desired secondary compounds accumulate within hours and days. In other words, the crop can be gently stressed to induce the synthesis of favourable secondary compounds, for example, at 1 or 2 days before harvest. However, this cannot be generalized, and case studies and metabolite-specific strategies are introduced in this textbook.

The reader is warned that the initial effect might be different, if an individual stressor interacts with other factors. For example, high irradiation often accompanies elevated temperature and drought stress (Selmar and Kleinwächter 2013). Thus, an advantageous ploy might sometimes be to enrich secondary compounds under totally controlled conditions. This is particularly the case with medicinal and pharmaceutical plants for which market requirements have to be fulfilled extremely precisely (Naik and Al-Kharyri 2016), e.g. in prescription medicines containing cannabinoids (Potter 2013). In addition,

even if environmental stressors can be well controlled, certain substances might nevertheless operate differently in isolated conditions than when acting together with other substances in the same plant (Bhatia and Bera 2015).

Next, we show an example of the way that pharmaceutically active secondary compounds, in particular cannabinoids, can be enriched in the medicinal plant cannabis (*Cannabis sativa* L.) by inducing controlled stress. Cannabis belongs to the Cannabaceae family and is an annual and dioecious plant (male and female flowers are sited on separate plants). After the pollination of female flowers, the male plants die (Flores-Sanchez and Verpoorte 2008). Several native species exist in Central Asia, but, nowadays, they are spread all over the world. They are a source of food, energy and fibre, and several components of the plant are used medicinally or pharmaceutically.

During the flowering period, cannabis plants produce many valuable unique metabolites including cannabinoids, terpenes and phenolic compounds. They protect the developing flowers from insects (sticky resinous oils and volatiles) and from excessive heat under shifting solar conditions. The predominant cannabinoids are Δ9-tetrahydrocannabinolic acid (THCA), cannabidiolic acid (CBDA) and cannabinolic acid (CBNA), followed by cannabigerolic acid (CBGA), cannabichromenic acid (CBCA) and cannabinodiolic acid (CBNDA) (Hazekamp et al. 2010; ElSohly et al. 2017). They are found in the secretory cavity of the glandular trichomes.

These acids are decarboxylated in the living plant, a process that is particularly induced upon heating, e.g. after harvest (Flores-Sanchez and Verpoorte 2008). Only thereafter can THC unfold its psychoactive properties (André et al. 2016). The concentrations of secondary metabolites depend on tissue type, age, variety, growth conditions (nutrition, humidity and light levels), harvest time and storage conditions (André et al. 2016). Their impacts on humans are observed as psychotropic, antinociceptive, antiepileptic, cardiovascular, immunosuppressive, antiemetic, appetite stimulating, antineoplastic, antimicrobial, anti-inflammatory and neuroprotective. Positive effects in psychiatric syndromes, such as depression, anxiety and sleep disorders, are well described (Kinghorn et al. 2017; Musty 2004; Cascio et al. 2017; Pertwee 2014). The precursors of cannabinoids are synthesized from the deoxyxylulose phosphate/methylerythritol phosphate (DOXP/MEP) pathway and the polyketide pathway (Flores-Sanchez and Verpoorte 2008; André et al. 2016).

Whereas, in general, the outdoor cultivation of cannabis is limited to one harvest per year, three to four crops are possible under controlled environment conditions (Thomas and ElSohly 2016). For indoor cultivation, protocols are available for horticulturists: with regard to photo-radiation, cannabis prefers high photosynthetic photon flux densities (≈ 1500 µmol/m^2/s) in order to exchange gas and water vapour efficiently between leaves and their surroundings. Several lamp types can be used, including high-pressure sodium (HPS) lamps and light-emitting diodes LEDs. During vegetative growth, an 18-h photoperiod is recommended, which is reduced to 12 h for the evocation of flowering. According to Gorelick and Bernstein (2017), UV-B light increases the THC content.

The amount, type and quality of cannabinoids rely on genetic background and can be induced by changing stressful conditions. Table 3.3 summarizes the effects of elicitors on cannabinoid production.

Table 3.3 Effects of selected biotic and abiotic elicitors on the production of cannabinoids

Stressor	Elicitation	Induced effect
Nutrients	Increased content of N, Ca, Fe, Mg	Increase of THC content
	Nutrient deficiency because of poor soil condition	Increase of cannabinoid content
	P deficiency	Increase of THC content
Drought	Drought stress	Increase of trichome density and thereby increase of cannabinoid production
	Increased humidity	Increase of THC content
Temperature	Increased temperature	Conflicting results about effects on cannabinoid content
Photo-radiation	Increased irradiance	Increase of THC concentration due to its defensive role against UV radiation
	Increased intensities of UV-B radiation	Increase of THC without changes of other cannabinoids
	Increased UV-C radiation	No effect on cannabinoid production but increase of stilbenes and cinnamic acid derivatives
Metals	Moderate concentrations of Cd, Ni, Cr	Tolerance regarding plant growth and physiology and only slight effect on THC content
Wounding	Insect herbivory	Increases cannabinoid and terpene content due to their role as natural insecticide
Pathogens	Fungal (e.g. *Phomopsis ganjae*) and bacterial (e.g. *Staphylococcus aureus*) pathogens	Modulate cannabinoid biosynthesis attributable to their antibiotic and antifungal properties
Hormones	Jasmonic acid, methyl jasmonate, salicylic acid	Increase of secondary metabolite production in cannabis cell suspension culture but no change in cannabinoid content
	Abscisic acid	Decrease of THC and CBD in vegetative plants but increase of THC content in flowering female plants

Modified after Gorelick and Bernstein (2017)

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Hydroponic Systems in Horticulture

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C.-M. Geilfus, *Controlled Environment Horticulture*,
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4.1 Drip System

In a drip system (Fig. 4.1), roots are allowed to grow into a growing medium, such as perlite or rock wool, and the nutrient solution is dripped onto the medium to provide nutrients and to keep the roots moist (Sheikh 2006). The nutrient solution is pumped through drip emitters to individual plants. The emitters are usually scheduled to run for approximately 10 minutes per hour. This, however, depends on plant variety, age and other factors such as ambient humidity, temperature or sun exposure. Plants are usually placed in a moderately absorbent growing medium so that the plants do not dry out (Sharma et al. 2018). Advantages: The drip system is easy to set up, with only a few components, and the nutrient and water supply is easy to control. Water and nutrients can be cycled, increasing use efficiencies. Disadvantages: Drip lines and emitters are susceptible to clogging by salt precipitation, sediment or algae formation. The pH level may become unstable because of the recycling of water and nutrients, and, thus, monitoring and adjustments are advised. The system is susceptible to power outages and pump failures; the lack of water can kill plants within a few hours.

4.2 Flood and Drain (Ebb and Flow) System

One of the most common hydroponic systems is the flood and drain approach (Fig. 4.2). Here, the seedlings are placed directly into growing trays filled with growing medium (Reshma and Joseph 2016). Plants are periodically flooded with the nutrient solution in such a way that it completely covers the growing medium for a period of time. To stop flooding, the solution is returned to the reservoir by opening a valve at the bottom of the growing tray. During each cycle (which is controlled by a timer), roots should not be flooded by the solution for more than 20 to 30 minutes, as this will provoke anoxia (Budye et al. 2018). Advantages: The flood and drain system is good for water-craving plants such as lettuces or the various types of spinach. It is energy-efficient and scales well. Disadvantages: Reservoir capacities need to be high, and high volumes of nutrient solution are required. The system is susceptible to power outages and pump or timer failures.

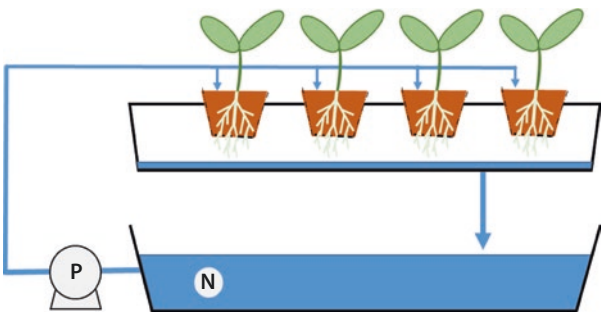

Fig. 4.1 Drip system, schematic drawing. (Modified according to Sharma et al. 2018); *P* water pump, *N* nutrient solution

4.3 Nutrient Film Technique

With the nutrient film technique (NFT) (Fig. 4.3), the plants are cultivated in canals or pipes that have a slight slope (2%) (Cooper 1979). The plants are usually fixed in substrate-filled net pots in a holder. The nutrient solution is pumped in a circulation system from the lighttight reservoir into the canal and then flows as a thin film down the canal and back into the basin by gravity. Optimally, air can be pumped into the nutrient solution (Van Os et al. 2008). The roots of the plants hang with their ends in the solution, so that some parts of the roots are always immersed in the flowing nutrient solution and some are surrounded by air. In this way, the plant is well supplied with a mixture of nutrients and oxygen. The size of the canals and the distance between plants must be adapted to the root growth. Otherwise, the canal could be blocked, and the flow of nutrient solution will become blocked. It is advisable to work with a slope of about 2%. As a general guideline, the flow rate for a channel should be 1 litre per minute. Advantages: The cost of building an NFT system is low. Water and nutrients can be reused. It is not prone to clogging. Disadvantages: Because of pH and nutrient fluctuations, monitoring and readjustment are required. For plants with short roots, top irrigation is required until the roots grow to the bottom of the canal. The system is susceptible to power outages and pump or timer failures.

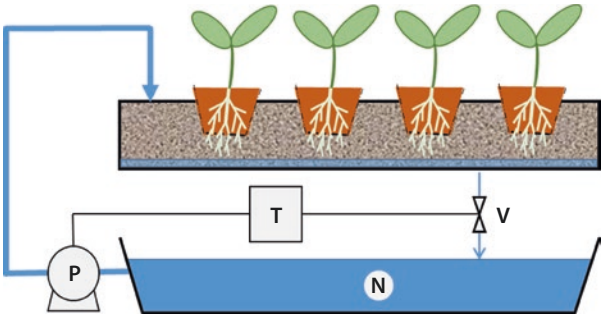

Fig. 4.2 Flood and drain system, schematic drawing. (Modified according to Sharma et al. 2018); *P* water pump, *T* timer, *N* nutrient solution, *V* valve

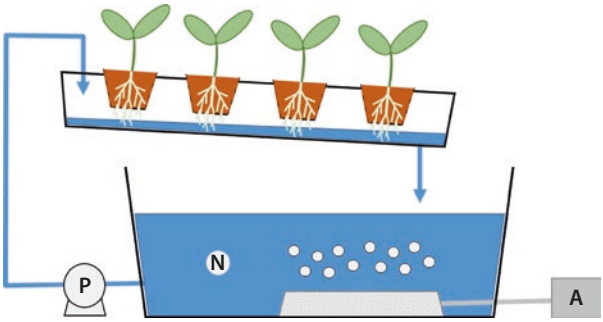

Fig. 4.3 NFT system, schematic drawing. (Modified according to Sharma et al. 2018); *P* water pump, *N* nutrient solution, *A* air pump

4.4 Deep Water Culture

With the deep water culture (DWC) method (Fig. 4.4), the plants are cultivated in substrate-filled net pots that are positioned in a tank filled with nutrient solution (Hoagland and Arnon 1950). The free-hanging roots of the plants grow directly into the nutrient solution. To prevent the roots from rotting and dying, oxygen is added to the water by means of a pump. For this purpose, fine-pored air stones are inserted into the basin. The tank should be lighttight, and the water surface should also be darkened to prevent algal growth and to prevent light from harming the roots. Advantages: The DWC system uses less water and nutrients than other methods and is great for water-loving, rapidly growing plants. It works well for organic hydroponics because there are no drip or spray emitters that might clog the system. Disadvantages: Plants can be prone to root diseases such as pathogens that spread easily.

4.5 Aeroponic System

With this system (Fig. 4.5), the roots of the plants hang in the air and thus receive sufficient oxygen. The plants are installed in such a way that roots are constantly wetted with an aerosol of nutrient solution pumped from a closed reservoir. The nutrient solution is located in a lighttight tank and is pumped to spray heads, where it is nebulized by using low-pressure, high-pressure or ultrasonic atomizers (Lakhiar et al. 2018). The spray mist should be as fine as possible. The nutrient solution can also be enriched with oxygen, although this is not necessary. Aeroponic systems are normally closed control loops capable of maintaining uniform climate and water values.

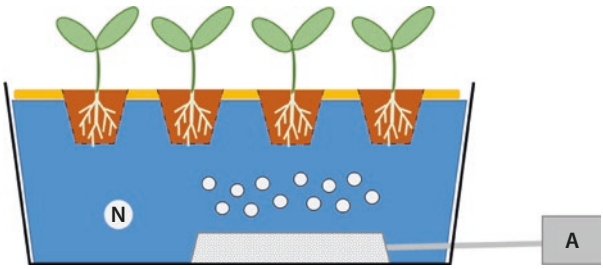

Fig. 4.4 DWC system, schematic drawing. (Modified according to Sharma et al. 2018); *N* nutrient solution, *A* air pump

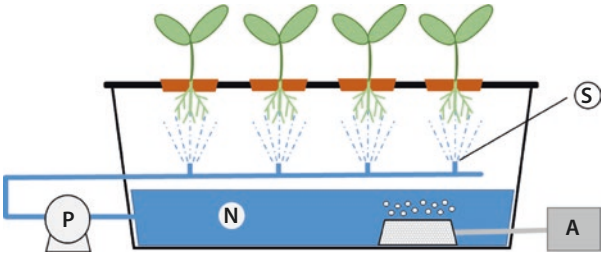

Fig. 4.5 Aeroponic system, schematic drawing. (Modified according to Sharma et al. 2018); *P* water pump, *N* nutrient solution, *S* spray heads, *A* air pump

Advantages: The aeroponic system provides excellent aeration of roots with constant high oxygen levels. Water and nutrients can be reused, and no growth medium is required. It uses water economically (Schröder and Lieth 2002). Disadvantages: The level of maintenance is higher compared with that of other systems. Spray heads clog easily, and cleaning of the root chamber is required to prevent root diseases (however, pathogens are unlikely to spread through the system via the waterways). The system is not cheap because of material costs.

4.6 Divergences of Hydroponic Systems

The following table (Table 4.1) gives an overview of the main advantages and disadvantages of various hydroponic systems.

Table 4.1 Divergences of hydroponic systems

System	Advantages	Disadvantages
Drip	Simple to build and use Low water usage Scales well	Clogging of drip lines pH and nutrient fluctuations because of cycling of nutrient solution Susceptible to pump failure and power outages
NFT	Low cost Little to no medium required No clogging	pH and nutrient fluctuations because of cycling of nutrient solution Limited space within the system for root formation Susceptible to pump failure and power outages
Flood and drain	Easy to build and to maintain Recycling of nutrients and water Works well with organic substrates (no clogging)	Requires large amounts of growing media pH and nutrient fluctuations because of cycling of nutrient solution Salts can accumulate in nutrient solution causing problems of toxicity Susceptible to pump failure and power outages
DWC	High tolerance to pump or electricity failures Scales well Allows for large root mass	Not suitable for larger plants or plants with a long growing period Root pathogens can easily spread diseases
Aeroponic	Excellent aeration of roots No grow medium required	Lowest tolerance to pump or electricity failures Spray heads clog easily Susceptible to pump failure and power outages

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Controllable Production Factors in Horticulture

The tightly controlled manipulation of environmental factors that influence plant physiology is a measure to shift plant metabolism towards the production of compounds that are favourable when they form part of a plant-based diet or are used plant-based pharmaceuticals. The second part of this textbook introduces the most important environmental factors, viz. production factors such as light, water or nutrients, that can easily be controlled by the horticulturist for changing the qualities of the plant-based products. Moreover, other factors are discussed such as wounding, inoculation with beneficial fungi or enrichment of the greenhouse atmosphere with CO_2. First, the physiological function of the production factor is explained. Second, an explanation is given regarding the way that the plant reacts when this factor is limited (e.g. nutrient, light) or present (wounding, enriched CO_2 greenhouse atmosphere). Third, the way in which this knowledge can be used to alter the pattern of health-promoting secondary metabolites is described. Lastly, examples are introduced from vegetable or medicinal plants.

Contents

Light

Contributions by Andreas Tilk (andreas.tilk@t-online.de).

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https://doi.org/10.1007/978-3-030-23197-2_5

Among controllable environmental production factors, light is essential because of its significance for photosynthesis in plants. What is visible light? Visible light is electromagnetic radiation that is visible to the human eye and that is measured in frequencies. Furthermore, light is defined as particle, called a photon, and its energy is referred to as quantum (Taiz et al. 2018).

Visible light ranges from 380 to 750 nm (see Fig. 5.1). The shortest wavelengths that humans perceive are light rays at about 380 nm (violet light) having high-energy photons, whereas the longest are at 750 nm (red light) having low-energy photons. Visible sunlight appears white because of the mixing of wavelengths that human eyes perceive. However, when passed through a prism, light waves become refracted into visible bands of colour producing a rainbow effect. The sun also emits gamma rays, X-rays and radio waves at various intensities (Bresinsky et al. 2013; Taiz et al. 2018).

Plants and some other living organisms, including cyanobacteria, need light for photosynthesis. Photosynthetically active radiation (PAR) ranges approximately from 400 to 700 nm. The various wavelengths in sunlight are not all used equally in photosynthesis (Bresinsky et al. 2013). Plants contain several pigments that absorb specific wavelengths of light while reflecting others. As shown in Fig. 5.2, the most important pigments in photosynthesis are chlorophyll a and chlorophyll b (the chlorophylls absorb blue wavelengths from 400 to 480 nm and red wavelengths from 600 to 700 nm) and the β-carotenoids (the carotenes and xanthophylls absorb violet to blue-green wavelengths from 400 to 550 nm, β-carotene reaching its maximal absorption peak at 480 nm). Carotenoids are found, for example, as red-coloured lycopene in tomato and yellow-coloured zeaxanthin in corn seed. Carotenoids not only capture light for photosynthesis but also help to absorb excess of light energy in order to dissipate it as heat when the plant is exposed too excessively to light. Another function is to attract animals for seed

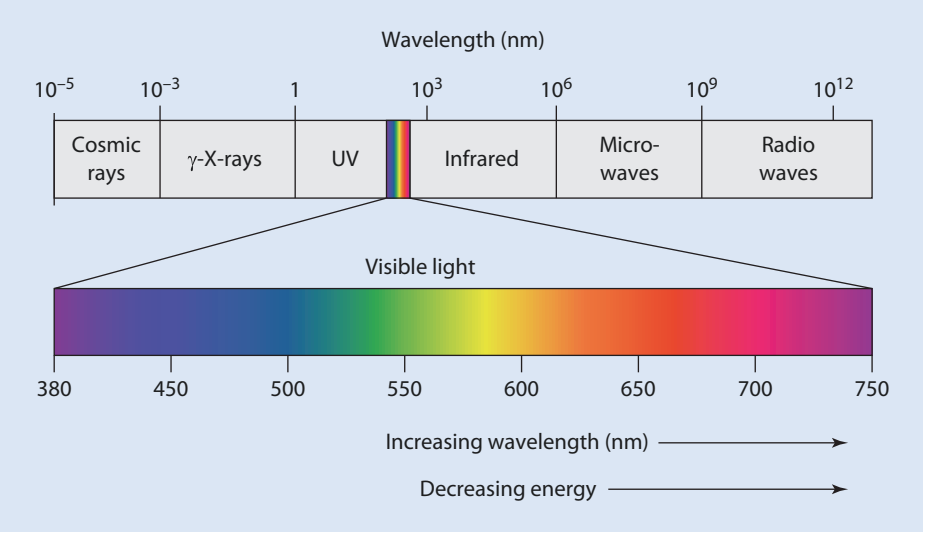

Fig. 5.1 Spectrum of electromagnetic radiation. (Bresinsky et al. 2013. Reprinted by permission from Springer Nature, license number 4406981227879 issued August 13, 2018)

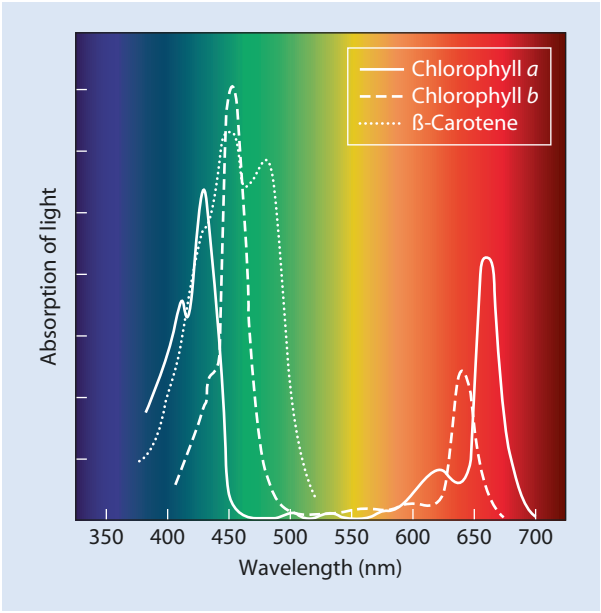

Fig. 5.2 Absorption spectra of selected plant pigments. ▶ https://www.khanacademy.org/science/biology/photosynthesis-in-plants/the-light-dependent-reactions-of-photosynthesis/a/light-and-photosynthetic-pigments [Accessed 13 August 2018]. (Image modified from 'The light-dependent reactions of photosynthesis: Figure 4', by OpenStax College, Biology (CC BY 3.0). Open-access material distributed under the terms and conditions of the Creative Commons Attribution license (▶ http://creativecommons.org/licenses/by/4.0/))

dispersal (Tanaka et al. 2008). Since green light is not absorbed by plant leaf pigments (but is reflected), the plant leaf appears green to the human eye. When a plant pigment absorbs a light particle (photon), it becomes energized, meaning that it becomes excited. As a result of excitation, these pigments now possess an electron that can be passed on to other photosynthetic acceptor molecules. This is the start of the photosynthetic electron transport chain. Here, chlorophylls are extremely important photosynthetic pigments because they are part of the light-catching 'antenna' complexes of photosystems (Bresinsky et al. 2013). With the help of the photosynthetic electron transport chain, plants transform physical energy (sunlight) into chemical energy during the so-called light reaction of the photosynthesis, producing adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate ($NADPH+H^+$). Both compounds fuel a plethora of energy-absorbing anabolic processes such as the Calvin cycle (the so-called dark reaction of photosynthesis). Here, the energy from ATP or $NADPH+H^+$ is used to assimilate carbon into carbohydrates, which are a long-term means of storage of chemical energy.

Light controls not only photosynthesis but also many other developmental or physiological processes, such as photomorphoses, phototropism or stomatal aperture. Here, light is perceived by light-sensitive molecules, mostly proteins, the so-called photoreceptors. As shown in Table 5.1, they detect light signals and are able to induce physiological and morphological processes, e.g. flowering or the control of stomatal widening.

Table 5.1 Examples of photoreceptors and the light-regulated events that they control

Photoreceptor type	Spectral sensitivity	Example of regulated process
Phytochrome class I	Red (blue) light	Photomorphoses induced in etiolated seedling by far-red (VLFR)
		HIR of photomorphogenesis in etiolated seedling
Phytochrome class II	Red light	HIR of photomorphogenesis in light
		Photoperiodic regulation of morphoses (e.g. flowering induction)
		Photoreversible red/far-red responses in weak light (LFR) (e.g. seed germination in those requiring light)
		Shade-avoidance reaction
		Photomodulation (e.g. day/night position of leaves)
Cytochrome	Blue, UV-A (320–390 nm) light	HIR response of photomorphogenesis of etiolated seedlings and photoperiodic regulation of morphogenesis
Phototropin	Blue light	Phototropism of higher plants
		Stomatal opening in higher plants
Sensor rhodopsin	Green light	Phototaxis of *Chlamydomonas* and other Chlorophyceae
Direct-light-sensitive transcription factors	Blue light	Carotenoid synthesis and sporulation of *Neurospora crassa*
Unknown	Blue light	Phototropism of *Phycomyces*
Unknown	Blue light	Phototaxis of *Euglena*

Modified after Bresinsky et al. (2013)
At the plant *Arabidopsis thaliana*, *VLFR* very-low-fluence response, *LFR* low-fluence response, *HIR* high-irradiance response

Whereas, on earth, the sun is the main source of natural light, multiple ways exist for the creation of artificial lighting for use in greenhouses or tunnel horticulture. The way that light directs plant growth and morphology (see Table 5.2) depends on its intensity, quality and photoperiod (Bresinsky et al. 2013).

Light intensity is the amount of light supplied to the plant. It is measured by the photosynthetic photon flux density (PPFD) and daily light integral (DLI). PPFD measures, in micromoles per square metre per second, the photons received by a plant and utilized for photosynthesis. DLI measures, in moles per square metre per 24 hours, the total daily number of photons received per growth area. The popular rule of thumb for

Table 5.2 Effect of photoperiod, light intensity and light quality on the production of plant secondary metabolites

Metabolite class	Metabolite name	Environmental factor	Concentration change	Plant species
Photoperiod effects on the content of various plant secondary metabolites				
Phenols	Caffeoylquinic acids	Short length of day	Decrease	*X. pensylvanicuim*
Phenols	Pelargonidin	Short length of day	Decrease	*P. contorta*
Phenols	Catechins	Long length of day	Increase	*I. batatas*
Phenols	Hydroxybenzoic acids	Long length of day	Increase	*I. batatas*
Phenols	Chlorogenic acid	Long length of day	Increase	*V. myrtillus*
Light intensity changes the content of various plant secondary metabolites				
Alkaloids	Camptothecin	27% full sunlight	Increase	*C. acuminate*
Phenols	Asiaticoside	Full sunlight	Increase	*C. asiatica*
Phenols	Chlorogenic acid	Full sunlight	Increase	*V. myrtillus*
Light quality changes the content of various plant secondary metabolites				
Phenols	Ferulic acid	Increase of red light	Decrease	*L. sativa*
Phenols	Kaempferol	Increase of red light	Decrease	*L. sativa*
Alkaloids	Catharanthine	UV-B	Increase	*C. roseus*
Phenols	Quercetin	UV	Increase	*F. esculentum*
Phenols	Catechins	UV	Increase	*F. esculentum*

Modified after Yang et al. (2018) and references therein

greenhouse growers is that 1% more light results in a 1% increase of yield (Peet and Welles 2005).

Light quality describes the wavelength of light, viz. its colour. In this context of photosynthesis, photobiologically active radiation (PBAR) includes light in the range of 280–800 nm (ultraviolet to far-red light) because it activates photosynthesis. Moreover, deep red light is important for photosynthesis, vegetative production and the stimulation of shoot development. Blue light induces the composition of anthocyanins, suppresses stem elongation and decreases the growth rate of plants. Far-red light acts positively on generative properties, flower formation and rooting (Taiz et al. 2018).

Photoperiod measures the duration of light that a plant receives throughout a day and influences growth, photosynthesis and morphology. Flowering plants frequently react sensitively to the light-darkness cycle (also called photoperiodism) (Taiz et al. 2018). There are day-neutral, long-day and short-day plants and mixed forms. However, the length of the dark period is relevant for morphological developments: long-day plants flower when the uninterrupted period of darkness falls below a critical time span, whereas short-day plants bloom when the critical time span of darkness is exceeded. Day-neutral plants can initiate flowering not depending on the length of darkness but after achieving a certain developmental stage or age.

The critical day length depends on the plant variety and the latitude, season and climate in which it finds itself (Bresinsky et al. 2013). *Spinacia oleracea* (spinach), a long-day plant with a critical day length of 13 hours, begins flowering when uninterrupted darkness falls below 11 hours. In contrast, *Euphorbia pulcherrima* (poinsettia), a short-day plant with a critical day length of 10 hours, begins flowering when uninterrupted darkness exceeds 14 hours. The manipulation of the photoperiod is used to elicit a change in plants from their vegetative to generative growth stage (when flowers or fruits are the horticulturist's main interest) or to extend the vegetative phase in order to increase the yield of the other plant organs (e.g. leaf vegetables). In the case of a long-day plant cultivated in CEH, flowering can be induced by interrupting the dark period.

5.1 Light Sources in CEH

In horticultural production, artificial light is used for three main purposes:

1. Under *replacement lighting*, solar radiation is completely substituted in indoor growth rooms and growth chambers (Kozai et al. 2016). Although sunlight offers a full spectrum of photosynthetically active wavelengths and its input is free of charge, some growers prefer to create completely artificial environments in order to control optimally all environmental factors and to improve quality parameters.
2. *Supplemental* or *production lighting* is used in greenhouses to supplement periods of low natural light (Schwend 2017).
3. *Photoperiodic lighting* is used to stimulate or influence photoperiod-dependent plant responses such as flowering or vegetative growth (Schwend 2017).

5.2 Types of Lamps

Despite the technical progress in light technology, no light source converts electrical energy entirely into light. All light sources also produce, to a certain extent, thermal energy. Hence, waste heat increases the production costs of the horticulturist. Therefore, when evaluating artificial light sources, several factors should be taken into account: lamp efficiency, intensity, spectral quality, cost, electrical requirements, maintenance demand and life span. Thermal radiation can cause heat stress during hot periods. Because of these reasons, a suitable type of lamp has to be determined for each individual application purpose. The most relevant types of lamp in horticulture are incandescent, low-pressure sodium, high-pressure discharge lamps and light-emitting diodes

(LEDs). In addition, plasma lamps might be relevant in the future. Whereas 'lamp' refers to the light source itself, the entire lighting fixture in horticulture, called the luminaire, comprises also reflectors, ballasts and other devices (Kozai et al. 2016).

Incandescent lamps emit light by the heating of a tungsten filament. Only around 6% of their energy is radiated in the PAR spectrum; 82% is emitted in the infrared, with the remaining 12% as thermal energy (Gendre 2017). Although they are not very efficient with respect to delivering light for photosynthesis, they are still useful for manipulating photoperiodic signalling (night-break and long-day lighting), since they produce large amounts of red and infrared radiation (Kozai et al. 2016).

Fluorescent lamps (belonging to the low-pressure discharge lamp type; Lister and Liu 2017) produce light from the excitation of low-pressure mercury vapour in a mixture of inert gases. Fluorescent lamps are more light-efficient than incandescent lamps, as they have a much longer life span and produce a more balanced wavelength range in the PAR spectrum if designed well. They are used in growth chambers and in multiple-tier applications, because they operate in relatively cool temperatures allowing them to be fixed close to plant canopies (Kozai et al. 2016).

High-pressure discharge lamps (or *high-intensity discharge* (HID) *lamps*) are based on the introduction of an electrical arc into an elemental gas mixture. In contrast to fluorescent lamps, no fluorescing powders are used on the lamp glass, and the gases are heated under much higher vapour pressures and temperatures. Thus, they are more light-intensive and efficient. Metal halide (MH) and high-pressure sodium lamps are still commonly used in horticulture. Their main difference is the spectrum of emitted light, which relies on the used gas. MH lamps produce a relatively full spectrum across the PAR region, with approximately 20% blue and 24% red light (Brown et al. 1995). According to Pinho and Halonen (2017), MH lamps are therefore suitable for facilitating vegetative plant growth, in general, and for the cultivation of leafy vegetables with compact morphological features, in particular. High-pressure sodium lamps are characterized by high electrical efficiency, high heat radiation and the poor quality of its spectral emission (mainly yellow-green and infrared). Because of the low red to far-red ratio and the low blue light emission, plants growing under high-pressure sodium lamps alone might suffer from excessive leaf and stem elongation (Pinho and Halonen 2017).

Plasma lamps belong to gas-discharge lamps. They are energized by radio frequency. Modern high efficiency plasma lamps stand out because of their high luminaire yield exceeding 90% and because of their life span. This technology provides a full continuous wavelength spectrum (including UV-A, UV-B, far-red and infrared radiation) and, thus, comes close to the solar spectrum (Tekstra 2012).

Electroluminescent lamps, e.g. LEDs, emit light by applying an electric field to a material. In general, LEDs are more advantageous compared with HID, fluorescent and incandescent lamps. LEDs are characterized by their long lifetime, their robustness and their stable output when an electric current is applied. In addition, they are compact and lightweight and turn on instantaneously, and the light output can be easily controlled. Finally, LEDs are available in several colour types allowing the control of the spectral distribution of emitted light (Kozai et al. 2016). Combining LED modules with different colour spectra (e.g. blue, red and far-red) allows an improved control of plant growth or production of secondary metabolites (Bantis et al. 2018; Magagnini et al. 2018). Moreover, the optimization of light spectrum could enhance energy efficiency as wavelengths being

less needed can be dimed. Additionally, the relatively low heat production makes LEDs suitable for heat-sensitive farming systems like vertical farming.

The high initial costs of mounting an LED or a plasma luminaire in a greenhouse are an important disadvantage compared with other lamp fittings (Kozai et al. 2016). However, with regard to operational costs and to other aspects relevant to users, LED lighting technology is more efficient than other conventional technologies (Darko et al. 2014; Karlicek et al. 2017). Based on economics and sustainability, LEDs are likely to substitute traditional lighting systems in horticulture, including both fluorescent and high-intensity discharge lamps, and revolutionize controlled environment horticulture (Dou et al. 2017). Organic LEDs (OLED) and Light Amplification by Stimulated Emission of Radiation (LASER) might be alternatives to inorganic LEDs in the future (Pinho and Halonen 2017). The reader should refer to Table 5.3 for a comparative overview of the major types of electric lamps.

5.3 Major Functions of Light: The Effect of Different Light Qualities on Plant Growth and Development

As shown in Table 5.4, light initiates many effects in plants. The elicitation of developmental processes by light is called photomorphosis, whereas the process of light-directed development is termed photomorphogenesis (Bresinsky et al. 2013). The photoperiod influences flowering, dormancy, growth rate, leaf drop and other processes. Light that controls developmental processes is perceived by photoreceptors that are present within plant cells (Carvahlho et al. 2011; Taiz et al. 2018).

Red light (660 nm) receptors include the phytochromes. Red light has an influence on flowering and seed formation. Plants can also perceive far-red light (730 nm). As starting signals for germination and flowering, the ratio of red to far-red light is relevant. Growers can delay flowering of certain plants, depending on their photoperiodic response, by keeping the plant exposed to red light during the dark period, e.g. *Arabidopsis* (Searle and Coupland 2004). By absorbing large amounts of red light while reflecting far-red light, plants gain information about the canopy in their immediate surroundings (Bresinsky et al. 2013). Moreover, red light supplemented with blue light increases the primary metabolism of herb species. Red, blue and UV light also induce an accumulation of essential oils and phenolic compounds in various herbs compared with artificial white light or sunlight. The same is true for plant antioxidant capacities, which can be increased by such light treatment (Dou et al. 2017 and references therein). Because increased radiation implies stress to plants, they react by the activation of protection mechanisms resulting, for instance, in the increased production of essential oils. Growers who wish to promote stem elongation and foliar expansion or to accelerate flowering will take into consideration low red to far-red light ratios. In contrast, higher red to far-red light ratios will enhance branching (Demotes-Mainard et al. 2016).

Blue light (450–520 nm, including violet and cyan) photoreceptors include the cryptochromes and phototropins. Especially in short-day seasons (autumn and winter in the northern hemisphere), blue light reduces the impact of the growth hormone auxin. Thus, the plant slows down its stem and root growth and its apical dominance; normally, the latter causes the stem to be dominant over side stems. For the induction of the development of

Table 5.3 Light emission principles and corresponding major electric lamps

Light emission principle	Electrical lamps/ devices	Luminous efficacy in lumens per watt	Wave-length spectrum in nm	CRI[a]	Lifetime in hours	Advantages	Inconveniences
Incandes-cence	Incandescent	13–17	450–5000	100	1000	Low purchase cost; no regulating equipment; halogen lamps more energy-efficient; light similar to sunlight	High operational costs; only <5% of energy is converted into light, with the rest going into heat; low energy efficiency; short lifetime
	Halogen incandescent	16–24	400–1200	100	2000		
Discharge light emission	Low-pressure discharge Low-pressure sodium Fluorescent	100–200 50–100	589 300–900	−44 82	18,000 6000–15,000	High energy efficiency; few end-of-life problems; suitable as grow lamps emitting primarily red and blue light	Require ballast (transistors); frequent on-and-off switching reduces lifetime; contain hazardous waste
	High-pressure discharge (HID) High-pressure mercury Metal halide High-pressure sodium High-pressure xenon	35–65 75–100 100–150 50	250–600 550–700 470 + 530–750 250–1100	n.a. 85 24 n.a.	24,000 6000–15,000 24,000 500–1500	Very high energy efficiency; useful in indoor horticulture; moderate to long lifetime	Require ballast and special fixtures; high initial costs; toxic mercury; UV-blocking filter required; negative end-of-life behaviour; require warm-up time; HPS without blue spectrum
	Plasma lamps	>90	300–850	94	30,000–50,000	Very high energy efficiency (90%)	No ballast but power supply required

(continued)

Table 5.3 (continued)

Light emission principle	Electrical lamps/ devices	Luminous efficacy in lumens per watt	Wave-length spectrum in nm	CRI[a]	Lifetime in hours	Advantages	Inconveniences
Electrolu-mines-cence	Injection electrolumines-cence devices Light-emitting diodes (LED)	90–150	380–780	>80	15,000–30,000	Flexibility for opting for monochromatic lights or a mix of various wave-lengths; emit little to no heat (= no cooling necessary); can be mounted closer to plants than other lights, thus space-saving; deliver entire PAR spectrum needed for growth and development; low operational costs; long life span	High initial costs; no ballast required; but power supply

According to Kozai et al. (2016) and Karlicek et al. (2017)

[a]Colour rendering index (CRI) measures how close an artificial light source reproduces the colours of an ideal or natural light source (e.g. sun, black body); highest value is 100

Table 5.4 Light-driven reactions in plants

Process	Definition	Examples
Photosynthesis	Conversion of light energy into chemical energy	Light reaction (PS I, PS II) Photophosphorylation (synthesis of ATP in PS I) Stomata opening during light phase for CO_2 uptake regulated by blue and red light
Photomorphogenesis Photomorphosis	Entire process of light-directed development Induction of developmental processes by light with the help of photoreceptors	Photoperiodic responses (effects caused by the duration of light phase with the help of photoreceptors), e.g.: Germination Induction of flowering and dormancy Growth rate Formation of storage organs Leaf drop Pigment synthesis
Phototropism (positive/negative)	Movement towards (positive)/away (negative) from the source of a light stimulus	Shoot growth towards the sun or lamp (positive), root growth away from light source (negative) Induced by phytohormones (auxin) and phototropin receptors
Allelopathy	Production of secondary metabolites affecting a plant community	By red light sensors (phytochrome), plants are able to detect their position relative to their neighbours helping them to compete with other plants
Stress response	Physiological avoidance and tolerance mechanisms, such as production of secondary metabolites	Synthesis of flavonoids, including anthocyanins, in dicot seedlings and apple skins induced by high irradiances and of ethylene in sorghum Photoinhibition Production of reactive oxygen species

Modified after Bresinsky et al. (2013) and Taiz et al. (2018)

side stems, viz. in order to obtain a more compact plant shape and a more robust structure, the plant needs to be exposed to blue light. Moreover, plants use blue light to determine the extent to which they should open their stomata (Wang et al. 2014). When exposed to increased quantities of blue light, plants open their stomata and accelerate their metabolism (if water is available). Further, blue light induces phototropism (encouraging leaves to grow towards the light), inhibits stem elongation and promotes pigment biosynthesis. Plants react to violet and indigo light similarly to their reactions to blue light (Taiz et al. 2018).

Green light (495–570 nm) is not absorbed by plants because they lack receptors for green colours (Terashima et al. 2009). Plants that are grown under green light will remain weak and may not survive for long.

Ultraviolet (UV-A and UV-B) light (200–300 nm) is also absorbed by plants. They receive UV light via the cryptochrome photoreceptor. Increased UV light induces the production of purple-coloured anthocyanins, which serve as a protection against UV radiation (Taiz et al. 2018). However, care must be taken by the horticulturist as an excess of UV light not only damages the DNA and cell membranes of plants but also disrupts photosynthesis (Kataria et al. 2014; Verdaguer et al. 2017).

5.4 What Happens Under Excess and Lack of Light?

Plant leaves are organized to absorb light in an optimal way. Chloroplasts change their position relative to the irradiation angle and intensity. Moreover, leaves and shoots follow the sunlight ensuring that leaves receive the optimal intensity of illumination while minimizing losses caused by reflection. This form of positive phototropism is called solar tracking (Taiz et al. 2018). If the radiation becomes too strong, the rate of photosynthesis will decrease, and the entire photosynthetic complex risks being damaged by the excess of light energy (Bresinsky et al. 2013). Even at 20–30% of full natural sunlight, photosynthesis is considered to be light-saturated.

Light intensities above saturation may cause damage to chloroplasts, as observed in *Arabidopsis thaliana* (Takahashi and Badger 2011), leading to a reduction in photosynthetic activity. Mechanistically, a burst of toxic and reactive oxygen species is considered to be responsible for such damage, which might be witnessed in the form of chlorotic or necrotic lesions (Cheng et al. 2016). This is called photoinhibition (Taiz et al. 2018; Schopfer and Brennecke 2010). Both shade-loving and sun-loving plants, when exposed to excess radiation, can show photoinhibitory damage. Shade-loving plants are light-saturated at approximately 100–500 μmol m^{-2} s^{-1}, whereas sun-loving plants are saturated at 500–1500 μmol m^{-2} s^{-1} (Bresinsky et al. 2013). Sometimes, the consequences of radiation stress are reversible depending on the extent of the damage. With regard to chloroplasts, plants can use protective and/or repairing mechanism to cope with light stress. Protective actions include the synthesis of secondary metabolites such as anthocyanins, which (1) act as a sun blocker by physically shielding the leave and (2) detoxify photoinhibitory-induced reactive oxygen species by functioning as scavengers for free radicals. Another line of defence comprises the synthesis of protective pigments including flavonoids and other phenolic substances, e.g. cutin (Schopfer and Brennecke 2010).

5.5 Strategies to Increase the Quality of Horticultural Crops by Lighting

According to recent state of the art (e.g. Ouzounis et al. 2015; Bantis et al. 2018), lighting systems are increasingly relevant in controlled environment horticulture. Afreen et al. (2005) have summarized experiments regarding the production of the secondary metabolite glycyrrhizin in *Glycyrrhiza uralensis* (Chinese liquorice). Glycyrrhizin is a

saponin and a main constituent of the Chinese liquorice root, which is used as a natural sweetener, in cosmetics and in traditional medicine. *Glycyrrhiza uralensis* was exposed to different wavelengths of light including red, blue, white and UV-B radiation in a controlled environment. Under red light, glycyrrhizin quantified in the root tissues showed the highest concentration in 1- to 3-month-old plants. Exposed to UV-B-radiation, the concentration of glycyrrhizin in the root tissues of 3-month-old plants was 1.5 times higher compared with that of the control plant under white fluorescent light treatment. The key message of this experiment is that growing plants under controlled environment conditions with special light treatment can accelerate the synthesis of secondary metabolites. This has also been observed in leaves of *Cannabis sativa* L. (hemp). When exposed to UV-B radiation, the leaves produce larger amounts of anthocyanins, which absorb most of the harmful UV radiation (Zwenger 2016). In turn, UV-B radiation is thought to stimulate the synthesis of Δ^9-tetrahydrocannabinol (THC), which accumulates in floral and vegetative tissues (Magagnini et al. 2018).

Exposing cannabis plants to UV-C radiation does not affect cannabinoid production but increases the concentration of bioactive stilbenes and cinnamic acid amide derivatives. Stilbenes belong to polyphenols (see ▶ Chap. 3) and have a positive impact on human health (Crozier et al. 2006). Resveratrol and pterostilbene, found in grapes and blueberries, may lower risks of cancer and cardiovascular diseases (Crozier et al. 2006). Cinnamic acids also have several health benefits, because of their antioxidant properties, their scavenging of free radicals and their antimicrobial activities (Sova 2012).

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Nutrient Deficiencies

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Important factors for the success of almost every plant growing system are the control and the adjustment of plant nutrients. No matter whether the system involves a traditional open field or a high-end indoor operation with hydroponics, proper nutrient management can help to optimize yields, costs of production, environmental impacts and the quality of horticultural products. In modern plant nutrition, 17 mineral elements are reported as nutrients (Table 6.1). To be counted among nutrients, an element has to fulfil three conditions. First, it must be needed by plants to complete their life cycle, i.e. a plant would not be able to produce offspring in the absence of this unique element. Second, the nutrient's function cannot be fulfilled by any other element. Specific elements that can replace some nutritional functions are known, but in general, they are not capable of substituting the whole spectrum of functions of the lacking nutrient. Third, a nutritional element has to be directly involved in the plant metabolism. In consequence, a nutrient deficiency leads to malfunctions in the plant's metabolism and, in turn, to a reduction of plant growth and development (Arnon and Stout 1939; Marschner 2012).

Nutrients are grouped into macronutrients or micronutrients (Table 6.1). Micronutrients are needed in a markedly smaller amount compared with macronutrients. On average, the concentration of a specific macronutrient is greater than or equal to 1 g kg^{-1}, whereas micronutrient concentrations only reach levels up to 100 mg kg^{-1} (Marschner 2012). The quantitative need of nutrients, however, can vary from plant species to plant species.

6.1 Nitrogen Deficiency

After carbon, nitrogen (N) accounts for the highest dry matter content (1–5%) of plant material among all established plant nutrients (Marschner 2012). Nitrogen is a constituent of many important molecules such as proteins, nucleic acids and amino acids and of signalling molecules. Nitric oxide (NO), for example, is known to be important for stomatal movement. It also plays a role in apoptosis, germination and many more mechanisms (Baudouin and Hancock 2014). Although the discovery of the Haber-Bosch process made inorganic N fertilizer available and affordable and enabled the intensive local application of large amounts of inorganic N onto fields, N deficiencies still occur during the cultivation of horticultural crop plants. The most common symptom of the N starvation of plants is the uniform chlorosis of the whole leaf blade of older leaves (Fig. 6.1). The green of the affected leaves becomes brighter until the leaves appear yellow. Under ongoing N starvation, the brightening process spreads out to the younger leaves, and the senescence of the older leaves begins (Uchida 2000).) Because N is a structural compound of chlorophyll, the described leaf discolouration is based on the decrease of the chlorophyll concentration in the leaf tissue. Under N deficiency, plants degrade not only chlorophyll but also proteins from the older leaves to reuse the assimilated N for the support of the younger parts of the plant and especially the generative plant organs (Kant et al. 2010). As a result, photosynthesis and growth are reduced, and the plant tries to invest in younger plant parts with the aim of finishing its life cycle.

However, apart from the negative effects of N deficiency on growth and yield, the limitation of the N supply can have positive effects on the quality of crops. Chishaki and

Table 6.1 Overview of plant nutrients with name, element abbreviation, available forms, classification into macro- or micronutrient, phloem mobility and some key functions according to Bergmann 1992; Marschner 2012; Behboudian et al. 2016

Nutrient	Element symbol	Available forms	Classification	Phloem mobility	Key functional areas (examples)
Carbon	C	CO_2, amino acids, carbohydrates (both with minor relevance for C-nutrition)	Macronutrient	High	Basic element of carbohydrates (e.g. glucose), fats (e.g. phospholipids) and amino acids (e.g. adenine)
Hydrogen	H	H^+, H_2O	Macronutrient	High	Proton gradients (e.g. ATPase), ion uptake (cotransport), redox reactions, basic element of carbohydrates, fats and amino acids
Oxygen	O	O_2, H_2O	Macronutrient	Not applicable	Oxidative phosphorylation, basic element of carbohydrates, fats and amino acids, general oxidation agent, respiration
Nitrogen	N	NO_3^-, NH_4^+, organic compounds	Macronutrient	High	Component of amino acids, DNA, RNA, chlorophyll, enzymes, membrane proteins, secondary compounds
Phosphorus	P	PO_4^{2-}, phytic acid, orthophosphate (both poor availability)	Macronutrient	High	Energy transfer (e.g. ATP), membrane structure, regulatory functions
Potassium	K	K^+	Macronutrient	High	Osmoregulation, photosynthesis, enzyme activation and protein biosynthesis
Magnesium	Mg	Mg^{2+}	Macronutrient	High	Central element of chlorophyll, also required for enzymatic activation, carbohydrate partitioning, chlorophyll/protein synthesis

(continued)

Table 6.1 (continued)

Nutrient	Element symbol	Available forms	Classification	Phloem mobility	Key functional areas (examples)
Calcium	Ca	Ca^{2+}	Macronutrient	Low	Cell wall and membrane structure, osmoregulation, cell extension, signalling
Sulphur	S	SO_4^{2-}, SO_2	Macronutrient	High	Lipids, glucosinolates, cysteine, methionine, glutathione, coenzymes
Iron	Fe	Fe^{2+}, Fe^{3+}	Micronutrient	Intermediate to low	Redox systems, photosynthetic and mitochondrial electron transport chains
Zinc	Zn	Zn^{2+}, $ZnOH^{+}$	Micronutrient	Intermediate to low	Enzyme component/activator, membrane structure, protein synthesis
Copper	Cu	Cu^{2+}	Micronutrient	Intermediate	Enzyme component, lignification, flower/fruit formation, photosynthetic and mitochondrial electron transport chain
Boron	B	H_3BO_3, $B(OH)_4^-$	Micronutrient	Intermediate	Cell wall and membrane structure, flower production, pollen production
Molybdenum	Mo	MO_4^{2-}	Micronutrient	Intermediate	Enzyme component (e.g. nitrate reductase), seed dormancy, photosynthesis
Manganese	Mn	Mn^{2+}	Micronutrient	Low	Enzyme component/activator, photosynthesis
Chlorine	Cl	Cl^-	Micronutrient	High	Osmoregulation, photosynthesis (oxygen evolution)
Nickel	Ni	Ni^{2+}	Micronutrient	Depends on species	Enzyme component, urease activity, seed germination

Fig. 6.1 Nitrogen deficiency symptoms exhibited by a tobacco plant (*Nicotiana tabacum*)

Horiguchi (1997) carried out an experiment in which they showed a correlation between phenolic metabolism and N deficiency. The experiment revealed that rice (*Oryza sativa* L.) seedlings exposed to nitrogen deficiency showed higher values of phenolic compounds, especially p-coumaric acid and ferulic acid. Bongue-Bartelsman and Phillips (1995) showed that N deficiency can lead to the expression of genes responsible for enzymes that play a role in flavonoid biosynthesis. This might also explain the findings of Stewart et al. (2002) and Ibrahim et al. (2011). Ibrahim et al. (2011) determined that the content of total flavonoids and phenolics becomes higher with decreasing N fertilization in the Malaysian medicinal herb *Labisia pumila* Benth, whereas Stewart et al. (2002) found a flavonol accumulation in the leaves of mature tomato plants (*Lycopersicon esculentum* cv. Chaser). These results indicate that N deficiency can be used as a tool for the quality improvement for leafy vegetables, as also shown by Galieni et al. (2015) who have reported that lettuces (*Lactuca sativa* L.) grown in pots show the highest polyphenol concentrations with no N fertilization. Furthermore, an N deficiency can induce the accumulation of anthocyanins as a reaction to photoinhibitory stress. The degradation of both chlorophyll and proteins

under N deficiency (Fig. 6.1) leads to the reduction of photosynthetic capacity. In consequence, the application of light to the leaves can induce oxidative damage, since reactive oxygen species are formed (see ▶ Chap. 8). In order to shield the leaf from sun energy, plants accumulate anthocyanins to absorb harmful UV radiation under N deficiency (Steyn et al. 2002). Additional details about anthocyanins and their role in high light protection are given in ▶ Chap. 5 and in the next section of this present chapter. Moreover, a reduction in N fertilization can cause a decline in the concentration of the amino acid asparagine (ASPN) and of reducing sugars (RS) in potato tubers. Both ASPN and RS are precursors of acrylamide, which is formed during the processing (e.g. deep-frying) of potatoes and is suspected to be carcinogenic. The effects of reduced ASPN and RS are even more pronounced when reduced N fertilization is accompanied with increased K fertilization. In contrast, under conditions of a high N and low K supply, free amino acids such as ASPN are thought to accumulate, whereas a sufficient K supply reduces free amino acids because of the increased metabolic capability of the plant. The concentration of RS is positively correlated with the level of N fertilization but is reduced under an increased K supply. The reduced concentration of RS under high K levels has been ascribed to the regulation of osmotic homeostasis. With a higher K content in the tissue, less RS is needed to maintain the osmotic pressure in the cell (Gerendás et al. 2007). Although the potentially carcinogenic effects (e.g. breast and ovarian cancer) of acrylamide have not as yet been validated and are still the focus of current studies, the acrylamide concentration in some deep-fried products is limited in the European Union (Pedreschi et al. 2013, Commission Regulation (EU) 2017/2158). Various concentrations are however legal in the European Union for a range of products such as ready-to-eat French fries (500 µg per kg^{-1}), potato chips (750 µg per kg^{-1}), wheat-based bread (50 µg per kg^{-1}) and roast coffee (400 µg per kg^{-1}).

6.2 Phosphorus Deficiency

Phosphorus (P) is a major plant nutrient and is essential for energy metabolism. Energy (from glycolysis, photosynthesis, etc.) is used to form P-rich molecules such as ATP and GTP, which provide energy for cell metabolism. Thereby, ATP can regulate primary metabolism by driving ion pumps or phosphorylating enzymes. P also plays an important role as a structural element. As part of the so-called phospholipids, P serves an essential role in cell membranes. P is also a structural component of nucleic acids, namely, DNA and RNA. To cope with a P deficiency, plants have evolved various strategies to maintain growth and generative propagation. As a result, plants change their morphological, physiological and metabolic processes. This set of changes is called the phosphorus starvation response (PSR) (Plaxton and Tran 2011). A well-known morphological adaption to P deficiency is the alteration of the plant root system. Plants suffering from P deprivation enhance their root growth in order to gain access to distant pools of P (Shen et al. 2011). The increased root growth is facilitated by a relocation of carbohydrates (mainly sucrose) from the leaves to the root. As a result, plants decrease their leaf expansion to save assimilates causing a slowdown in their development leading to a higher root-to-shoot ratio (Shen et al. 2011). The reduced leaf expansion also causes the leaf to appear darker green or even bluish-

Fig. 6.2 Trichomes of a hemp plant (*C.sativa*, *L. sativa*) containing cannabinoids and aromatic compounds

green, because of an increase in the number of chloroplasts per leaf area. The consequence is a higher reflection of green light from the same leaf area because of a higher chlorophyll concentration (Valentinuzzi et al. 2015). Nevertheless, the yellowing of leaves and interveinal chlorosis followed by necrotic lesions are also known in a variety of cultivars. The P deficiency results in insufficient P for the relevant cotransporter (phosphate antiporter) for the export of triose phosphate in exchange. Therefore, the carbohydrates produced during photosynthesis within the chloroplast cannot be exported into the cytosol (Schleucher et al. 1998). This gives rise to oxygen-derived radicals (atoms or molecules with a free electron), because electrons that are excited from magnesium in photosystem II cannot be transferred to oxidized reduction equivalents or ferredoxin. Radicals can damage cellular structures and molecules. To avoid the formation of these radicals, the plant produces and accumulates anthocyanins under P deficiency (Liu et al. 2015). Anthocyanins absorb harmful UV radiation and act as scavengers of free radicals, such as reactive oxygen species (ROS). In this context, anthocyanins are assumed, on the one hand, to scavenge ROS and, on the other hand, to absorb UV light, thus protecting the photosystems from photoinhibitory damage. The accumulation of anthocyanins can cause the red to purple colouration of plant leaves, stems and petioles (Fig. 6.2), which is a symptom often described during P deficiency (Hernández and Munné-Bosch 2015). The accumulation of anthocyanins has been reported in various plant species undergoing P deficiency. For example, P starvation induces anthocyanin accumulation in tomatoes (*Lycopersicon esculentum*) (Ulrychová and Sosnová 1970) and Chinese kale (*Brassica alboglabra* Bailey). (Chen et al. 2013). Stewart et al. (2002) detected an increase of flavonol in P-deficient tomato fruits at early ripening. A controlled P deficiency can also boost the content of alkaloids and phenolics in some plants. In an experimental cell culture of *Catharanthus roseus* (Knobloch and Berlin 1983), a rise in alkaloids such as tryptamine, indole alkaloids and phenolic was determined under P deficiency. This is especially interesting as *C. roseus* is known to contain anti-carcinogenic alkaloids (vincristine and vinblastine). An increase in phenolics has also been found in rice seedlings suffering from P deficiency (Chishaki and Horiguchi 1997). The enrichment

of horticultural food products with secondary metabolites such as anthocyanins is desirable as these compounds considered to confer health benefits when part of the human diet (Khoo et al. 2017). For instance, anthocyanins are assumed to have anti-carcinogenic (e.g. cancer of liver and breast), neuroprotective or metabolism-improving (e.g. against diabetes mellitus) effects on the human body (Hoensch and Oertel 2015; Daotong et al. 2017). As a positive side effect, the attractive colouration of, for example, apples or strawberries attributable to increased anthocyanin synthesis is a quality-improving feature that appeals to the consumer (Jezek et al. 2018). Anthocyanins can also improve the storage properties of horticultural products. For example, the postharvest damage to tomatoes caused by *Botrytis cinerea* is significantly reduced if they contain higher anthocyanin concentrations. The fruits can remain in storage for longer, as the anthocyanins reduce the ROS in the fruits (Zhang et al. 2013).

6.3 Potassium Deficiency and Other Nutrient Deficiencies

Although only a few examples of quality improvements in horticultural crops have as yet been established because nutrient deficiencies are usually accompanied by yield losses, some hints regarding effective nutrient deficiencies have been reported. Potassium (K) is a crucial element for the growth, development and reproduction of plants. K is relevant for the generation of turgor, for the activation of many enzymes (e.g. for the carbon metabolism), for the source-to-sink transport of metabolites or for the adjustment of guard cell aperture (Cakmak 2005; Hafsi et al. 2014; Behboudian et al. 2016; Zörb et al. 2019). Troufflard et al. (2010) have observed the induction of the biosynthesis of oxylipins (OL) and glucosinolates (GS) in the model plant *Arabidopsis thaliana*. Both OL and GS are assumed to accumulate as a reversible storage for nitrogen (N) and sulphur (S), which cannot be assimilated during K deficiency (Abdin et al. 2003; Barrelet et al. 2006; Armengaud et al. 2009). Both metabolites, namely, OL and GS, are thought to promote human health (Traka and Mithen 2009; Zivkovic et al. 2011). Gorelick and Bernstein (2017) report that K deficiency is correlated with increased tetrahydrocannabinol content in wild hemp *(Cannabis sativa* L. *sativa*) (Fig. 6.2), whereas Gremigni et al. (2001) have shown significant increases in alkaloid content in several varieties of lupins (*Lupinus angustifolius*). Another hint of alkaloid increase has been presented by Khan and Harborne (1991) who have witnessed alkaloid accumulation in *Atropa acuminata* during K deficiency in a hydroponic experiment.

Calcium (Ca) plays a crucial role as a structural element in cell walls and membranes and acts a counter ion for anions in the vacuole. Ca also acts an intracellular messenger and is thereby responsible for various responses to environmental factors (White and Broadley 2003). Chishaki and Horiguchi detected a rise in p-coumaric acid in the Ca-deficient seed coats of broad beans (*Vivia faba* L.). In addition, Tavares et al. (2013) have established that an enrichment of anthocyanins occurs in S-deficient grapevine (*Vitis vinifera* L.) plantlets. S has various functions in plants from pathogen defence to structural functions in enzymes and further to building aromatic compounds.

6.4 Practical Note

The challenge for the horticulturist is to implement nutrient deficiencies without yield losses. This can be achieved in two ways. The indirect method employs soils poor in nutrients from the beginning of cultivation. This requires accurate cost calculations and a suitable choice of crops in advance. Nevertheless, many uncontrollable factors are involved with regard to soil cultures. Therefore, the use of nutrient deficiency techniques in hydroponic cultures is highly recommended as these enable the gardener to control the nutrient solution directly. To avoid yield and quality losses, nutrient deficiencies should be performed only for short time periods and at intervals. The plants should be exposed to the nutrient deficiency only for 12 h every third to fourth day in the last 2 weeks prior to harvest. This can be achieved by omitting a nutrient when the nutrient solution is prepared. Another way of provoking nutrient deficiency is by decreasing a nutrient's availability via the changing of the pH of the solution to an unfavourable point. For example, the plant-available forms of P in a hydroponic solution reach a maximum at a pH of around 5, whereas the availability significantly starts to decrease with a pH of 6 and higher, thereby causing a P-deficient environment for the plant (Trejo-Téllez and Gómez-Merino 2012). The choice concerning which nutrient to pick for deficiency techniques strongly depends on the crop being cultured and those secondary metabolites that are desired to be accumulated.

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Salt Stress

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The vast majority of our horticultural crops are so-called glycophytes. This means that these plants show optimal growth only under moderate salt concentrations. If salt concentrations are too high, plants will suffer. Only a few, but economically insignificant, horticultural crops such as *Salsola soda* (agretti), *Salicornia bigelovii* (samphire), *Beta maritima* (sea beet) or *Portulaca oleracea* (common purslane) are halophytes that need salty water for growth and development.

The growth of all glycophytic horticultural crops is slowed down by excessive concentrations of sodium (Na^+)-, chlorine (Cl)-, ammonium (NH_4^+)- or sulphate (SO_4^{2-})-containing salts. Extreme salt concentrations may even lead to plant death (Hasegawa et al. 2000; Geilfus 2018a). However, an optimal supply of these ions will promote growth, as chlorine (Cl), nitrogen (N) and sulphur (S) are plant nutrients. Na^+ does not belong to the group of plant nutrients. However, a moderate supply can induce beneficial growth effects (Marschner 2012).

Toxicities, which are a result of the excessive application of NH_4^+ and SO_4^{2-} salts, rarely occur in horticultural production systems for two reasons. First, fertilizer products containing these ions are expensive. Therefore, there is an economic awareness to avoid using fertilizer rates that go beyond the promotion of plant growth. Second, the concentration of NH_4^+ and SO_4^{2-} in the growth medium (e.g. hydroponic nutrient solution) can be easily monitored, either sporadically and less accurately (quick test) or continuously with high precision (ion-selective electrodes).

In horticultural practice, Cl concentrations in the growing media are usually not monitored. This is because of the limited awareness among growers that plant development can be endangered by a too high dose of Cl. Cl is a micronutrient that is only required in traces. Depositions of Cl from dust or water, such as irrigation water or water for preparing the hydroponic nutrient solution, are often sufficient to meet the demand of the plant. Moreover, the seeds of plants contain enough Cl to supply the plants for days or even weeks after germination (White and Broadley 2001). Because of this low requirement, horticultural crops are, in most instances, supplied with more Cl than is necessary under glass/foil production systems. One reason is that, in fertilizer products, the anion of Cl, namely, chloride (Cl^-), is frequently the counterion of cationic nutrients. Therefore, fertilization with potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}) and NH_4^+, given together with Cl^- as the counter anion (KCl, $CaCl_2$, $MgCl_2$ and NH_4Cl) or applications of kainite ($KMg(SO_4)Cl{*}3H_2O$), contributes to the input of Cl. Although the need for Cl is covered, more and more Cl is supplied during the growth period via those fertilizer products as the demand of the plant for the macronutrients K^+, Ca^{2+}, Mg^{2+} or NH_4^+ is high. In other words, the horticulturist intends to fertilize plants with these cationic macronutrients but does not take into account that these measures can stress plants by exposing them to excessive amounts of Cl.

Na^+ is a beneficial ion because moderate amounts can increase the turgor of the plant, thereby promoting growth (Maathuis 2013). However, too high concentrations are toxic. Care must be taken because water (irrigation water or water for preparing the hydroponic nutrient solution) can contain considerable amounts of Na^+. This is certainly the case in coastal areas but is also a problem in areas with high concentration of Na^+ in parental bedrock material that is prone to weathering. During the growth period, the concentration of Na^+ in hydroponic tanks is likely to increase if the water is not fully replaced regularly. Whereas mineral nutrients are taken up by the plant, the

uptake of Na^+ is restricted by the plant as the plant avoids Na^+ uptake. In consequence, the concentration of Na^+ increases in the tank because the water is steadily reduced because of the plant's water consumption, i.e. by cellular water uptake and transpiration. Refilling the tank with fresh Na^+-contaminated water will further increase the concentration of Na^+ in the growing media. If a certain Na^+ is exceeded, plants cannot avoid the uptake of Na^+, which will then accumulate excessively in the cells where it causes toxicities.

Mineral ($NaNO_3$) or organic fertilizers can also contribute to the input of Na^+. Organic fertilizer (manures, slurries) contain considerable amounts of Na^+ (Diez et al. 2004), as Na^+ is a nutrient for mammals and is present in gastrointestinal secretions from our livestock. This is also true for the excreta from humans, as used in some regions of the world to supply plants with mineral nutrients such as phosphorus. The horticulturist should be aware of these 'hidden' sources of Na^+ as otherwise Na^+ might accumulate excessively.

7.1 Salt Toxicity Effects

A plant that is suddenly treated with a stressful dose of NaCl will lose cell turgor within the first few minutes (please see ▶ Chap. 8 for a definition of turgor). Plants with filigree leaves such as basil appear limp. The reason for the lack of cellular water is that the addition of salt decreases the osmotic potential of the soil solution (e.g. hydroponic nutrient solution), making it harder for the plant to take up water (Munns 2002) (please see ▶ Chap. 8 for a definition of osmotic potential). Within the first few minutes, the loss of turgor is transient because the cells restore their turgor. This is possible because plants close their stomata minutes after experiencing a drop in the osmotic potential of the solution that harbours the roots (Geilfus et al. 2015a).

After a couple of days of exposure to high salt stress, leaf growth is hampered, which is attributable to a reduction in cell elongation and in the cell division rate (◘ Table 7.1). This is the so-called osmotic stress phase (Munns and Tester 2008). The reasons for the growth reduction during the osmotic stress phase remain to be elucidated. One explanation is that the plant shifts its metabolic activity from growth processes to the synthesis of so-called osmotically active plant metabolites (osmolytes) that decrease the osmotic potential of the cellular water (Zörb et al. 2018). The lower the osmotic potential in the cell, the easier it is to take up water. Osmolytes are highly water-soluble organic compounds that do not interfere with normal metabolic reactions, even at high cellular concentrations (Flowers et al. 1977). Examples are proline (amino acid), glycine betaine (quaternary ammonium compounds) or inositol and mannitol (sugar alcohols). However, the production of osmolytes means that large amounts of the photoassimilates are channelled into the production of these metabolites and are no longer available for supporting growth. Thus, metabolic measures for stress tolerance and adaptation compete energetically and materially with growth (◘ Fig. 7.1).

Another school of thought considers that stress-related changes in the rheological, viz. fluid, properties of the cell wall contribute to the salt stress-induced growth reduction during the osmotic salt stress phase. In a fully grown cell, the cell wall is rigid and stiff. This property is important to protect the cell from the ingress of aphids

Table 7.1 Plant response to salinity at various timescales. The effects on a salt-tolerant plant are basically identical to those attributable to soil water deficit

Time	Water stress effects (Observed effect on growth of a salt-tolerant plant)	Salt-specific effects (Additional effects on growth of a salt-sensitive plant)
Minutes	Instant reduction in leaf and root elongation rate and then a rapid partial recovery	
Hours	Steady but reduced rate of leaf and root elongation	
Days	Leaf growth more affected than root growth; reduced rate of leaf emergence	Injury visible in oldest leaf
Weeks	Reduced final leaf size and/or number of lateral shoots	Death of older leaves
Months	Altered flowering time, reduced seed production	Younger leaves dead, plant may die before seed matures

Taken from Munns (2002), with permission from Wiley, license number 4414591176211 issued August 23, 2018

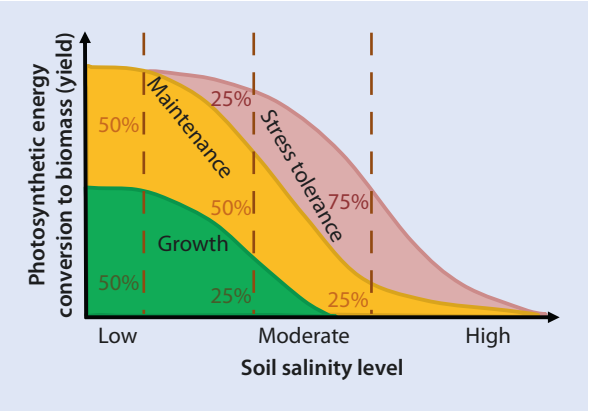

Fig. 7.1 Scheme of energy gain and energy use of crops under salinity stress. The proportion of energy used for maintenance, growth and stress defence is portrayed (given as percentages). The relative proportions will change depending on salt stress intensity. For instance, if the soil salinity level is low, no energy is required for metabolic adjustments that confer stress tolerance. This changes as a function of the soil salinity level. (Taken from Zörb et al. (2018), with permission from Wiley, license number 4414040808618 issued August 22, 2018)

Fig. 7.2 Detail of the a stem of maize (*Zea mays* L.) showing incrustation with NaCl

or phytopathogenic fungi. A high cell wall rigidity is also crucial because, otherwise, the cell would burst, as the cellular turgor pressure is too high for the thin plasma membrane to counteract the internal forces generated from the cytoplasm (Keegstra 2010). The process of growth-induced cell elongation, however, requires that the cell wall relaxes during the period of cell elongation. This cell wall loosening allows the turgor to push the microfibrils of the cell wall apart. By these means, the cell grows (elongates) and newly synthesized cellulose fibrils are segregated into the extracellular matrix to fill the gaps (Cosgrove 2005). However, during the osmotic phase of the salt stress, this cell wall loosening, which is mediated by the class of expansin proteins, seems to be impaired. As a result, the cell wall remains stiff, the cells cannot elongate, and plant growth is hindered (Fricke et al. 2006; Geilfus et al. 2010).

After days and weeks of exposure with NaCl, more and more salt ions (i.e. Na^+ and Cl^-) are taken up by the plant (Fig. 7.2), finally exceeding cellular thresholds and causing cellular damage (Munns 2002). First, the salt ions stream into the roots and move radially via the apoplastic or symplastic pathway towards the root xylem. The root xylem is a transport tissue in vascular plants that channels the flow of water and nutrients from root to shoot. The uptake of ions into the xylem, however, is selective. This means that ions cannot access the xylem in an uncontrolled manner. Uptake is mediated by transmembrane proteins (ion transporters) that control the transport of

required ions in useful amounts (i.e. the uptake of excess ions is restricted) (Pitman 1977; Barbier-Brygoo et al. 2011). However, when excessive amounts of Na^+ and Cl^- reach the root xylem under conditions of salinity, this selectivity of uptake can no longer be maintained, which means that too much Na^+ and Cl^- are transported into the shoot, causing toxicities. In the case of Na^+, the reason is that Na^+ when highly concentrated leaks through uptake proteins for K^+ into the xylem (Blumwald et al. 2000). The situation with regard to Cl^- is similar. The plant requires only the smallest of amounts of Cl^-, e.g. for photosynthesis. However, since Cl^- has a similar charge and ion radius to those of nitrate (NO_3^-), large quantities can slip through NO_3^- channels (N is a macronutrient, and hence plants take up large amounts of NO_3^-) (Geilfus 2018b).

Once Na^+ and Cl^- have reached the xylem, both ions will be transported upwards towards the shoot where they are released into the leaf. After influx into a leaf cell (i.e. mesophyll cell), both ions can be sequestered into the vacuole. This has the advantage that the ions do not accumulate in the cytosol. Cytosolic concentrations of Na^+ and Cl^- that are too high are most probably toxic for the plant because an increasing salt strength (concentration) in the cytosol is thought to hamper the functionality of many enzymes. This severely impairs metabolism. Moreover, excessive Na^+ damages the integrity of membranes, thereby causing the leakage of nutrients and metabolites between the cytoplasmic compartments or between the symplast and apoplast. Electric membrane potentials are then disturbed (Flowers et al. 2014; Geilfus 2018b).

Furthermore, the uncontrolled influx of Na^+ and Cl^- into the chloroplast or mitochondria can disturb photosynthetic or respiratory electron flow, as Na^+ and Cl^- can either damage the structural integrity of the chloroplast or mitochondria or affect the enzyme activities therein. In consequence, photosynthetic electron transport becomes over-reduced, electrons spontaneously reduce O_2 to the superoxide radical ($O_2{\cdot}^-$), and reactive oxygen species (ROS) are released (Miller et al. 2010). Likewise, mitochondrial respiration is inhibited and enhances ROS production in respiratory electron transport (Jacoby et al. 2010). Excessive ROS concentrations are dangerous because they damage chloroplastidial or mitochondrial proteins and membranes. For this reason, the plant has mechanisms to break down the superoxide radical. The enzyme superoxide dismutase mediates the partitioning of $O_2{\cdot}^-$ to H_2O_2. However, the presence of an excess of H_2O_2 in the chloroplast or mitochondrion is also highly critical because H_2O_2 will be converted into the destructive hydroxyl radical (·OH) through transition-metal-mediated pathways (Dietz et al. 2016; Bose et al. 2017). Both chloroplasts and mitochondria are relatively rich in transition metals because copper, iron, manganese and zinc are cofactors in metalloenzymes and metalloproteins, all of which are needed for electron transport in photosynthesis and respiration (Yruela 2013). The resulting radicals spontaneous oxidize pigments, electron acceptors, (endo-)membranes, proteins, metabolites, DNA or structural components of the compartments, leading to structural disorganization, to chlorotic lesions and later to necrotic spots (Fig. 7.3). As a result, major metabolic processes cannot work properly (Fig. 7.4), which delays crop growth and development (Fig. 7.3).

Fig. 7.3 NaCl-stressed maize. Control without NaCl **a**; 100 mM NaCl leads to a reduction in growth and biomass **b**; necrotic leaf area, as indicated by *black arrow* **c**

7.2 Adaptation Strategy to Mitigate Burst of ROS Under Salinity Stress

In order to adapt to salinity, the plant endeavours both to detoxify ROS and to avoid their production. Among many strategies and mechanisms involved in the adaptation to excess ROS, the production and tissue accumulation of the water-soluble anthocyanin are of utmost significance (Eryılmaz 2006). Anthocyanins are red, purple or blue vacuolar water-soluble plant pigments that are relevant for plant performance under various stresses. They belong to the class of flavonoids and are synthesized in the cytosol via the phenylpropanoid pathway. Anthocyanins are glucosides of anthocyanidins, and

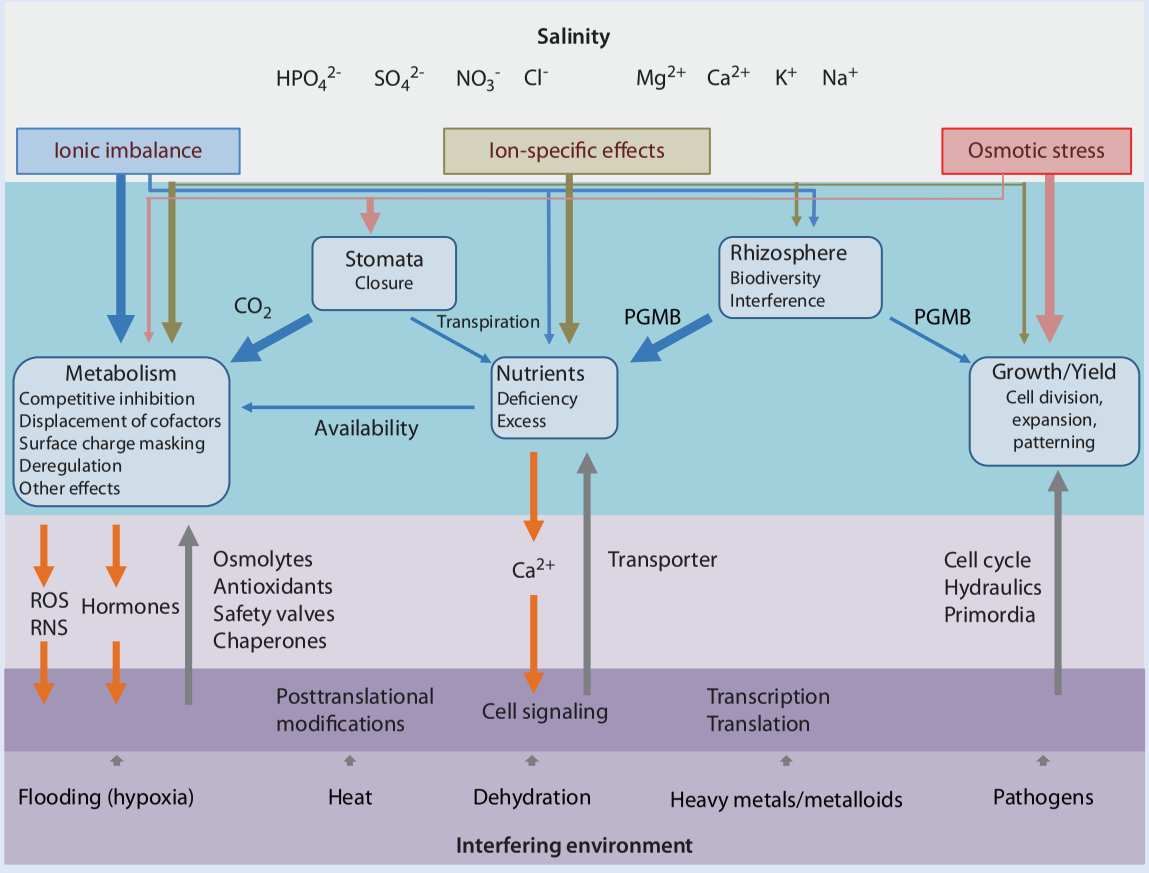

Fig. 7.4 Interaction network of salinity stress with cellular processes. Ions, entering the plant organs in excess, interfere with ion balance, exert ion-specific effects and establish osmotic stress. The *lines* and their *thickness* illustrate how these stress components distinctly affect metabolism, stomatal function, nutrient homeostasis, processes in the rhizosphere and biomass development. (Taken from Zörb et al. (2018), with permission from Wiley, license number 4414040808618 issued August 22, 2018)

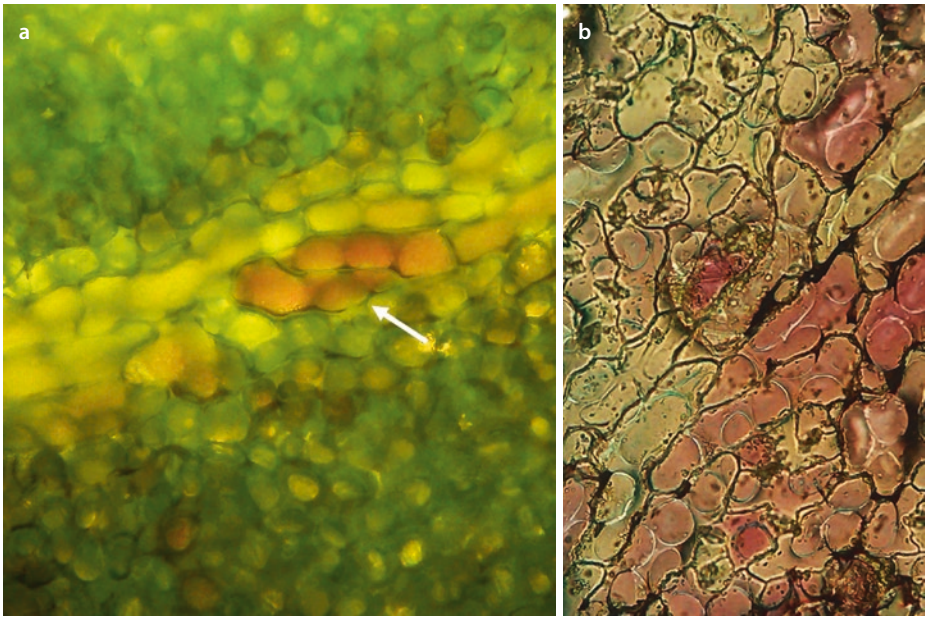

Fig. 7.5 Pak Choi leaves (*Brassica rapa chinensis*) accumulate anthocyanins under stress. Microscopic view of the leaf surface shows detail (*white arrow*) with epidermal cells that have turned red **a**. Leaf epidermal peel with anthocyanin-filled epidermis cells **b**

their general structure consists of a flavylium ion backbone that is linked to hydroxyl and methoxy groups (Jezek et al. 2018). Additional linkages to sugars and organic acids diversify the complexity and define their physiological functions by affecting various properties such as colour, stability and bioactivity (Kovinich et al. 2014; Miguel 2011).

Anthocyanins act in a photoprotective way via light attenuation and/or via their antioxidative properties (Landi et al. 2015). They have the potential to mitigate photooxidative injury in leaves, both by shielding chloroplasts from excess high-energy quanta and by scavenging ROS (Neill and Gould. 2003). Anthocyanins absorb visible light mainly in the green region of the light spectrum. Thus, they can efficiently protect leaves from light stress by absorbing excess photons that would otherwise burden chlorophyll molecules (Gitelson et al. 2001). In other words, they act literally as a sunblock, which is particularly helpful when electron transport is over-reduced because of salt stress. The finding that anthocyanins are mainly localized in epidermis cells (Fig. 7.5) or in mesophyll cells below the epidermis supports the assumption that anthocyanins function as light shields.

In addition to their role in light attenuation, some anthocyanins scavenge ROS because of their antioxidant activity (Kytridis and Manetas 2006). Hence, anthocyanins alleviate photooxidative damage by scavenging ROS (as ROS can destroy biological structures via oxidation). However, anthocyanins often reside only in the vacuole of the epidermis cells. Therefore, the pigments are not optimally localized to scavenge ROS that are produced primarily in the chloroplasts and mitochondria of the mesophyll cells. Hence, the relevance of anthocyanins as scavenging agents is thought to be of minor

relevance for ROS that are primarily localized in those compartments (Hernandez et al. 2009). With regard to the detoxification of H_2O_2, anthocyanins might be more relevant, as H_2O_2 is more durable than other ROS species. Because of this property, H_2O_2 diffuses throughout cells and compartments, entering the vacuole where it encounters anthocyanins (Bienert et al. 2006; Fini et al. 2011). Upon arrival in the vacuole, vacuolar peroxidases reduce, viz. detoxify, H_2O_2 into water by using flavonoids such as anthocyanins as electron donors (Bienert et al. 2006). Hence, anthocyanins may be efficient ROS scavengers, provided that they are in close vicinity to the source of ROS production. However, notably, not all anthocyanins exhibit the same potential to act as ROS scavengers, as this greatly depends on their chemical structure.

In summary, anthocyanins are relevant for the adaptation of plants to salty environments. Excessive salt ions cause cellular toxicities, ultimately causing the release of ROS. Anthocyanins play an important function in both the detoxification and avoidance of salinity-induced formation of excess ROS.

7

7.3 Enriching Bioactive Compounds in Crops by Exposing the Plants to Salt Stress

Anthocyanins not only protect the plant tissue against stressors such as high radiation but also have a health-promoting potential when included in the human diet. Because of their antioxidant and anti-inflammatory properties, the dietary intake of anthocyanins lowers the risk of cardiovascular diseases and diabetes (Ghosh and Konishi 2007; Seeram 2008; Miguel 2011).

As explained above, anthocyanins are important secondary plant components that increase the plant fitness under conditions of salt stress. This knowledge can be utilized in plant production strategies to produce horticultural crops that are enriched with these human health-promoting metabolites. In their study, Guo et al. (2014) manipulated the anthocyanin content in the sprouts of broccoli. For this, plants were grown on vermiculite treated with 40 or 80 mM NaCl solution by watering the plants every 12 hours with the salt solution. After 4 days of treatment, plants were analysed. The salt treatment was shown to increase the anthocyanin content in the sprouts. Salt stress can also be applied in a controlled manner to enrich tomato fruits of the anthocyanin-accumulating tomato genotypes 'Sun Black' (Borghesi et al. 2011) in anthocyanins.

This chapter is strongly focused on the synthesis of anthocyanins as an adaptive response to salt stress. However, the adaptation to salt stress requires complex metabolic adjustments, involving primary and secondary metabolic pathways (Fig. 7.4) (Geilfus et al. 2015b). In other words, many more secondary metabolites are involved here. For instance, Jaleel et al. (2008) have shown that the content of the anticarcinogen alkaloids vincristine and vinblastine can be increased in the medicinal crop of Madagascar periwinkle (*Catharanthus roseus*) by means of salt stress. Salt stress was induced by presoaking the seeds for 12 h in 50 or 100 mM NaCl, before the plants were grown with no further NaCl. In response to the treatment with 100 mM NaCl, the alkaloid content increased, whereas biomass decreased. In the light of this biomass reduction, care must be taken in horticultural practise that the production of these alkaloids remains profitable.

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Drought Stress

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8.1 Introduction

Drought is considered the most limiting factor to crop production worldwide (Boyer 1985; Costa et al. 2007). Water is an increasingly scarce resource in many important regions for vegetable production, for example, in California, USA; in the Guanzhong Plain, China; or in the south of Spain (Cabello et al. 2009; Tang et al. 2013; Olen et al. 2016; Coyago-Cruz et al. 2017). Drought stress events are anticipated to increase in the light of the changing climate. Competition for water will also increase as the world population is growing. Therefore, the more efficient use of water for irrigation is an important target in agriculture (Somerville and Briscoe 2001).

Notably, a mild and short water deficit can improve the quality of some crops. Under controlled conditions, a precisely regulated, crop-adapted water supply can result in a considerable rise of valuable components such as antioxidants, enzymes, sugars, acids and minerals (Sanders and Arndt 2012; Acevedo et al. 2013; Albert et al. 2016; Bogale et al. 2016; Coyago-Cruz et al. 2017; Hazrati et al. 2017). This implies a great potential to improve both the efficiency of water use and the quality of our horticultural plant products (Costa et al. 2007). This chapter aims to explain physiological adaptation strategies of plants to drought and to present methods for the successful application of this knowledge to produce vegetables and medicinal plants that are enriched in the desired metabolites.

8.2 Function of Water in Plants

Water is the most important substance in plants: the water content of nonwoody plant tissue is about 70–95%, and all physiological processes are dependent on the presence of water (Lambers et al. 2008). Water is the transporting medium for nutrients and metabolites, allowing transport between the various plant organs and the cells. In other words, water is the solvent that enables cellular organization and homeostasis. It is taken up from the soil by the roots and transported to the above-ground parts of the plant through the xylem. The smallest and mostly insignificant amounts of water can also enter the plant through (1) opened stomata or (2) epidermal water exchange. The absorbed water is not pure but contains many dissolved nutrient ions and some small organic substances that are required in various metabolic processes inside cells. Last but not least, water cools cells under heat stress (Farooq et al. 2012).

Cellular membranes are semipermeable, which means that ions, large charged metabolites and other compounds cannot disperse freely. For this reason, specialized proteins inside cell membranes, the so-called transporter proteins, actively regulate the influx and efflux of ions and metabolites across the membranes. Water, however, can cross cell membranes with much less restriction, partly flowing directly through the membranes and partly through aquaporins. The aquaporins are specialized channels (proteins inside the cell membranes) that allow the rapid exchange of water in plant cells. In an isothermal system (i.e. when the temperature on both sides of the membrane is equal), water diffuses through a semipermeable membrane from a region with the smaller solute (e.g. ion or metabolite) concentration to a region with the higher solute concentration, aiming to balance the solute concentration per volumetric unit of water

across the membrane, even though this target can rarely ever be achieved in practice because of the dynamics of metabolism. The more solutes dissolved in the water, the stronger is its chemical potential. This is called the osmotic potential (Ψ_π). It is measured by the pressure unit called the megapascal (MPa), which describes the pressure that is needed to press the water out (Nabors 2004). The osmotic potential always has negative values.

To make water diffuse from the xylem into the cell, the solute (e.g. ion) concentration inside the cell must be higher than the solute concentration in the xylem. In other words, the osmotic potential inside the cell must be lower (more negative) than that outside, viz. in the xylem, so that the water is 'attracted' and flows from the xylem into the cell.

Because of the lower osmotic potential, the pressure exerted by the water inside the cell is higher than that outside, pressing the outer cell membrane (the plasma membrane) against the cell wall. As a result, the cell is kept in shape. This force is called the turgor pressure. Typical turgor pressures in plants range between 1.0 and 5.0 MPa (Lambers et al. 2008). An appropriate turgor pressure is essential for regular cell functions, metabolism and growth, as it ensures the connectivity between adjacent cells and thus the stream of the cytosol (the aqueous phase between the plasma membrane and the cellular compartment) through the plasmodesmata (channels connecting cells) and thus enables transport. A loss of turgor will result in the loss of cell stability, a visible effect that we call wilt.

Only 1–5% of the water taken up by the roots is finally kept by plants, as the rest is lost via transpiration through the stomata (Kramer and Boyer 1995; Lambers et al. 2008). This may seem inefficient. However, transpiration is the main driving force for the water stream into/through the plants, enabling nutrient uptake and distribution, and serves as a temperature regulator inside the plant (Kögler and Söffker 2017). Without transpiration, the leaf temperature can rapidly rise to lethal values.

Clearly, in the case of a lessening water supply, the plant has to react rapidly because almost all physiological processes are affected under drought (Kögler and Söffker 2017). By 'react', we mean that the plant has to induce mechanisms that help it (1) to take up water via its roots and (2) to maintain cell turgor (i.e. avoid the loss of water).

8.3 What Happens in Plants During Drought Stress?

In general, drought stress is an imbalance between the water supply and the plant's water demand (Tardieu 1996). It occurs when the water demand of the plant cannot be fulfilled, i.e. when too little water is available or less water is taken up than is needed for optimal growth and development (Brouwer et al. 1989). This can be the case when the transpiration rate from the leaves surpasses the water uptake by the roots, e.g. because of (1) insufficient precipitation, (2) too little soil water content or (3) the retention of water held in small pores at large suction tensions (Salehi-Lisar and Bakhshayeshan-Agdam 2016; Lambers et al. 2008).

As a reaction to a drying soil, the roots produce the phytohormone abscisic acid (ABA) (Avolio et al. 2018). ABA is transported through the xylem up to the leaves, where it accumulates as drought continues. High concentrations of ABA lead to a loss of

water in the guard cells, so that the stomata close (Nabors 2004; McAdam et al. 2010). Taking only some seconds to some minutes, the closing of the stomata is a fast reaction of plants to reduced water availability (Kuromori et al. 2018). In some instances, stomata close even before any change occurs in the plant's water status (Karuppanapandian et al. 2017). Thus, fast mechanisms must be present that sense and perceive changes in soil water content.

However, the stomata are not only the exit for transpiring water but also the entry for CO_2, the basic material for carboxylation after the light reaction (see ▶ Chap. 14). This is the reason that the closure of the stomata results in markedly lower carbon assimilation (Flexas et al. 2004). What happens to photosynthesis if there is not enough CO_2, as may occur under drought conditions when the guard cells close the stomata? Photosynthesis comprises two main reactions: the light reaction and the dark reaction (Calvin cycle). During the light reaction, chloroplastidial chlorophyll pigments absorb energy from the sunlight. This energy is used to split water, a process that releases a free electron. The electron is passed through redox proteins (the electron transport chain), finally providing the energy for the enzymatic reduction of $NADP^+$ to $NADPH+H^+$. $NADPH+H^+$ is a strong reductant, viz. a storage for electrons, which are needed to energize a myriad of anabolic reactions. The photosynthetic transfer of electrons also energizes the phosphorylation of ADP to ATP. ATP acts a long-term storage for energy. $NADPH+H^+$ and ATP are used in the dark reaction, the Calvin cycle, by providing the energy for the incorporation of inorganic carbon (CO_2) into organic C-skeletons (e.g. triose phosphates). In C_3 plants, triose phosphate is the first stable carbohydrate, being the precursor for the synthesis of all other metabolites in the plant. Hence, a water deficit can reduce CO_2 concentrations in the photosynthetic plant cells because of stomatal closure. By this means, a water deficit can result in stunted growth as less carbon is assimilated.

8.4 Plant Reactions to Drought Stress

8.4.1 Adjusting the Osmotic Potential (Ψ_π)

Some species are able to maintain photosynthetic activity and plant growth under a reduced water supply. This is because they manage to maintain their cellular turgor (Avolio et al. 2018). How do they do this? These plants can make a so-called osmotic adjustment: this means that under drought stress, they actively accumulate certain osmotically active substances in their cells to lower the osmotic potential (i.e. to make it more negative) (see also ▶ Chap. 7). The accumulation of such highly soluble and almost electrically neutral compounds (called osmolytes or osmoprotectants) happens mainly in the chloroplasts and in the cytosol (Zivcak et al. 2016). This leads to water being attracted from the surroundings, e.g. the vasculature or the apoplast, ultimately driving uptake from the soil solution (Kramer and Boyer 1995; Sanders and Arndt 2012). As a result, turgor pressure is maintained, allowing normal cellular homeostasis. The stomata can remain opened, enabling the uptake of a sufficient amount of CO_2 so that photosynthesis and the Calvin cycle can run normally and growth is not reduced (Zivcak et al. 2016). Simultaneously, an upregulation of genes occurs that encode enzymes for the

synthesis of aquaporins, leading to a higher number of aquaporins in the cell membranes so that the water uptake of plant cells is facilitated (Avolio et al. 2018).

By implementing a short and well-controlled water shortage, the horticulturist can induce this process of osmotic adjustment with the aim of changing the sugar to acid ratio in a plant. This is because many of the osmotically active compounds (osmolytes) that accumulate during osmotic adjustment are soluble sugars (sucrose, hexose, trehalose) (Sanders and Arndt 2012). The sugar content in an aqueous solution can be measured with a refractometer and is expressed in degrees Brix (°Brix; see ▶ Chap. 19) (Kuscu et al. 2014). Thus, the accumulation of sugars during the process of osmotic adjustment normally results in a sweeter taste, e.g. in tomato fruits (see ▶ Chap. 19).

In addition to sugars, some other substances contribute to the osmotic adjustment. Among them are organic acids, amino acids or K^+, Na^+ and Cl^- (Lemoine et al. 2013; Zivcak et al. 2016). Valine, leucine, isoleucine, glutamic acid, aspartic acid, threonine and, importantly, proline (Pro) belong to the amino acids that are produced under conditions of drought. Pro is one of the most relevant osmotically active compounds (Girousse et al. 1996). The Pro concentration typically increases when drought stress becomes more severe (Hazzoumi et al. 2015; Khan et al. 2015; Slama et al. 2011). Pro exerts a protective effect on cell structures and has been shown to be an effective scavenger of reactive oxygenic species. As its concentration rises during numerous stress situations, it can be used as an indicator for the extent of stress in plants (Avolio et al. 2018; Kanayama and Kochetov 2015). Since Pro confers an acid taste (Yahia et al. 2011), the content of Pro and other drought-inducible acids is relevant for the modulation of the taste, viz. the sugar to acid ratio in plant-based food.

However, the composition and the quantity of osmotically active compounds vary widely not only between different species but also between cultivars. The ability to actively adjust the osmotic potential as a reaction to drought stress seems to be genetically determined (Acevedo et al. 2013; Albert et al. 2016). The reader should also note that the adjustment of the osmotic potential is limited to some extent. If the drought stress becomes too severe, turgor loss and wilting cannot be avoided. However, mild drought stress conditions can have a positive effect on product quality as described above.

8.4.2 Rise of Antioxidants in Drought-Stressed Plants

Furthermore, drought stress induces an increase of the oxidative capacity, which is of potential interest for the horticulturist if they intend to improve the quality of vegetables and medicinal plants.

Stomata close when the plant suffers a water deficit. This closure results in a limited CO_2 supply entering the plant and thus in a reduced photosynthesis rate. Apart from the reduced biomass accumulation, a disturbance of the photosynthetic reactions has another important consequence (◘ Fig. 8.1).

Because of the lower availability of CO_2, the activity of the Calvin cycle is reduced, and less $NADPH+H^+$ is oxidized to $NADP^+$. However, electrons are continuously delivered via the photosynthetic electron transport chain to reduce $NADP^+$ to $NADPH+H^+$. As a smaller number of them can be bound to $NADP^+$, more free

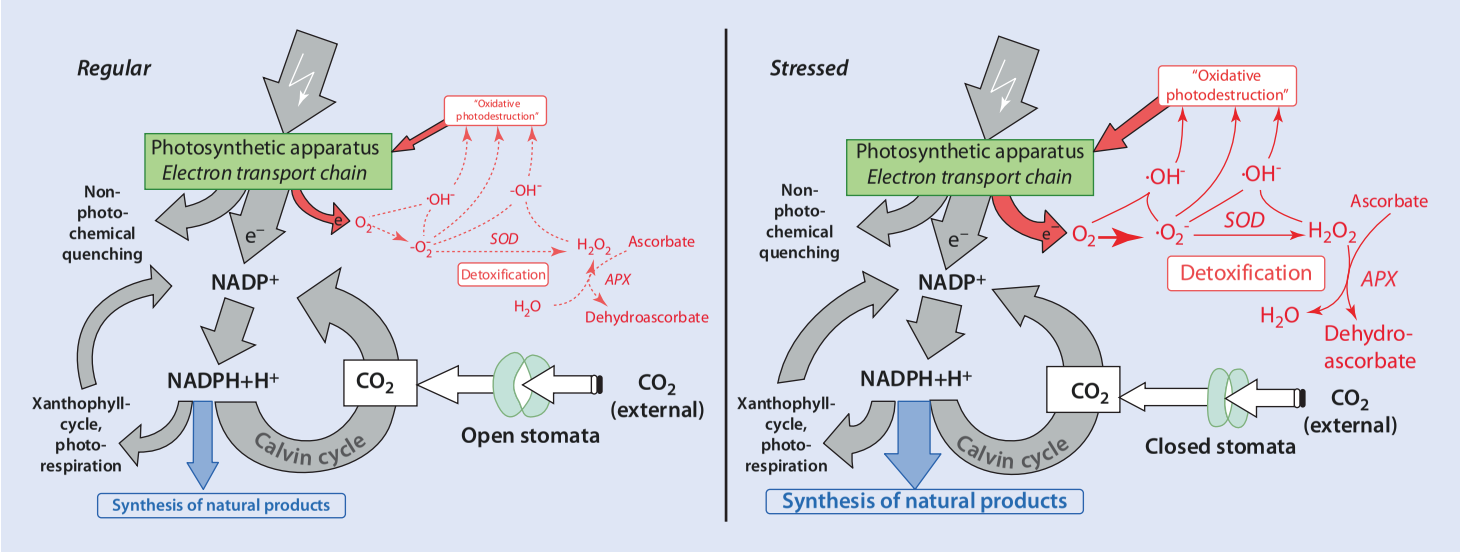

Fig. 8.1 Energy dissipation in plants under regular and stressful conditions. Closed stomata result in a lower CO_2 supply and, hence, in reduced Calvin cycle activity. The surplus of energy is used to enhance the activity of the xanthophyll cycle and for the synthesis of secondary plant compounds. On the other hand, a rise of reactive oxygenic species (ROS) occurs, which is followed by a higher synthesis of antioxidants. (Diagram taken from Selmar and Kleinwächter (2013a), with permission from Elsevier, license number 4417080028213, issued August 27, 2018)

electrons spontaneously reduce molecular oxygen and form oxygen radicals that give rise to the formation of excessive reactive oxygenic species (ROS) (see also ▶ Chap. 7) (Selmar and Kleinwächter 2013a; Saed-Moucheshi et al. 2014). The main ROS are singlet oxygen ($^1O_2$), the superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and the hydroxyl radical ($HO^{\bullet}$). When photosynthesis is blocked, these substances are mainly produced in the chloroplasts. ROS such as H_2O_2 can cross biological membranes and function as signalling molecules in plants (Mittler 2017). However, when their level exceeds a certain threshold, ROS cause damage. Especially the hydroxyl radical ($HO^{\bullet}$), which is the highest reactive oxygenic radical, can have very destructive effects on cellular components, damaging not only proteins and lipids but even RNA and DNA (Mittler 2017; Saed-Moucheshi et al. 2014). As a reaction to this undesirable process, the plant synthesizes antioxidants to scavenge the ROS and to avoid damage (see ◘ Fig. 8.1). The main function of these antioxidative agents is to provide an electron that is transferred to the radical. By this means, the radical is detoxified and cannot randomly oxidize other cellular structures.

The ROS-scavenging antioxidants can be enzymatic and non-enzymatic (Grant 2012). Among the enzymatic antioxidants, the enzyme group of the superoxide dismutases (SODs) can transform the superoxide anion ($O_2^{\bullet-}$) to O_2 and H_2O_2, the latter of which is then detoxified by other enzymes (catalases, ascorbate peroxidise (APX), guaiacol-type peroxidases and enzymes of the ascorbate-glutathione cycle) (Mittler 2002; Saed-Moucheshi et al. 2014). Among the non-enzymatic antioxidants, ascorbic acid (vitamin C) is particularly important because of its capacity to quench not only $O_2^{\bullet-}$ and $^1O_2$ but also even hydroxyl radicals ($HO^{\bullet}$). Furthermore, carotenoids, flavonoids, anthocyanins and tocopherols play a role in ROS scavenging (Saed-Moucheshi et al. 2014).

In addition to their importance in intrinsic defence mechanisms, antioxidants are known for their positive effect on human health (e.g. as anticancerogens, antiproliferatives) and are highly desired substances in food (Foyer and Fletcher 2001; Kunwar and Priyadarsini 2011). Therefore, the accumulation of antioxidants in food crops implies an improvement of food quality.

The reduced carbon assimilation under drought stress also results in the accumulation of human health-promoting carotenoids. This is because a reduced activity of the Calvin cycle results in a surplus of photosynthetic energy that is stored either in fully reduced reduction equivalents ($NADPH+H^+$) or in ATP. The energy is channelled to the xanthophyll cycle, which synthesizes, among other compounds, carotenoids (◘ Fig. 8.1). Carotenoids are accessory pigments that are able to dissipate the surplus in light energy and thus to protect leaves from photobleaching (Gruszecki and Strzałka 2005). The plant has another advantage when energy is consumed by those processes: $NADPH+H^+$ is oxidized into $NADP^+$, which can again accept electrons from the photosynthetic electron chain. This helps to avoid the formation of ROS as electrons do not react with molecular oxygen since they are transferred to $NADP^+$. Additionally, the enhancement of the reductive power (electron surplus) caused by drought stress gives rise to the synthesis of highly reduced secondary plant metabolites such as isoprenoids, phenols or alkaloids (see ▶ Chap. 3). Especially in medicinal plants such as sage (*Salvia officinalis*), these secondary metabolites confer beneficial attributes to the plant (Selmar and Kleinwächter 2013a, b). However, if the horticulturist is aiming to enrich these

compounds by the induction of drought stress, care must be taken, because if the drought stress becomes too severe, any excessive production of ROS cannot be sufficiently buffered by the production of the antagonistic antioxidants. In consequence, the ROS will damage the membranes resulting in destroyed chloroplasts and accelerated leaf senescence.

The reader should further note that the described energy dissipation processes also take place under regular circumstances (i.e. when stomata are opened), but to a minor degree (Fig. 8.1), because plants usually gain much more energy from light than is needed for CO_2 fixation (Wilhelm and Selmar 2011).

8.5 Additional Effects of a Deficient Water Supply

Of course, a water deficiency-induced lack of CO_2 will lead to decreased plant growth, as it directly affects the amount of assimilated triose phosphate, which is the precursor for all metabolites. However, for fruit crops, this is not necessarily a disadvantage. The whole vegetative part of the plant might however be smaller, as the plant aims to maintain the development of the generative parts, viz. the fruit production. For instance, drought-stressed tomato plants are known to allocate a higher share of photoassimilates to their fruits (Lemoine et al. 2013; Albert et al. 2016). Albert et al. (2016) observed that, in general, plant vigour (measured in stem and leaf size) and yield were both reduced under drought but that yield was less reduced than plant vigour. This suggests that tomato plants limit their vegetative growth more severely than their fruit production (generative growth) under water scarcity. Additionally, Nitsch et al. (2012) assumed that ABA stimulates cell enlargement in tomato fruits. As ABA is accumulated in the shoot during drought stress, this might also be a reason for the maintenance of fruit size, despite the stressful conditions.

Furthermore, although plants under drought stress may reduce their above-ground biomass accumulation, their root growth is less reduced and, in some cases, is even increased because, under drought stress, certain plants 'invest' in root growth to acquire new water pools. As a consequence, they show higher root-to-shoot ratios under drought, e.g. tomato (*Lycopersicon esculentum*), melon (*Cucumis melo*) or alfalfa (*Medicago sativa*) (Khan et al. 2015; Lemoine et al. 2013; Sharma et al. 2014; Slama et al. 2011). A larger root system, however, implies a greater potential for not only water uptake but also nutrient uptake and can thus contribute to a higher nutrient concentration inside the plant (Nangare et al. 2016).

Moreover, a lack of cellular water is associated with a so-called concentration effect of bioactive and flavouring compounds in plants. Fruits or leaves might be smaller according to the lower water content, but the taste is much more intense as the concentrations of the flavour-active components are higher. Apart from the active accumulation of osmolytes, the metabolites concentrate passively because of the reduced fruit enlargement and the continuous water consumption as, for example, in tomato (Kanayama and Kochetov 2015). The horticulturist should keep this in mind when facing consumer demands, since the nutrient content itself and the taste are important quality parameters of plant-based foods.

In addition to the improving of the quality of plants and their products, a deficit in irrigation is associated with the potential to save a considerable amount of water. The water-use efficiency of plants is generally higher under a deficient water supply (Nangare et al. 2016). Zwart and Bastiaanssen (2004) state that 20–40% of irrigation water can be saved if deficit irrigation is applied properly. For tomato plants, Linker et al. (2016) have calculated a saving potential of 30% [60%] of irrigation water when accepting a 5% [10%] decline in maximal yield. Cabello et al. (2009) have shown that melon (*Cucumis melo* cv. Sancho) can be grown under moderate deficient irrigation (90% ETc) without losses in yield and quality. Lobos et al. (2016) have reported that the postharvest quality (firmness, titratable acidity, soluble solids, antioxidant activity) of highbush blueberries (*Vaccinium x corymbosum* cv. Brigitta) is not affected by a mild water deficit treatment (replacing 75% of actual evapotranspiration) that is started during flowering, i.e. 1–2 weeks before the full bloom stage, and ended after harvest is complete.

8.6 Methods of Creating a Controlled Water Deficit for Plants

Drought stress can be induced either by regulated deficit irrigation (RDI) or by partial rootzone drying (PRD). In the RDI treatment, the complete rootzone is exposed to a water deficit during certain noncritical phenological periods (for a further explanation, see below). In consequence, vigour is lower so that plants consume less water (Galindo et al. 2018). The plant water status must be kept within narrow limits (Jones 2004), which is hard to achieve in the open field but is possible in Controlled Environment Horticulture (CEH). In general, RDI should be applied when fruit growth is minimal, i.e. during the stage between the fruit cell division and the fruit enlargement stage when vegetative parts are growing rapidly (Goodwin and Boland 2002; see also below).

On the contrary, during PRD, only a part of the root system is exposed to drought, while the rest is fully irrigated. In certain intervals (e.g. 3–5 days for tomato) depending on a critical soil water content, the irrigation treatment is exchanged between the root zones, so that each part is alternatively exposed to drought and re-watering (Bogale et al. 2016; Galindo et al. 2018). By applying this method, the drying root part induces ABA production, sending a systemic drought stress signal to the shoot, an event that is followed by osmotic adjustment (Xu et al. 2011). At the same time, the fully irrigated root part ensures a favourable plant water status, thus reducing the risk of a severe (harmful) water deficit (Galindo et al. 2018). With PRD, a minimization of excessive vegetative growth is possible, which is essential, especially in modern high-density plantations, whereas yield can be maintained and even be of higher quality at a relatively low risk to damage (Goodwin and Boland 2002). Furthermore, root growth is increased as soon as the dried root parts are rewetted. Mingo et al. (2004) have observed a 55% larger root biomass of tomato plants after PRD treatment (compared with RDI). The resulting mild drought stress induces an accumulation of osmotically active compounds and/or antioxidants, synthesized by the mechanisms explained above. The same is true under RDI.

The effect of the irrigation treatments also depends on species and cultivar. Therefore, species-specific knowledge is essential before RDI or PRD can be applied. First, the producer has to be aware of the specific water demand of the crop. For many species,

values for crop evapotranspiration (ETc) are available according to the various developmental stages (e.g. FAO 2018). However, specific circumstances (growth conditions) can cause a divergence from these values. The measurement of relevant parameters in situ is more exact, for example, the measurement of the soil moisture or soil water potential, the real evapotranspiration, the plant tissue water status (e.g. the relative water content (RWC) of the leaves), the stomatal conductance (using porometer) or the water content (thermal sensing, balance of pots or sap-flow sensors). Examples of the imposition of a controlled drought stress are given in ◘ Tables 8.1, 8.2, 8.3, 8.4 and 8.5.

◘ Table 8.1 Leaf water potentials under control and drought stress conditions for tomato and mung bean

Species	Well-watered	Mild drought stress	Severe drought stress	Values obtained in	Reference
Tomato (*Solanum lycopersicum* L.)	−0.2 to −0.7 MPa	−1.0 to −1.2 MPa	–	Greenhouse	Coyago-Cruz et al. (2017)
Mung bean (*Vigna radiata* (L.) Wilczek var. B1)	–	−0.5 MPa	−1.0 to −1.5 MPa	Petri dishes, plastic boxes	Das and Kar (2013, 2017)

◘ Table 8.2 Crop evapotranspiration (ETc) under well-watered and dry conditions

Species	Well-watered	Mild drought stress	Severe drought stress	Values obtained in	Reference
Tomato (*Solanum lycopersicum* L. cv. 'Matina', 'Cochoro'), genetic overall variability	100%	50–40%	–	Green-house	Ripoll et al. (2016), Albert et al. (2016), Bogale et al. (2016)
Tomato (*Solanum lycopersicum* L. cv. Ryna®)	100%	80%	60%	Field, India	Nangare et al. (2016)
Melon (*Cucumis melo* L. cv. Sancho)	100%	90%	60%	Field, Spain	Cabello et al. (2009)
Pear-jujube trees (*Ziziphus jujube* Mill.)	100%	50%	–	Solar green-house	Feng et al. (2017)
Peach trees (*Prunus persica* cv. Golden Queen)	100%	40%	–	Field, Australia	Goodwin and Boland (2002)

Table 8.3 Soil water potential under well-watered and dry conditions for *Ilex paraguariensis*

Species	Well-watered	Mild drought stress	Severe drought stress	Values obtained in	Reference
Ilex paraguariensis (cv. San Isidro 49)	−0.04 MPa	−1.0 MPa	−2.0 to −3.0 MPa	Controlled environmental conditions	Acevedo et al. (2013)

Table 8.4 Relative soil humidity under well-watered and dry conditions for tomato

Species	Well-watered	Mild drought stress	Severe drought stress	Values obtained in	Reference
Tomato (*Solanum lycopersicum* L.)	65%	30–25%	–	Greenhouse	Albert et al. (2016)

Table 8.5 Percentage field capacity (FC) indicating various degrees of drought stress in various species

Species	Well-watered	Mild drought stress	Severe drought stress	Values obtained in	Reference
Alfalfa (*Medicago sativa*)	100%	33%	–	Greenhouse	Slama et al. (2011)
Aloe vera (*Aloe vera*)	100%	60%	–	Greenhouse	Hazrati et al. (2017)
Cassia (*Cassia obtusifolia* L.)	100%	70%	40%	Pot/field	Xue et al. (2018)
Hot pepper (*Capsicum annuum* L.)	100%	70% throughout the season, 90% during late fruit bearing	–	Greenhouse	Yang et al. (2017)
Parsley (*Petroselinum crispum* L.)	100%	50%	30–10%	Pot	Najla et al. (2012)

Knowledge about the developmental stages of the crop is of utmost importance when the aim is to adjust metabolism by the induction of drought stress because, in certain stages, water deficiency can lead to severe yield losses and a reduction of fruit quality. Moreover, this stage-dependent sensitivity is not the same among the species. Fruity crops can be extremely sensitive to drought stress during certain developmental phases, especially during flowering.

This is the reason that, for example, with regard to tomato plants, the right time to begin with RDI is the developmental stage after flowering (Albert et al. 2016; Coyago-Cruz et al. 2017). When drought-stressed during flowering, tomato plants react with flower abortion, resulting in high yield losses (Zegbe-Dominguez et al. 2003). Moreover, the fruit setting stage in tomato plants is also sensitive to drought stress (Harmanto et al. 2005; Nangare et al. 2016).

Hot pepper (*Capsicum annuum* L.) has been demonstrated to have improved fruit quality (increased content of total soluble solids and vitamin C and better fruit firmness) and only a slight yield reduction when the soil moisture is kept at 70% of field capacity (FC) during the growth season and at 90% during late fruit bearing and the harvesting stage (Yang et al. 2017). For citrus species, a slight water deficit during the ripening phase (summer and autumn) results in an increase of total soluble solids and acidity (Pérez-Pérez et al. 2008; Okuda et al. 2008).

In contrast, for *Aloe vera*, a medicinal plant whose leaves are the plant organ that is harvested, deficient irrigation can be adopted from the moment when plants have grown to a certain size threshold (>20 cm) until harvest, resulting in higher concentrations of anthocyanins (Hazrati et al. 2017). The leafy culinary herb parsley (*Petroselinum crispum*) has also been shown to react to deficit irrigation with an increased production of chlorophyll, β-carotenes, vitamins and anthocyanins when submitted to water stress treatment (50% FC) beginning 2 months after sowing until harvest (Najla et al. 2012). Rowland et al. (2018) assume that, for numerous other herb crops (basil, coriander, parsley, mint, thyme, lemongrass), a controlled mild water stress can contribute to improved quality (in terms of, for example, essential oils and antioxidant capacity).

In a study with potato plants, tuber yield was increased when plants were submitted to a PRD treatment during the early season, although yield was reduced when PRD was applied throughout the season, because of the reduced leaf size (source for carbohydrates accumulating in the tubers) (Xu et al. 2011).

The choice of the cultivar is another critical factor. With regard to tomato, the pattern of accumulated bioactive compounds can vary considerably among cultivars (Bogale et al. 2016; Albert et al. 2016; Coyago-Cruz et al. 2017). For example, whereas the content of total carotenoids increased in certain cultivars ('Summerbrix' and 'Lazarino', both cherry varieties), a decrease was observed in others (Coyago-Cruz et al. 2017). The vitamin C and lycopene content increased in the cultivar 'Matina' and decreased in the cultivar 'Cochoro' under RDI and PRD treatments (Bogale et al. 2016). Albert et al. (2016) reported that the variable reaction of the diverse tomato cultivars to drought is mainly caused by the genotype. Among the 141 accessions tested, 50 showed improved fruit quality while maintaining yield. Overall, drought-induced fruit size reduction was concluded to be more pronounced for common tomato cultivars than for cherry tomato cultivars (Albert et al. 2016).

A cultivar-specific response to drought has also been described for melon (*Cucumis melo* L.) (Sharma et al. 2014), alfalfa (*Medicago sativa*) (Slama et al. 2011) and parsley (*Petroselinum crispum* L.) (Najla et al. 2012).

Apart from drought stress, a lower water availability is often associated with salt and heat stress for plants, so that the impact of drought on plants cannot always be distinguished from other abiotic stress factors (Vicente-Serrano et al. 2012; Selmar and Kleinwächter 2013a; for further information, see ▶ Chap. 7 for salt stress and ▶ Chap. 9 for heat stress).

Finally, the economic impact of drought-induced yield reductions should be calculated, as the improvement of the quality must at least counterbalance the eventual losses in yield quantity (Santos Pereira et al. 2002; Zegbe et al. 2006). For example, in *Aloe vera*, the highest aloin and anthocyanin contents are produced under the most severe drought stress; however, the leaf yield and plant growth are negatively affected by drought, so that the best overall results are obtained when the drought treatment is kept at a moderate level (60% of FC) (Hazrati et al. 2016, 2017; see ◘ Table 8.5). Nangare et al. (2016) have observed that a mild water deficit (80% of crop evapotranspiration (ETc), viz. the daily water requirement) does not decrease the marketable fruit yield of tomato, whereas a stronger water deficit (60% of ETc) results in a yield loss of about 25%.

Although, to date, deficit irrigation is still sparsely applied, it is likely to gain more importance in the near future, as it provides a useful option both for coping with the anticipated water scarcity attributable to climate change and for improving the quality of several horticultural products.

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Thermal Stress

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9.1 What Is Thermal Stress?

Plants are exposed to external environmental changes that control their development (Rodriguez et al. 2015). According to Sutcliffe (1977), the relatively low heat production of plants in relation to their mass means that they are ectothermic organisms, i.e. their temperature can vary dramatically with external environmental temperature changes. Temperature variation is a core factor that drives plant phenology and that therefore limits the geographical distribution and productivity of many important crops including fruit and nut crops and many cereals (Rodriguez et al. 2015).

When the higher or lower threshold is exceeded, temperature is a major abiotic environmental stress factor that can limit plant growth, productivity and yield stability by impeding metabolism and that can thus potentially affect the productivity of the simplest crops, such as salad varieties, to complex fruiting crops including legumes, berries and aggregate crops such as strawberries. Therefore, extreme temperatures are a threat to plant production worldwide. Every plant has an ideal temperature range that is optimal for its growth processes. At temperatures above or below this optimal range, growth begins to diminish and eventually, at high extremes, will result in plant death (Guy 1999; Rodriguez et al. 2015). Therefore, the response to temperature variations differs significantly among crop species. In addition, because plant growth and development are essentially triggered by phenological responses and seasonal cycles, optimal ranges can vary with regard to the developmental stage of the plant. For instance, in most plant species, including maize (*Zea mays* L.) and rice (*Oryza sativa* L.), higher optimum temperatures are required for vegetative development than for reproductive development (Hatfield and Prueger 2015). Hence, for optimal growth, species-specific temperature ranges must be considered and tailored according to the developmental stage, with plans for suitable controlled growing conditions for such stages. Controlled horticulture is a type of crop production allowing the temperature to be controlled.

An important factor to consider in relation to temperature stress and plant development is the availability of water (see also ▶ Chap. 8). Water enables a plant to carry out its key metabolic processes and therefore to cope with the stress that is affecting it. Additionally, water can assist in the cooling of a plant's organs, for example, enabling leaf cooling during high-temperature extremes through increased transpiration. In the instance of cold weather extremes, water is often unavailable, being trapped in ice formations. This results in plants being exposed to 'frost drought'. During these conditions, relative air humidity is extremely low, driving water loss through (cuticular) transpiration.

9.2 Protected Cultivation: Methods of Thermal Regulation

The thermal adaptation of our environment for crop production requirements is facilitated by protective structures. These can be either active (e.g. with additional heating or cooling being provided via a fan or the use of water circulation) or passive (e.g. by using natural solar energy) and can come in a variety of forms from small simple cold frame constructions to large complex greenhouse systems. The suitability and performance of these various systems depend on the grower's requirements, local climate conditions, location and the system itself and its size (Sethi and Sharma 2008).

Mulch: plastic and natural Mulching is the addition of a material to the soil surface, such as plastic sheet mulching or natural straw mulching. Natural mulches can intercept solar radiation and therefore modify soil temperature, facilitating cooling under hot conditions. By keeping thermal energy in the soil, mulching aids warming under cool conditions (Facelli and Pickett 1991; Chalker-Scott 2007). Natural mulches are more effective temperature-moderating media than plastic sheet mulching (Ramakrishna et al. 2006; Singer and Martin 2008). Singer and Martin (2008) have found that, in warm regions, natural mulches can reduce soil temperatures by 2.5 °C. Martin and Poultney (1992) have reported that, in geographical regions with high temperatures, organic mulches can reduce soil temperature by nearly 10 °C compared with bare soils. This is largely because mulching retains soil moisture and water content in the root zone, resulting in increased water availability for plant uptake. Mulching additionally improves quality, as shown by Ni et al. (2016) who demonstrated that nutrient uptake in tea olive (*Osmanthus fragrans*) was greater in plants grown in mulched soils than in unmulched ones. Additionally, although proline levels were unchanged, soluble sugar content increased up to 37.5%, which suggested that temperature stress resistance in plants was stimulated by mulching. Additionally, Ni et al. (2016) found that the chlorophyll a content increased up to 46.3% with various mulching methods. This indicates that the photosynthetic rate of plant leaves grown under mulching conditions is enhanced.

Cold frames and row tunnels Growing structures such as cold frames and polytunnels enable the extension of the growing season by modifying the growing temperature and therefore environment (Adams and Todd 2014). Cold frames are the smallest and most affordable growing structure available. They are generally passive and are used for an early start in propagating crops enabling producers to extend the growing season by 4–6 weeks (Larson 2009; Singh 2012). By increasing the nighttime air temperatures around plants via the slow release of the captured daytime solar energy from the soil, cold frames can decrease cold injury events in cold seasons (Adams and Todd 2014). High tunnels or row tunnels perform the same function as a cold frame by trapping solar heat energy; however, they are much larger, are utilized mainly for commercial purposes and are covered by polyethylene plastic (Lamont 2005). These methods can assist in increasing the productivity and quality of multiple crops. A study in which sweet cherry fruit trees were covered early in spring by polythenal plastic to force early flowering revealed that the weight and size of the fruit were increased 6–14% (Overbeck et al. 2016). Both hydrophilic (water soluble) and lipophilic (lipid- or oil-soluble) fractions in the soluble extracts gained from sweet cherry fruit possessed greater antioxidative potential. Fruits under polythenal plastic cover also had higher phenolic values, thus increasing the health value of the cherries. In addition, flavonoids, carotenoids and anthocyanins increased significantly, and phenolics were increased by an average of 14%.

Greenhouses Greenhouses, among other purposes, provide various means of plant protection to adverse climate and therefore temperature conditions, enabling maximum returns from cultivation to be achieved through the creation of better growing conditions (Bouadilla et al. 2014). They have become increasingly sophisticated, enabling growth factors (e.g. light, temperature, humidity and air composition) to be controlled optimally (Elsner et al. 2000). A greenhouse can enable higher yield crop production outside of the cultivation season (see ► Chap. 2) or the geographical region of a specific species (Sethi and Sharma 2008).

Temperature within greenhouses can be controlled through (1) additional heating, (2) heat loss prevention via the use of appropriate cover materials or (3) active cooling via ventilation or artificial cooling (Elsner et al. 2000). One main constraint, of course, is the energy requirement of the active systems. Additionally, active greenhouse temperature control systems are largely reliant on fossil fuels. Thus, we need to consider the use of appropriate systems powered by passive and renewable energy, the environments in which greenhouses are used and their sustainability. Therefore, the transition of active systems away from fossil fuel reliance is an important consideration for the fulfilment of some of the Global Goals for Sustainable Development as set by the United Nations General Assembly in 2015.

9.3 Thermal Stress

Thermal stress involves both cold (e.g. frost) and heat stress. Plants display differential sensitivity to temperature and its fluctuations depending on the duration and severity of the stress and on the developmental stage of the plant. Additionally, temperature stress is often linked to low air humidity and reduced water availability and uptake and is therefore linked to water scarcity and drought. Hot desert climate species possess a higher optimum temperature than those adapted to cold or moderate climates. Photosynthesis within cold environment-adapted plants can function between 0 and 30 °C, whereas warm season crops require 7–40 °C and hot environment plants (tropical species and summer species from areas such as the Mojave Desert) need 15–45 °C (Bunce 2000; Sage and Kubien 2007). At temperatures near the extremes of the optimal range, injury will occur to the plant affecting its rate of photosynthesis, and the plant will be irreversibly impaired (Sage and Kubien 2007). As a result, pleiotropic aspects of sugar and starch metabolism are affected (Wilhelm et al. 1999).

9.4 Heat Stress: Core Effects on Plant Growth

Initial effects of heat stress include temporarily reduced and altered photosynthetic and respiratory activity and mechanisms, all of which can cause a shortened plant life cycle and reduced productivity. Such effects if prolonged can become irreversible, as moderate heat stress reduces photosynthesis reversibly, whereas increased and prolonged heat stress can cause irreversible damage to the photosynthetic apparatus of plants and potentially results in plant death (Song et al. 2014; Sharkey and Zhang 2010). Photosystem II (PSII), which absorbs light used in the reduction of plastoquinone and oxidation of water, is the most heat-sensitive photosynthesis apparatus of a plant (Song et al. 2014). PSII can be irreversibly damaged by high temperatures (Berry and Björkmann 1980; Sharkey and Zhang 2010). As a result, plant growth is reduced, for example, in maize plants (Aiqing et al. 2018); however, the content of secondary metabolites that affect citrus (*Citrus* L.) quality has recently found to be significantly altered in citrus cultivars, with variation occurring between species of the Carrizo citrange (*Poncirus trifoliata* L. Raf. x *Citrus sinensis* L. Osband *C. mandarin* (*Citrus reshni* Hort. Ex Tan.)) (Zandalinas et al. 2016). This includes flavonoid-related compounds, such as apigenin, with higher levels occurring in the *C. mandarin*. Additionally the accumulation of kaempferol derivatives is higher within *C. mandarin*.

Cellular changes When higher plants are exposed to temperatures at least 5 °C higher than their optimal limit, plants display characteristic metabolic and cellular responses required for the survival of the plant (Guy 1999). These include changes (1) in cellular structure including that of the cytoskeleton; (2) in membrane functions and organelles such as subtle changes in lipids affecting membrane properties involving fluidity, thickness, and permeability; and (3) in the reorganization of microtubules (Weis and Berry 1988; Bita and Gerats 2013). Early effects of heat stress include structural changes to chloroplast protein complexes and reduced enzyme activity (Ahmad et al. 2010). Moreover, a decrease occurs in the synthesis of normally present proteins, and an acceleration takes place in the translation and transcription of heat shock proteins (HSPs) (Bray et al. 2000; Bita and Gerats 2013). Additionally, cell membranes are injured, microtubules and the cytoskeleton are reorganized (Weis and Berry 1988), and membrane fluidity, permeability and cell differentiation are affected, all of which changes cell expansion, elongation and differentiation (Smertenko et al. 1997; Orvar et al. 2000; Bita and Gerats 2013). In order to maintain the stability of membranes, cellular homeostasis and regular cell functions in the case of heat stress, plants initiate the synthesis of HSPs as these 'molecular chaperones' assist in preventing proteins from misfolding or aggregating (Gray and Brady 2016).

Shoot morphology and habitus Plant injuries from extreme and prolonged heat stress can result in a decrease in plant productivity. This includes leaf senescence (ageing) and abscission (loss or shedding), the scorching of stems and leaves, the inhibition of root and shoot growth and fruit damage (Vollenwieder and Günthardt-Goerg 2005; Bita and Gerats 2013). The architecture of the plant also changes, with petioles and hypocotyls elongating in a similar morphological response to that of shade avoidance (Tian et al. 2009; Bita and Gerats 2013). Plant growth overall is affected because of alterations of shoot assimilation rates, which therefore affect the plants total dry weight (Wahid et al. 2007).

Root development Elevated temperature stress can result in increased primary root angle branching, causing a shallow and broad root system (McMichael and Quisenberry 1993; Nagel et al. 2009). This therefore alters the architecture and shape of the root system and primary root length, both of which can negatively affect crop development and success because of unfavourable effects on core root functions such as respiration and nutrient uptake (Awal et al. 2003; Gray and Brady 2016). When root development is affected, nutrient and water acquisition and therefore plant developmental can in turn also be affected. A plant's ability to maintain good root growth is dictated by carbon fixation during photosynthesis and the amount of carbohydrates provided to the root area. Therefore, any interruption of the root-based carbohydrate metabolism is seen as a key factor responsible for the inhibition of growth and root functions within plants grown under high-temperature stress conditions (Du and Tachibana 1994). Nevertheless, superior root thermo-tolerance in some C_3 perennial grass species has been identified, for instance, in hair grass (*Agrostis scabra*) and creeping bentgrass (*Agrostis stolonifera*) (Huang et al. 2012). Studies into these species have found that the efficient protein and carbon metabolism of these plants assists in root thermo-tolerance. This root thermo-tolerance is linked to such plants possessing an increased capacity to use respiratory acclimatization to control respiratory costs. In other words, thermo-tolerance lowers a plant carbon investment in maintaining protein turnover and assists with the provision of carbon to various metabolic areas and respiration pathways (Huang et al. 2012).

Reproductive development A plant's developmental phase is largely dictated by temperature thresholds that assist and trigger the transition, for instance, from vegetative to reproductive growth. Therefore, a plant's optimum temperature shifts with its respective tissue developmental stage (Gray and Brady 2016). This can differ significantly within individual species. For example, in sorghum (*Sorghum bicolor*), the optimum temperature for vegetative growth ranges from 26 to 34 °C, whereas reproductive growth ranges from 25 to 28 °C (Maiti 1996). An inflorescence is the formation and arrangement of a cluster of flowers on the axis or stem, with a coordinated timing of flowering. In thale cress (*Arabidopsis thaliana*), the whole inflorescence is aborted at heat stress treatment levels of 36 °C (Warner and Erwin 2005). Therefore, although a plant's developmental stage is largely linked and triggered by cyclic temperature changes, the final impact of temperature stress on reproductive fitness and yield depends on the developmental stage at which the stress occurs (Warner and Erwin 2005).

Heat stress and elevated temperature can therefore impact reproductive development by altering the timing of the event or by resulting in heat stress damage to the reproductive structures. According to Gray and Brady (2016), heat stress can result in (1) an earlier flowering transition time, (2) the increased abortion of the inflorescence, (3) the decreased viability of pollen, (4) the premature abortion of tapetal cells, (5) a reduction in the growth of the pollen tube, (6) the increased expression of HSPs in pollen tubes and grains, (7) a reduction in the number of ovules and (8) an increased abortion rate of ovules. These effects can have a vast impact on crops that are largely reproductive organ-based, such as corn, soya and rice. A study undertaken by Wilhelm et al. (1999) in order to assess the way that heat stress affected maize (*Zea mays* L.) revealed that it lengthened the duration of grain filling but reduced the growth rate of kernels leading to a 7% loss of dry mature kernel weight. Even short events (hours to days) with hot dry air can significantly reduce yields if occurring during bloom.

Metabolism and quality Reproductive growth and therefore grain filling are considered to be affected by numerous factors including sucrose availability and the activity levels of enzymes involved in plant starch and sugar metabolism (Wilhelm et al. 1999). Aiqing et al. (2018) have recently found evidence for a certain strain of wheat (*Triticum aestivum* L.) being particularly sensitive to heat stress. When the crop is exposed to high temperatures during the flowering phase, a significant reduction occurs in starch (25%) and protein (21%) content, although this is accompanied by a 17% increase in proline concentration. Additionally, in a study undertaken by Du and Tachibana (1994) on the effect of heat stress on cucumber plants (*Cucumis sativus* L. cultivar 'Sharp I'), root respiration has been shown to increase significantly associated with a temperature increase from 35 °C to 38 °C. High temperatures have also been found greatly to increase the soluble sugar content, especially of raffinose, in the cucumber roots, although the pectin content decreases. According to the study, this decrease of root pectin results from high root temperatures. All of this evidence supports the hypothesis that the disturbance of the carbohydrate metabolism in roots resulting from high temperatures is the main cause of the growth inhibition of cucumbers and that this results in an increased sugar content. An overview of adaptation strategies and injuries resulting from heat stress on vascular plants is given in ◘ Table 9.1.

Table 9.1 Summary of injuries and adaptations that can occur within plants in response to extreme temperature stress

Description	Impact
Temporarily altered photosynthetic and respiratory activity and mechanisms[a]	Injury
Irreversible damage of PSII[b]	Injury
Cellular structure altered including cytoskeleton, membrane functions and organelles[c]	Adaptation
Synthesis of HSPs initiated to assist in preventing proteins from misfolding or aggregating[c]	Adaptation
Leaf senescence and abscission, scorched stems and leaves, inhibition of shoot growth and fruit damage[c, d, e]	Injury
Negative impact on reproductive organ-based crops such as corn, soya and rice through aborted inflorescence and impeded pollen development[f, g]	Injury
Increased primary root angle branching resulting in altered root system architecture[h]; this can alter nutrient uptake and respiration[i]	Adaptation
Interruption of carbohydrate metabolism in the root system inhibits growth and root function[j]	Injury
Sugar and starch metabolism adjusted[k]	Adaptation
Increase in secondary metabolites[l]	Adaptation
Reduced germination, stunted seedlings, leaf chlorosis and wilting[m]	Injury
Reproductive development affected[m]	Injury
Severe membrane damage and cell rupture because of acute dehydration[m]	Injury
Transformation of plasma membrane from a semifluid to semicrystalline state[m]	Adaptation
Production of secondary metabolites from primary metabolites[n]	Adaptation
Development of increased polyamine levels as plants develop tolerance to extreme temperature stress[o]	Adaptation

Sources: [a]Song et al. (2014)
[b]Sharkey and Zhang (2010)
[c]Bita and Gerats (2013)
[d]Vollenwieder and Günthardt-Goerg (2005)
[e]Wahid et al. (2007)
[f]Warner and Erwin (2005)
[g]Gray and Brady (2016)
[h]McMichael and Quisenberry (1993)
[i]Koevoets et al. (2016)
[j]Du and Tachibana (1994)
[k]Wilhelm et al. (1999)
[l]Zandalines et al. (2016)
[m]Yadav (2010)
[n]Ramakrishna and Ravishanka (2011)
[o]Krasensky and Jonak (2012)

9.5 Frost Stress: Plant Sensitivity and Effects on Plant Growth

Low temperature, together with reduced water availability, is considered to be the most important factor limiting the geographical distribution and productivity of plants worldwide (Galiba and Toth 2017).

Plant sensitivity to cold is defined by the limit at which metabolic processes can continue functioning or the point at which permanent injuries occur resulting in plant death. The cold tolerance of a plant species is therefore dependent on the original evolutionary climate of the plant.

Chill-sensitive/chill-susceptible plants are those displaying a loss of viability and/or injury at temperatures between 0 °C and 12 °C, such as rice (Tajimi et al. 1983; Guy 1999). Cold-sensitive plants are those that can tolerate low non-freezing temperatures but that will die or be injured when ice formation within the plasma occurs, such as potato (*Solanum tuberosum*) (Sukumaran and Weiser 1972; Guy 1999). Frost-resistant plants include those that can withstand tissue ice formation but that die in low subzero temperatures (−6 °C to −1 °C), such as members of the *Citrus* genus (Yelenosky and Guy 1989). Some plants can survive temperatures from −10 °C to −30 °C, including many cereal crops and fruit-producing trees (Scorza et al. 1983). The last group, i.e. the most hardy, can survive −30 °C to −50 °C and include many alpine and temperate trees, for example, *Robinia pseudoacacia* (Sakai and Yoshida 1968).

Therefore, plants possess two major strategies: stress avoidance and stress tolerance. According to Krasensky and Jonak (2012), stress avoidance is an adaptive protective mechanism that is inherited and stable and that delays or prevents the potentially negative impact of the stressful conditions. Stress tolerance on the other hand is a plant's potential to acclimatize and adjust to stressful conditions. These processes are enabled by metabolic adjustments leading to morphological and physiological adaptations. For example, exposure to cold conditions can induce hardening and, therefore, result in the acclimatization of plants to survival in extreme temperatures. Plants can thus increase their tolerance to various stresses. A greater number of metabolic processes alter under cold stress conditions than under heat stress indicating the stronger impact of cold on a plant's metabolism (Krasensky and Jonak 2012). By inducing thermal stress in a controlled way, the horticulturist can induce metabolic processes in the plant resulting in the accumulation of metabolites that are favourable when part of a plant-based diet.

Shoot morphology and habitus Injury arising from cold stress may appear after as little as 10 minutes of cold stress (Yadav 2010). Cold stress can result in a variety of phenotypic plant symptoms including reduced germination, stunted seedlings, leaf chlorosis (yellowing), wilting, a reduction in leaf expansion and the possible death (necrosis) of plant tissue (Ahmad et al. 2010). Additionally, cold stress severely affects the reproductive development of plants and induces severe membrane damage because of freezing-associated acute dehydration (Gray and Brady 2016). Cold stress at the reproductive stage of plants will result in pollen sterility (Suzuki et al. 2008).

Transformation of plasma membrane and apoplast The formation of ice within plant tissues during cold stress results in dehydration and possible cell rupturing, which is noted as the main cause for plant damage resulting from cold stress (Yadav 2010). This is caused by damage that arises from a transformation of the plasma membrane from a semifluid state into a semicrystalline state (Steponkus et al. 1993; Yadav 2010). A plasma membrane consists of proteins and lipids (unsaturated and saturated fatty acids) (Steponkus et al. 1993). The concentration ratio of these fatty acids within a plasma membrane denotes a plant's cold hardiness, as saturated fatty acids solidify faster (Thaku and Nayyar 2013). Cold-sensitive plants therefore possess more saturated fatty acids in their plasma membrane, whereas cold-resistant plants possess more unsaturated fats and therefore have a lower transition temperature. The reason is that unsaturated fatty acids have lower melting points than saturated fatty acids of the same length. Thus, the unsaturated state enhances the fluidity of the fatty acids in cold environments (Berg et al. 2002). An example from the animal kingdom is helpful: membranes from artic fish contain more unsaturated fatty acids than membranes from fish that live in the South Seas. This is because the organism endeavours to keep membranes liquid in the cold environment. Plant health can be impaired because of cell rupture. As ice has a lower vapour pressure than water, the apoplast ice formation creates a vapour pressure gradient between the surrounding cells and the apoplast (Thaku and Nayyar 2013). This then results in the creation of more ice crystals in the apoplastic space causing mechanical strain on the plasma membrane and cell wall, a strain that in turn causes the cell to rupture (Yadav 2010).

Secondary metabolites Secondary metabolites are crucial for plants developing adaptations and acclimatizing to stressful temperature conditions and are produced from primary metabolites such as amino acids, lipids and carbohydrates. They also contribute to the colours, tastes and odours of plants and are therefore important for the pharmaceutical industry and for the food industry for use as food additives and flavours (Ramakrishna and Ravishanka 2011) (see ▶ Chap. 3). Although a complete overview of the effect of cold stress on secondary metabolite production within higher plants is beyond the scope of this chapter, we need to understand that stress tolerance and therefore the cold acclimatization of plants will produce a variety of results and increase secondary metabolite production. Of course, this varies for every plant species.

For example, the polyamines, a stable group of organic compounds that are formed in all living organisms during normal metabolic processes and that are considered to have an indispensable role in the human metabolism system, have been linked to various positive human health benefits, such as positive carcinogenic effects and tumour growth reduction (Moret et al. 2005). Polyamines such as cadaverine, spermine, spermidine and putrescine are involved in nearly every step of protein, RNA and DNA synthesis and are therefore crucial for cell growth and reproduction (Bardócz 1995). Putrescine has also been found to assist in increasing the shelf life of pears (*Pyrus communis* cv. Spadona) when utilized in a preharvest treatment during cold storage (Hosseini et al. 2017). Additionally, with regard to plant development, polyamines are linked to various physiological stages, including senescence, stress responses and development. These compounds are also involved in membrane protection and oxidative stress alleviation.

High polyamine levels are positively correlated with plants developing tolerance to environmental stresses. Therefore, cold stress-tolerant plants such as the Brassica family possess a greater ability to increase polyamine biosynthesis as a response to temperature stress (Krasensky and Jonak 2012) and therefore to increase the content of these positive health-benefiting compounds.

9.6 Controlled Environment Case Studies

Heat stress Controlled environments such as greenhouses or growth chambers enable the maintenance of optimum growth temperatures (Sutcliffe 1977) and can be utilized in the scientific examination of various temperature stress regimes on plant development. *Panax quinquefolius*, American ginseng, is an endemic understory herb found within eastern deciduous North American forests. These plants are highly sensitive to their external growth conditions. As previously mentioned, when plants are stressed, their production of secondary metabolites may increase. *Panax quinquefolius* can produce steroidal saponin secondary metabolites called ginsenoides. Ginsenoides act as antimicrobial and antifungal agents and are found in all organs of the plant (Jochum et al. 2007). Ginsenoides have been found to be highly beneficial in the treatment of cardiovascular diseases and are therefore an important medicinal component (Lee and Kim 2014). Jochum et al. (2007) grew 3-year-old *Panax quinquefolius* plants within greenhouses at elevated temperatures of 25/20 °C (day/night) and 30/25 °C and analysed the storage root ginsenoides between the two treatments. Within the higher-temperature treatments, the plants had less biomass (53%) than plants grown at the lower-temperature treatments, but storage-root ginsenoides were significantly higher (49%) within those plants grown at higher temperatures. Thus, an increased temperature can improve the required content of medicinal compounds of certain crops.

Cold stress Hasdai et al. (2006) studied the effects of extreme chilling and freezing tolerance on *Arabidopsis* ecotypes to explore variation within plant species. Their study involved the use of controlled growth chambers, whereby plants were first placed within a chilled room for a 4-day incubation period following germination before being introduced to the controlled growth chamber. Plants were grown at a chilling temperature (14 °C) and under cold (6 °C). Overall, those plants grown under lower-temperature conditions were found to possess longer inflorescences. Additionally, a decrease in chlorophyll content was associated with an increased accumulation of anthocyanins in plants grown at 6 °C. Anthocyanins are water-soluble antioxidant vacuolar pigment flavonoids that arise as a plant's defence mechanism to stress. Their pigmentation can provide a plant with protection by acting as a block against unwanted UV radiation, for example, against UV-B. Moreover, they can assist a plant to deal with free radicals that arise from stress by binding with them and making them inactive. Anthocyanins in plant-based food are currently also being investigated with regard to their assistance in the prevention of cardiovascular diseases and cancer and are considered as being healthy for consumers. In the same study (Hasdai et al. 2006), morphological changes have been observed at seedling developmental stages, such as hypocotyl elongation, the second pair of true leaf angles and the lengths of petioles. Inflorescences elongate but result in a lower seed yield. Therefore, spe-

cific plants grown at lower temperature extremes might enhance the accumulation of desirable and health-benefiting secondary metabolite flavonoids. Nevertheless, Hasdai et al. (2006) have demonstrated that this varies according to ecotype, supporting the hypothesis that each individual species of plants has its own ideal temperature ranges and, therefore, species-specific based research is important. This example was based on the model plant *Arabidopsis*. However, various other horticultural crops react with a similar accumulation of anthocyanins, as is evident with certain vegetables and crops under cold stress (Cisneros-Zevallos 2003; Lo Piero et al. 2005), e.g. red orange (*Citrus sinensis* L. Osbeck) (Piero et al. 2005) and "BetaSweet" purple carrots (Cisneros-Zevallos 2003).

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Wounding

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In nature, plants experience mechanical damage and biological injuries. As immobile organisms firmly set in substrate, they cannot move to avoid herbivore attacks. Therefore, plants have had to come up with alternative defence strategies:

- Physical barriers such as a cuticle, trichomes, thorns and other specialized organs
- Chemical defence substances such as phenolics and terpenoids (please see ▶ Chap. 3)
- Plant-plant communication via volatiles to 'warn' neighbouring plants
- Plant-insect interactions, e.g. extrafloral nectar secretion from wounds of bittersweet nightshade (*Solanum dulcamara*) to attract ants to defend the plant against herbivory by flea beetle larvae (Léon et al. 2001; Heil and Ton 2008; Bhattacharya et al. 2010; Steppuhn et al. 2016)

As our interest lies in improving the inner quality of crops, this chapter will focus on stimulating the wound-induced chemical defence of the plant in order to obtain substances beneficial to human health (◘ Table 10.1). Responses to wounding can be local, systemic or both. Healing and repair are locally activated reactions, whereas herbivore deterrence can be locally and systematically activated. Wound-induced responses

◘ Table 10.1 List of effects of jasmonic acid, methyl jasmonate and salicylic acid on selected crops

Treatment	Crop	Effects	Comment	Reference
JA	Madagascar periwinkle (*Catharanthus roseus*)	Stimulation of alkaloid biosynthesis		Van der Fits and Memelink (2000)
	Hemp (*Cannabis sativa*)	Increase of carotenoid, α-tocopherol and THC; decrease of CBD	10 μM JA (α-tocopherol), 5 μM JA (THC)	Salari and Mansori (2013)
MeJA	Norway spruce trees (*Picea abies*)	Increase of volatile terpenes	10 mM, foliar spray	Martin et al. (2003)
	Romaine lettuce (*Lactuca sativa*)	Increase of antioxidant activity and phenolics		Kim et al. (2007)
	Chinese bayberries (*Myrica rubra*)	Increase of antioxidant activity and phenolics		Wang et al. (2009)
	Red raspberries (*Rubus idaeus*)	Increase of flavonoids	0.01 mM, vapour	Flores and Ruiz del Castillo (2014)
	Peach (*Prunus persica*)	Increase of sucrose levels	10 μM, vapour	Yu et al. (2016)

Table 10.1 (continued)

Treatment	Crop	Effects	Comment	Reference
	Pomegranate (*Punica granatum*)	Increase of phenolics and anthocyanins	0.01–0.1 mM, vapour	Sayyari et al. (2011)
	Grapes (*Vitis vinifera*)	Increase of anthocyanins and phenols	1.78 mM, vapour	Flores et al. (2015)
	Blueberry (*Vaccinium* sect. Cyanococcus)	Increase of anthocyanins	0.01–0.1 mM, vapour	Huang et al. (2015)
	Blackberry (*Rubus* spp.)	Increase of anthocyanins and phenolic acid	0.1 mM, vapour	Wang et al. (2008)
	Strawberry (*Fragaria ananassa* Duch. cv. Allstar)	Increase of anthocyanins and phenolic acid	0.1 mM, vapour	Ayala-Zavala et al. (2005)
SA	Tomato (*Lycopersicon esculentum* cv. Baraka)	Increase of ascorbic acid, soluble solids, titratable acidity; decreased chilling injury	Foliar spray 3 weeks before harvest, + postharvest fruit dipping	Baninaiem et al. (2016)
		Increase of lycopene, carotenoids, phenolics and free amino acids	Dipping of mature green tomato fruit	Kant et al. (2016)
	Apple (*Malus domestica* Borkh. cv. Red Delicious)	Increase of phenolics, antioxidant activity and anthocyanins	Enhanced values only in early stages of cold storage	Hadian-Deljou et al. (2016)
	Banana (*Musa acuminata*)	Delayed ripening	During storage	Srivastava and Dwivedi (2000)

include changes in metabolic processes and in the expression of wound-inducible defence genes. Gene expression underlies the generation, perception, translocation and transduction of signals. Many structurally different molecules are involved in the regulation of wound signalling. Examples are:

- The oligopeptide systemin
- Oligosaccharides of the damaged cell wall

- Several phytohormones such as salicylic acid (SA) and jasmonic acid (JA), which have a central role in wound signalling (Bishop et al. 1981; Farmer and Ryan 1992; Pearce et al. 1991).

Deliberate injury to the entire plant stock to induce stress reactions would certainly not be practical in order to increase the concentration of desired metabolites. Thus, we have to come up with another strategy based on intra-plant communication and plant-to-plant communication. Instead of wounding the plant itself, we simply apply the wound signals themselves to stimulate physiological wound reactions.

10.1 Jasmonic Acid

Jasmonic acid (JA) is a well-recognized plant hormone known to activate many defence responses. JA is synthesized from its precursor alpha-linolenic acid. Farmer and Ryan (1992) noted that the application of linolenic acid (and of other JA precursors) to tomato leaves induced the expression of the same set of genes as JA itself. Van der Fits and Memelink (2000) found a transcription factor in Madagascar periwinkle (*Catharanthus roseus*) responding to exogenous JA by the expression of genes encoding alkaloid biosynthesis. This also indirectly affects alkaloid synthesis by the activation of primary metabolism pathways, which provide precursors for alkaloid formation. *Catharanthus roseus* is known to contain several anticancerous alkaloids such as vinblastine and vincristine (Arora et al. 2010). JA treatment of hemp (*Cannabis sativa)* results in increases of carotenoid, α-tocopherol and tetrahydrocannabinol (THC) (see also ▶ Chap. 3), whereas the cannabidiol (CBD) content decreases (Salari and Mansori 2013). Although the carotenoid increase and CBD decrease do not depend on the JA concentration, α-tocopherol shows an increase at 10 and 100 µM jasmonate, whereas THC levels are considerably enhanced at concentrations of 1 µM and 5 µM, with the 5 µM solution being more effective. However, despite both THC and CBD being the main secondary metabolites for medicinal uses, they have very distinct effects. Hence, JA application must be evaluated with regard to the sought medical treatment. Auxins have been demonstrated to have a negative effect on wound-induced gene expression, presumably to limit the extent and duration of the wound responses (Thornburg and Li 1991; Rojo et al. 1998). This should therefore also be considered when using phytohormones for quality improvement.

10.2 Methyl Jasmonate

Methyl jasmonate (MeJA) is another phytohormone produced after wounding. It is derived from JA by methylation through jasmonate-methyl-transferase and is a volatile form of jasmonate. MeJA is used for internal defence and as a communication signal between plants (Farmer and Ryan 1992). Exogenous MeJA stimulates volatile terpene biosynthesis in Norway spruce trees (*Picea abies*). Martin et al. (2003) detected a two-fold increase in terpene accumulation in needles of young saplings after MeJA treatment. The treatment involved the use of 150 ml of a 10 mM solution of 95% pure (w/w) MeJA in distilled water applied as a spray to saplings (40–50 cm) in a ventilated fume

hood for 30 min. After being sprayed, the saplings were left under the fume hood for another 1–2 h until the needles were dry. Maximum values of volatile terpene levels were observed at 15 days after treatment, which declined within the next 5 days to control levels. An increase in antioxidant activity and phenolic compounds in romaine lettuce (*Lactuca sativa)* and Chinese bayberries (*Myrica rubra*) after MeJA application has also been noted (Kim et al. 2007; Wang et al. 2009). Flores and Ruiz del Castillo (2014) documented the promotion of phenylalanine ammonia lyase in red raspberries (*Rubus idaeus*) leading to an increase of health-promoting compounds including quercetin and myricetin. Yu et al. (2016) showed that MeJa treatment influences the quality of peaches (*Prunus persica*) even after harvest. During cold storage, the peach fruit increased their sucrose levels, which promoted a sweeter taste. Additionally, enhanced postharvest anthocyanin levels after MeJA application have been recorded for many crops including apple (*Malus pumila* Mill. var. *domestica* Schneid.) and pomegranate (*Punica granatum*) (Kondo et al. 2001; Sayyari et al. 2011). Aromatic compounds in grapevines (*Vitis vinifera*) and volatile organic compounds in strawberry (*Fragaria ananassa*) and mango (*Mangifera indica*) show increased values after MeJA exposure and thus an enhanced taste intensity in their fruits (Lalel et al. 2003; D'Onofrio et al. 2009; De la Peña et al. 2010). Notably, a preharvest application of MeJA is more effective than one postharvest because of the better reception of the fruit (Li et al. 2010).

10.3 Salicylic Acid

Another important wound signal for plant defence is salicylic acid (SA). SA is a phenolic compound and acts as an antioxidant defence system and as a plant growth regulator (Khan et al. 2003). Baninaiem et al. (2016) showed that foliar application of SA to tomato plants (*Lycopersicon esculentum* cv. Baraka) 3 weeks before harvest plus postharvest fruit dipping retains quality traits such as ascorbic acid content, total soluble solids and titratable acidity. It also has been demonstrated to delay ripening in several fruits including banana (*Musa acuminata*) during storage (Srivastava and Dwivedi 2000; Zhang et al. 2003). Furthermore, Kant et al. (2016) documented a delay in the biosynthesis of phytochemicals, e.g. lycopene, carotenoids, phenolics and free amino acids, after the dipping of mature green tomatoes in SA (*Solanum lycopersicon* L. cv. Pusa Rohini and Pusa Gaurav). These results show that the application of SA improves marketing quality indirectly by maintaining fruit quality during storage. 'Red Delicious' apples (*Malus domestica* Borkh. cv. Red Delicious) exhibit an increase in total phenolics and antioxidant activity in early stages of cold storage after SA treatment (2 mM) (Hadian-Deljou et al. 2016). Additionally, the anthocyanin content gradually increases until day 60 of storage and then immediately decreases.

10.4 Food Safety

With regard to safety concerns for human health, jasmonates are considered safe, and there are no restrictions for postharvest treatment. Experiments even indicate health-promoting effects. Jasmonates have been documented to possess selective cytotoxicity towards cancer

cells (Fingrut and Flescher 2002; Kniazhanski et al. 2008). Thus, jasmonates inhibit the reproduction of cancer cells and induce apoptosis (cell death) in various cancer lines, e.g. breast, prostate and melanoma. Moreover, experiments by Umukoro et al. (2011) have suggested antidepressant effects of MeJA. Nevertheless, Wiesner et al. (2014) have recorded strongly (20-fold to control) mutagenic activity in juices from steamed pak choi (*Brassica rapa chinensis*) sprouts treated with MeJA. Therefore, the application of jasmonates must be evaluated and adjusted to the crop. SA is widely accepted as a health-promoting substance and extensively used because of its antipyretic and analgesic effects. Lethal doses are well-known but are negligible when plant fruit treatment is carried out correctly. Responses to JA and SA treatments depend on crop, phenological stage and dose.

Dedicated by J.J. Jones To my mother. To Lisa. To Oskar.

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Mycorrhiza

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11.1 Interaction Between Mycorrhizal Fungi and Host Plants

Most land plants on earth live in symbiotic relationships with mycorrhizal fungi. The main benefit arising from this symbiosis is that fungi provide plants with a physical extension of their root system (Bahram et al. 2014). As a result, the plant receives soil nutrients from the fungus in exchange for photosynthates (Manzoor 2014). Research into mycorrhizae has expanded rapidly in recent years, focusing on the stabilization of yields in a changing world in which environmental stress, biodiversity depletion, deforestation or decreasing soil fertility are just some of the urgent challenges (Ahmad 2014).

Mycorrhiza designates the symbiosis between a fungus species and the roots of host plants wherein the formation of the mycorrhiza is called mycorrhization (George 2017). On a global scale, 86–94% of all land plants live in symbiosis with mycorrhizal fungi (Brundrett 2009). Such fungi can be found in various habitats, including aquatic ecosystems, deserts, lowlands and tropical forests. According to Van der Heijden et al. (2015), Albert Bernhard Frank was one of the first scientists to discover the association between plants roots and mycorrhizal fungi. He classified four major types of mycorrhiza, with most of them growing (1) inside the cortex (outermost layer) of plant roots, (2) around the epidermal cells of the root or (3–4) on the root surface:

(i) Arbuscular mycorrhiza (AMF) comprises more than 200,000 host plant species such as herbs, grasses, many trees, hornworts and liverworts (Young 2012).
(ii) Ectomycorrhiza has around 6000 host plant species including the pine family (Pinaceae), angiosperms and some liverworts (Sharma 2017).
(iii) Orchid mycorrhiza includes 20,000–35,000 host orchid species (Van der Heijden et al. 2015).
(iv) Ericoid mycorrhiza has 3900 host plant species from the Ericaceae family and some liverworts (Van der Heijden et al. 2015).

The symbiosis process takes place in the rhizosphere, which is defined as the narrow part of soil that is influenced by living roots and that is characterized by intensive microbial growth activity. This region is also called the mycorrhizosphere because of the presence of mycorrhizal fungi that form a symbiosis with plants (Bonfante 2018). Plant root exudates play an important role in the communication with rhizosphere-inhabiting microorganisms and are relevant for the host plant to be able to attract the fungi. For this communication, the plant roots release soluble sugars, amino acids or secondary metabolites to attract the fungus (Chaparro et al. 2013). Upon successful establishment of the symbiosis, the plant receives soil nutrients such as phosphorus (P) and nitrogen (N) from the mycorrhizal fungi in exchange for photosynthates (Manzoor 2014; Prasad et al. 2017). As much as 5–21% of the photosynthetically fixed carbon can be channelled to the fungi (Chaparro et al. 2013).

11.2 Beneficial Effects of Plant-Arbuscular Mycorrhiza Fungi Association

Arbuscular mycorrhiza fungi (AMF) are obligate biotrophic organisms that live symbiotically inside the roots of plant and that cannot grow apart from their hosts. It is estimated that more than 80% of terrestrial plants can carry out such symbiosis, including

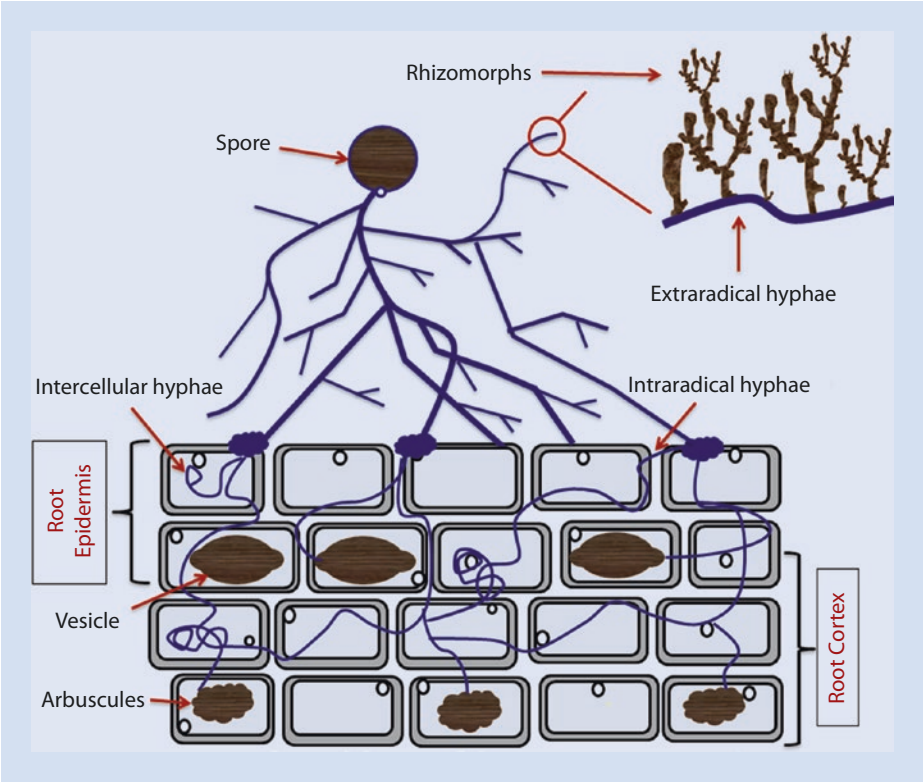

Fig. 11.1 Root colonization by AMF. (Adapted and modified from Teotia et al. 2017; Sharma 2017)

angiosperms, gymnosperms and pteridophytes (Brundrett 2009; Moore et al. 2011). The AMF in the root is dependent on the supply of carbohydrates (photoassimilates) from the host plant. These fungi occur mainly in warm and dry climates where P availability is low. Distinct AMF communities among various host plant species have also been found in semiarid prairie ecosystems and temperate grasslands (Valyi et al. 2015).

The spores of the arbuscular mycorrhiza (AMF) germinate in the soil and form penetrating hyphae. These proliferate in the plant root cortex and form characteristic structures such as (1) vesicles, which are terminal bumps having a storage function (Sullia 1991), (2) intraradical hyphae (within a plant root) and (3) intercellular hyphae (see Fig. 11.1). The intercellular hyphae develop within the air-filled spaces (apoplast) between the cortical cells and then invade the cells with short side branches to form arbuscules (Giovannetti et al. 2010). The arbuscule is where the nutrient exchange between the host plant and fungus takes place and is crucial for the maintenance of AMF symbiosis (Leonie and Giles 2017). After root colonization, the AMF grows out of the root cortex and spreads in the soil around the root, producing an extensive mycelia network (extraradical hyphae). Each AMF hypha can extend up to 20 cm away from the root surface. Finely branched hyphae provide a large surface and facilitate the plant's uptake of water and mineral elements, such as P or N, from the soil, particularly when

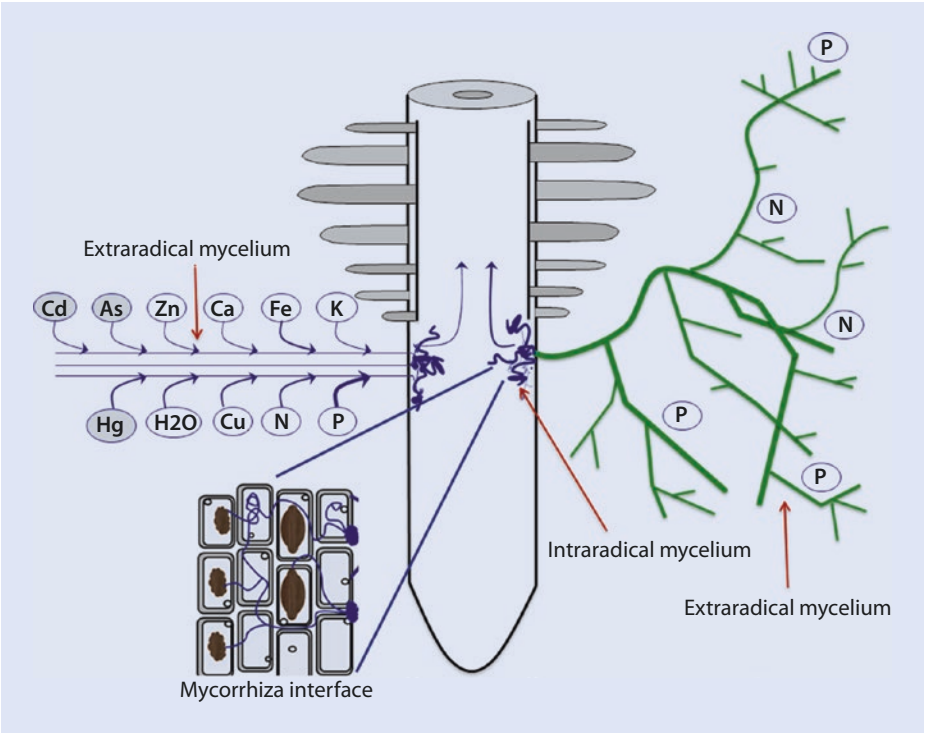

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Fig. 11.2 Plant nutrient (*open elipse*) and toxicant (*gray elipse*) acquisition by AMF association in root surface. (Adapted and modified from George 2017; Bücking et al. 2012)

the soil fertilization level is low (Pallardy 2008). The colonization of AMF can actually alter plant root architecture, fortifying the existing mycorrhizal symbiosis and reducing pathogen colonization (Berdeni et al. 2018; Nadeem et al. 2014).

AMF improve the uptake of both water and mineral nutrients, mainly P, nitrogen (N), sulphur, potassium, calcium, iron, copper and zinc, from the soil to the plant roots, thereby improving the growth and productivity of the host plants (Li et al. 2006; Phillips et al. 2013; Sharma 2017). The AMF might provide non-nutritional benefits to the host, including resistance against pathogens and pests plus tolerance to abiotic stress (Nadeem et al. 2014; Sikes 2010). Many publications affirm that the symbiosis between fungi and host plant can increase water stress tolerance by the direct uptake and translocation of water via the hyphal network (see Fig. 11.2).

The nutrients are taken up and channelled to the plant through the fungal hyphae of the AMF, which are in direct contact with the surface of the root, building up an extraradical mycelium. This mycelium competently explores the soil, locks up sparse nutrients around the root and transports obtained nutrients to the host plant (Fig. 11.2) (Bücking and Kafle 2015). Each hypha forms rhizomorphs, which are intricate lineal multi-hyphae arrangements, their principal beneficial effects being nutrient uptake and transport, especially the uptake of N and P from soil particles that are inaccessible to the plant roots (Bücking et al. 2012; Behie and Bidochka 2014). Furthermore, colonization

with AMF has been shown to decrease heavy metal-induced stress. Smith et al. (2010) have demonstrated that AMF relieves arsenic toxicity in crop plants, thereby reducing heavy metal content in fruits, because AMF immobilize toxic elements (Cd, As, Hg) at the root level, reducing their translocation to the aerial biomass and, thus, decreasing the concentration in the soil or plant biomass (Spagnoletti et al. 2017).

11.3 Improving Crop Quality by Mycorrhization with Regard to Human Health

In medicinal plant production, the synthesis of diverse secondary metabolites has been reported as being linked to mycorrhization (Sbrana et al. 2014). For instance, in the leaves of Brittlewood (*Claoxylon australe*) and the seeds of Australian chestnut (*Castanospermum australe*), the alkaloid 'castanospermine', which has an anti-inflammatory and immune-suppressive activity in the human body, is induced because of mycorrhization. Under AMF inoculation, the content of castanospermine shows an increase at the seedling stage (Zubek and Blaszkowski 2009). Growing seedlings inoculated with AMF (*Glomus intraradices*) under greenhouse conditions show an increase in growth, in P content and in the yield of castanospermine in the leaves (Abu-Zeyad et al. 1999). Inoculation with AMF in wild mint (*Mentha arvensis*) and basil (*Ocimum basilicum*) plants increases plant height, the fresh and dry biomass and the production of total essential oil (especially α-terpineol, which is widely used in the pharmaceutical and herbal industry) in comparison with non-mycorrhizal plants (Grupta et al. 2002; Freitas et al. 2004). Accumulation of α-terpineol is also associated with a significantly larger number of peltate glandular trichomes in the leaves of inoculated plants (Copetta et al. 2006).

Some studies have demonstrated that AMF inoculation influences the synthesis and accumulation of some alkaloids, depending on the plant stage and organ examined (Andrade et al. 2012; Raei and Weisany 2013). For instance, in the leaves and roots of Madagascar periwinkle (*Catharanthus roseus*) plants, several monoterpene indole alkaloids (MIAs) are found as the result of successful mycorrhization (El-Sayed and Verpoorte 2007). Some of these alkaloids have pharmaceutical importance and a human health interest, such as the anticancer drugs 'vinblastine' and 'vincristine' and the antihypertensive drug 'ajmalicine' (Guirimand et al. 2010). Under AMF inoculation, *C. roseus* shows higher mass yields attributable to mycorrhization (Ratti et al. 2010; Cartmill et al. 2008), and, in the study of Andrade et al. (2012), AMF inoculation results in an increasing amount of ajmalicine in the roots. In the cases of vinblastine and vincristine, the accumulation is higher in young completely developed leaves than in not very well-developed younger leaves (Roepke et al. 2010). A possible reason for these enhanced concentrations could be the influence of AMF association on the biosynthesis pathways of defence-related plant hormones (Andrade et al. 2012; Vanstraeken and Benková 2012).

The inoculation of vegetable crops with AMF can be profitable, and commercial inoculation products are available. Raiola et al. (2015) found that use of a commercial inoculant containing AMF can increase the antioxidant activity in some crops: in strawberries (*Fragaria x ananassa*), the antioxidant activity was increased by 37.50%, in giant

lentils (*Lens culinaris*) by 29.17% and in durum wheat (*Triticum durum*) by 63.63%. However, the treatment caused a decrease of the antioxidant activity by 31.81% in kiwi (*Actinidia deliciosa*) and by 19.81% in grape (*Vitis vinifera*).

Hart et al. (2014) inoculated tomato (*Solanum lycopersicum*) fruits with two different AMF strains (*Rhizophagus irregularis*, *Funneliformis mosseae*) to test their impact on the mineral content of fruits. The result showed an increased amount of minerals, mainly N, P, Ca and Cu, compared with non-mycorrhizal fruits. Moreover, the inoculation of tomato with *G. mosseae* before sowing increased the percentage of extra-large fruit, whereas co-inoculation with *T. harzianum* and *G. mosseae* increased tomato lycopene (important antioxidant) content (Nzanza et al. 2012, Giovannetti et al. 2012). Ulrich et al. (2008) confirmed that tomato plants inoculated with AMF had higher contents of lycopene and β-carotene, which are potential compounds for the prevention of some types of cancer and are related to a reduced risk of cardiovascular diseases (Böhm 2012; Calvo 2005; Rao and Rao 2007).

Cucumbers (*Cucumis sativus*) possess terpenoid compounds that have an anti-inflammatory and pain-relieving activity in the human body (Dahm and Golinska 2010; Sharma et al. 2017). AMF inoculation by the application of a 53-µl sieving from a soil inoculum significantly increased the level of these compounds in the roots and enhanced terpenoid accumulation in the fruit (Akiyama and Hayashi 2002). Lettuce (*Lactuca sativa*) has healthy properties, for instance, because of the presence of antioxidant compounds such as vitamins C and E, carotenoids and polyphenols (Baslam et al. 2013). Several studies have demonstrated the beneficial role of AMF in the production of these compounds (Gianinazzi et al. 2010); moreover, mycorrhization increases the yield and improves P uptake in many leafy vegetables, mainly lettuce (Bumgarner et al. 2012; Smith and Smith 2011). According to Baslam et al. (2012), β-carotene was the most accumulated carotenoid in leaves of greenhouse-grown red lettuces under AMF inoculation. The levels of carotenoids in the outer leaves were four to eight times higher than those found in non-inoculated plants (Baslam et al. 2013). Similar levels of carotenoids were obtained by applying mycorrhizal inoculum to the green leaf lettuce (Baslam et al. 2012). The amount of total ascorbate and tocopherol (vitamin C and E components) increased in leaves of lettuce plants under optimal irrigation when inoculated with AMF. Inoculation with a mixture of *G. mosseae* and *G. intraradices* proved to be a very good measure to enhance levels of vitamins C and E (Baslam et al. 2011, 2012).

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Microbial and Plant-Based Biostimulants

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Biostimulants are substances or microorganisms that promote and/or improve plant growth, development, metabolism and/or tolerance to abiotic stress without being fertilizers or pesticides (Zhang and Schmidt 1997; Kauffman et al. 2007; European Biostimulants Industry 2013). The definition of plant biostimulants is flexible, and they are mainly defined by what they are not. Important to mention is that the substances stimulate the plant even in minute amounts (the smallest amounts). In consensus with scientists, regulators and stakeholders, some generally acknowledged main categories of biostimulants have been drawn up (du Jardin 2015; Calvo et al. 2014; Halpern et al. 2015), namely:

- Chitosan and other biopolymers
- Protein hydrolysates and other nitrogenous molecules
- Humic substances
- Seaweed extracts and botanicals
- Beneficial bacteria
- Beneficial fungi
- Inorganic compounds (i.e. Al, Co, Na, Se, Si)

In this chapter, we will only discuss microbial and plant-based biostimulants and their effects on crop quality (for a quick overview, see ◘ Tables 12.1 and 12.2).

◘ **Table 12.1** List of effects of chitosan and protein hydrolysates on selected crops

Biostimulant	Crop	Effects	Application form	References
Chitosan	Peppermint (*Mentha piperita*)	Increased menthol values	Added to cell culture	Chang et al. (1998)
	Oregano (*Origanum vulgare* ssp. *hirtum)*	Increased polyphenol concentration	Foliar spray, 2 weeks before flowering	Yin et al. (2012)
	Sweet basil (*Ocimum basilicum*)	Increased polyphenols, terpenoids and antioxidant activity	Seed-soaking and root-dipping before transplantation	Kim et al. (2005)
	Tomato (*Solanum lycopersicum L.*)	Increased polyphenolic compounds	Applied to wounds	Liu et al. (2007)
	Apricot (*Prunus armeniaca L.*)	Increased total phenols and antioxidant activity	Postharvest fruit coating	Ghasemnezhad et al. (2010)

Table 12.1 (continued)

Biostimulant		Crop	Effects	Application form	References
		Cherry (*Prunus avium L.*)	Enhanced vitamin C synthesis, increased anthocyanin and total phenolic content	Postharvest fruit dipping	Kerch et al. (2011)
		Citrus (*Citrus x nobilis* 'W. Murcott')	Improved titratable acidity, ascorbic acidity and water content	Postharvest fruit dipping	Chien et al. (2007)
Protein hydrolysate	Chicken feather	Banana (*Musa paradisiaca* L.)	Increased proteins, reduced sugars, amino acids, phenols and flavonoids	Irrigation and foliar	Gurav and Jadhav (2013)
	Plant-derived	Grapevine (*Vitis vinifera* L.)	Increased soluble solids, total phenols and anthocyanins	Foliar	Boselli et al. (2015)
	Alfalfa-derived	Pepper (*Capsicum chinensis* L.)	Increased several secondary metabolites	Foliar	Ertani et al. (2014)
	Alfalfa-derived	Pecan (*Carya illinoinensis*)	Increased kernel protein content	Foliar	Ashraf et al. (2013)

Table 12.2 List of effects of humic substances and seaweed extracts on selected crops

Biostimulant	Crop	Effects	Comment	References
Humic substances (humic acid; HA)	Cucumber (*Cucumis sativus* L.)	Increased total soluble sugars, reducing sugars	Organic production, greenhouse	Karakurt et al. (2009)
HA	Tomato (*Solanum lycopersicum* L.)	Increased soluble solids, ascorbic acid		Yildrim (2007)
HA	Pepper (*Capsicum chinensis* L.)	Increased soluble sugars		Karakurt et al. (2009)

(continued)

Table 12.2 (continued)

Biostimulant	Crop	Effects	Comment	References
HA	Grape (*Vitis vinifera*)	Improved titratable acidity and soluble solids values	Foliar application at various stages	Ferrara and Brunetti (2010)
Fulvic acid	Lemon tree (*Citrus limon*) on *C. macrophilia* rootstock	Increased juice pH and vitamin C	Grown on calcareous soil	Sánchez-Sánchez et al. (2007)
Seaweed extracts	Broccoli (*Brassica oleracea* var. *italica)*	Increased antioxidant activity, flavonoids	*Ascophyllum nodosum* extract	Lola-Luz et al. (2014)
		Increased phenols and isothiocyanate	*A. nodosum* and *Durvillaea potatorum* extract	Mattner et al. (2013)
	Cabbage (*Brassica oleracea convar. capitata* var. *alba)*	Increased flavonoids and phenols	*A. nodosum* extract	Lola-Luz et al. (2013)
	Gram mung bean (*Vigna radiata*)	Increased total protein, carbohydrate and lipid content	*Sargassum wightii* extract	Ashok-Kumar et al. (2012)
	Spinach (*Spinacia oleracea)*	Increased antioxidant activity, flavonoid and phenol content and Fe-chelating ability	*A. nodosum* extract	Fan et al. (2013)
	Olive (*Olea europaea* L.)	Increased oil content and linolenic and oleic acid; decreased palmitoleic, stearic and linoleic acid	*A. nodosum* extract	Chouliaras et al. (2009)

12.1 Chitosan

Chitosan is the deacylated derivate of chitin. Chitin is a biopolymer that is a natural compound of fungal cell walls, insect exoskeletons and crustacean shells. Chitosan is easily made by the saponification of chitin and has a better solubility than chitin. Sources are usually food production, i.e. crab or shrimp shell waste, which is demineralized and deproteinized (Rinaudo 2006; Younes and Rinaudo 2015). Chitosan elicits plant defence responses to wounding (Doares et al. 1995) and pathogen infections (Bhaskara Reddy et al. 1999; Bautista-Baños et al. 2003; Yu et al. 2012). The application

of chitosan triggers the oxidative burst response with the production of H_2O_2, which induces phenylalanine ammonia lyase (PAL) activity (Lee et al. 1999; Zhao et al. 2007). PAL is a plant defence enzyme and is an important enzyme in phenolic compound biosynthesis (Camm and Towers 1973). The increase in phenolic compounds after chitosan treatment has been reported for several horticultural crops including basil (*Ocimum* spp.), grape (*Vitis vinifera L.*) and tomato (*Solanum lycopersicum L.*) (Kim et al. 2005; Meng and Tian 2009; Liu et al. 2007; Badawy and Rabea 2009). Kim et al. (2005) applied chitosan to *Ocimum basilicum* by seed-soaking and root-dipping in a 1% (w/v) solution before transplantation. The treatment leads to increased growth and secondary metabolite content together with a great increase in the levels of human health-promoting rosmarinic acid and eugenol. Eugenol is also an important compound for perfumes. Additionally, polyphenol oxidase (PPO) activity is recorded to be delayed after such treatment (Jiang and Li 2001; De Reuck et al. 2009; Badawy and Rabea 2009). Inhibited PPO activity has several positive effects: a prolonged shelf life attributable to a decreased respiration rate and the inhibited degradation of organic compounds (Qi et al. 2011). This results in a delay of browning and the prevention of weight loss. The most common method to prolong shelf life is fruit dipping/coating. Responses to the application of chitosan depend on the concentration and quality of the product and on the plant species and developmental stage.

12.2 Protein Hydrolysates

Protein hydrolysates (PH) consist of blends of poly—any oligopeptides and amino acids produced from various protein sources by using partial hydrolysis (Schaafsma 2009). Sources can be animal- or plant-derived and are then treated by enzymatic and/or chemical hydrolysis. Therefore, protein hydrolysates are a sustainable solution for agro-industrial waste disposal, e.g. feathers and blood or hay and vegetable by-products (Maini 2006; Kasparkova et al. 2009; du Jardin 2015). PHs stimulate iron (Fe) and nitrogen (N) metabolism, nutrient uptake and water and nutrient use efficiency. This is attributable to the higher soil microbial and enzymatic activity, the improved micronutrient mobility and solubility, the modifications of root architecture and an increase in specific enzymes (Cerdán et al. 2009; Ertani et al. 2009; García-Martínez et al. 2010; Colla et al. 2014; Halpern et al. 2015; Lucini et al. 2015). Plant-derived PHs also modify the phytohormone balance, eliciting auxin- and gibberellin-like effects by specific peptides and phytohormone precursors such as tryptophan (Colla et al. 2014). Many reports have been presented regarding the way that PH can raise the concentrations of human health-promoting phytochemicals such as carotenoids, flavonoids and polyphenols (Parrado et al. 2007; Paradikovic et al. 2011; Ertani et al. 2014). Gurav and Jadhav (2013) reported an accumulation of total phenolics, flavonoids and proteins and an increased antioxidant activity in banana (*Musa* ssp.) after using degraded feather products. In berries of red grapevines (*Vitis vinifera*), increased phenolic (+22%) and anthocyanin (+76%) values have been reported after the treatment of the plants with an enzymatic vegetable extract. This effect is attributable to the phytohormones and nutrients in the extract (Ban et al. 2003; Jeong et al. 2004; Parrado et al. 2007). Ertani et al. (2014) treated pepper (*Capsicum chinensis L.*) with alfalfa (*Medicago sativa*) PH (25 or 50 ml/l). This

led to high concentrations of chlorogenic acid, p-hydroxybenzoic acid and p-coumaric acid in green pepper fruits and to an increase of capsaicin in red pepper fruit. Application of PH from red grapes additionally improved aroma-influencing compounds such as glucose and ascorbate. The results are related to the stimulation of secondary metabolism by an increase of gene expression of phenylalanine ammonia lyase (PAL). PH upregulate the expression and activity of PAL, which promotes flavonoid biosynthesis (Schiavon et al. 2010; Ertani et al. 2011). Ertani et al. (2013) noted a consistent increase in the flavonoid content in hydroponically grown maize under saline conditions after treatment with alfalfa PH in comparison with control plants. Ertani et al. (2013) also described an increase of phenolics induced by salinity (NaCl) but a decrease of phenolics by the PH treatment. Furthermore, Lucini et al. (2015) reported that lettuce (*Lactuca sativa*) grown under saline conditions showed increased terpenes and glucosinolates (among other health-enhancing secondary metabolites) after the application of plant-derived PH. Treatment of lemon balm (*Melissa officinalis L.*) with 2 l/ha of an amino acid mixture via foliar application resulted in enhanced concentrations of several terpenoids (Mehrafarin et al. 2015). In addition, Liu and Lee (2012) have found that PH reduce undesired compounds, e.g. nitrates, in leafy vegetables such as arugula (*Eruca sativa*) and spinach (*Spinacea oleracea*).

A large difference exists between plant- and animal-derived PH with regard to phytotoxicity. Cerdán et al. (2009) have demonstrated no phytotoxic effects of plant-derived PH on tomato (*Lycopersicum L.*), even at the highest concentrations, whereas phytotoxic and growth-depressing effects of animal-derived PH have been reported for fruiting crops after repeated application (Cerdán et al. 2009; Lisiecka et al. 2011). An unbalanced amino acid composition, high concentrations of free amino acids and higher salt concentrations seem to be responsible for the potential detrimental effects of animal-derived PH (Oaks et al. 1977; Moe 2013; Colla et al. 2014). Another negative effect is that root nitrate uptake is repressed because of strong phloem loading with free amino acids (Ruiz et al. 2000). This especially affects plants with a low N supply. The most optimized effects will be achieved by very low dosages. This however depends on the cultivar, environment, phenological stage, time and mode of application (Kauffman et al. 2007; Kunicki et al. 2010; Ertani et al. 2014).

12.3 Humic Substances

Humic substances (HS) are natural compounds of soil organic matter. HS arise from the decomposition of plant, animal and microbial debris and from microbial metabolism. Originally, HS were classified into humins, humic acids and fulvic acids categorized according to their molecular weight and solubility. Schiavon et al. (2010) reported an enhanced expression of PAL that was attributable to HS application and that was accompanied by increased phenol values in leaves of maize (*Zea mays* L.). Stimulation of other compounds linked to the shikimic pathway (alkaloids, tocopherols) has also been noted. A foliar treatment with humic acids (HA) from peat leads to an increase of pyruvic acid in garlic (*Allium sativum*) (Denre et al. 2014). In this case, pyruvic acid is an indicator of pungency and therefore aroma. Moreover, a foliar spray of HA on grape (*Vitis vinifera* L. cv. Italia) improves titratable acidity and soluble solid content and, hence, improves

the taste (Ferrara and Brunetti 2010). As a side effect, berry size is also increased. As HS also carry carboxylic and phenolic functional groups, they are capable of complexing toxic heavy metals and therefore of reducing heavy metal content and mobility in plants (Zeng et al. 2002). Shahid et al. (2012) noted a dose-dependent reduction of Pb^{2+} uptake by fava bean (*Vicia faba* L.) but only showing high effectiveness when high concentrations of HS were used. HS might also have an indirect effect on fruit quality. A lower incidence of plant disease has been reported, as has the capability of using HS as a carrier for microbial inoculants (Zaller 2006; Singh et al. 2010; Naidu et al. 2013; Canellas et al. 2013). The optimum dosage in general depends on the cultivar and mode of application (foliar spray or soil drench). Additionally, in some cases, the efficacy depends on the source or quality of the HS (Lulakis and Petsas 1995; Azcona et al. 2011).

12.4 Seaweed Extracts

Seaweed extracts are made from marine macroalgae. Several compounds are exclusive to the algal source, and the chemical composition of the extract depends on the extraction method, which in turn affects its biological activity (Kim 2012; Khairy and El-Shafay 2013). In general, the extracts contain polysaccharides, micro- and macronutrients, sterols, betaines, hormones and vitamins and their precursors (Blunden et al. 1985; Berlyn and Russo 1990; Craigie et al. 2008; Khan et al. 2009; Craigie 2011). Of importance to consider, the activity of seaweed extracts is similar to that of phytohormones. At low concentrations, growth increases, but, at high concentrations, growth is inhibited (Provasoli and Carlucci 1974; Khan et al. 2009). Chalcone isomerase (CHI) has been noted to increase after seaweed extract treatment. CHI is a key enzyme for the synthesis of flavonoid precursors. Fan et al. (2011, 2013) have reported enhanced antioxidant activity and flavonoid and phenolic content and improved storage quality in spinach after the application of common brown algae (*Ascophyllum nodosum*) extract. The fatty acid profile of olive oil can also be modified by *A. nodosum* extracts: Chouliaras et al. (2009) noted a significant increase in the health-promoting linolenic and oleic acids and an ample decrease in the rather unhealthy palmitoleic, stearic and linoleic acids. Extract of *A. nodosum*, when sprayed at 10 l/ha/month, has also been found to raise the phenolic and flavonoid content in onion (*Allium cepa*) (Lola-Luz et al. 2014). Lola-Luz et al. (2014) further noted an increase in total phenolic, total flavonoid and total isothiocyanate content in broccoli (*Brassica oleracea* var. *italica*) after such treatment.

An extract of several seaweeds combined (*Sargassum*, *Laminaria*, *A. nodosum*) can be successfully be used as a postharvest treatment that is superior to $CaCl_2$, which only maintains the actual quality. Fruit dipping in a 4% seaweed extract results in a significant improvement of sweetness attributable to increases of total soluble solids and sugars and reducing sugars in navel oranges (*Citrus sinensis* (L.) *Osbeck*) when they are stored at ambient temperature or in cold storage (Omar 2014). Liquid extracts can be applied by irrigation/fertigation or as a foliar spray (Rao 1991; Fornes et al. 2002; Selvaraj et al. 2004; Haider et al. 2012). Foliar application is best performed in the morning when the stomata are open (see ► Chaps. 13 and 20). Responses to seaweed extracts are crop-specific with regard to concentration and frequency of application (Battacharyya et al. 2015).

12.5 Botanicals

Botanicals are products derived from plants, algae, fungi or lichens. Sánchez-Gómez et al. (2016) studied effects of extracts from white grape (*Vitis vinifera* cv. Airén) vine-shoot residues as a viticultural biostimulant on grapevine. Results showed an increased varietal aroma typical of the Airén variety (norisoprenoids and terpenes) after foliar application combined with a wetting agent. A positive modulation of the phenolic composition (especially of hydroxycinnamic acid) was also achieved. Moreover, French oak (*Quercus robur*) extract has been noted to influence grape berry volatile organic compounds (VOCs). According to Martínez-Gil et al. (2011), the grapes (*Vitis vinifera* cv. *Petit Verdot*) store the VOCs primarily as non-volatile precursors, some of which are released after winemaking. The effects were evident only after alcoholic fermentation sampling. Therefore, the results are mainly interesting for young wines, which are bottled immediately after fermentation and clarification. Pardo-García et al. (2013a, b) also found a modulation of the phenolic composition of red wine grapes (*Vitis vinifera* cv. *Monastrell*). The foliar oak extract treatment resulted in a concentration of polyphenols such as garlic acid, hydroxycinnamoyltartaric acids, acylated anthocyanins, flavonoids and stilbenes in the berries. The application also led to less alcoholic and acid wines, therefore improving taste with a higher colour intensity and deeper shade. Application of lavandin hydrolat (*Lavandula hybrida*) to Petit Verdot grapes exhibited an impact on their wine aroma compounds. Martinez-Gil et al. (2013) showed an unusual increase of camphor in wines after repetitive foliar spraying (5 ×) at weekly intervals, when using 250 ml hydrolat per plant starting at 7 days after the half-veraison. A wetting agent at 0.5 ml/l was added to the hydrolat formulation to ensure sufficient adhesion to the leaves. Additionally, the aroma of the wines was positively modified 6 months after malolactic fermentation. A higher stability of some compounds, i.e. esters, was also discovered.

12

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Mineral Biofortification

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The idea behind fortification is to add certain minerals to plants and therefore to increase the nutritional value of crops. Fortification can also describe measures to enrich plants with health-promoting secondary plant compounds; however, this subject will not be covered here. Humans, domestic animals and plants require severe mineral elements, usually in small amounts from 1 to 2500 mg per day, depending on the mineral and species (Soetan et al. 2010). All of these minerals can be supplied to humans by an appropriate plant-based diet. Nevertheless, over half of the world's population, especially women and preschool children, are deficient in one or more nutrients of the following: calcium (Ca), iron (Fe), iodine (I), magnesium (Mg), selenium (Se) or zinc (Zn) (Graham et al. 2007; White and Broadley 2005; Nestel et al. 2006). This deficiency is not only present in developing countries. Observations have been made that some people in well-developed countries suffer chronic malnutrition of micronutrients caused by an unbalanced and poor diet, in combination with low levels of micronutrients in crops/products attributable to micronutrient-deficient soil. Deficiencies of micronutrients can cause several illnesses and imbalances and are one reason for hidden hunger.

The provision of a nutrient via foliar application into a plant organ is relatively easy. The mineral is dissolved in water, and this solution is sprayed together with a surfactant (wetting agent) onto the plant surface. The surfactant is critical for allowing the minerals to access the cells and the apoplastic spaces. It ensures that the solution remains for a longer time period (retention) on the leaf in moist conditions and breaks the surface tension of the solution resulting in a better solution spread over the entire leaf surface. This facilitates the introduction of the minerals into the leaf tissue. However, a substantial amount of the minerals will also be lost because of the residue collection on the outside of the plant being washed off by rainfall precipitation. Nevertheless, the plant tissue will be enriched with these elements (Fernández and Eichert 2009; Fernández et al. 2013; Neuhaus et al. 2013; Jezek et al. 2015). Alternatively, plants can be biofortified with minerals via normal application into the soil. Both strategies, either application into the soil or onto the leaf surface, are do-able. However, the efficiency of the fortification of plant tissues with essential (for humans) mineral micronutrients depends on several factors: the application method (soil versus leaf application; formulation additives), precipitation (rain, fog, relative humidity), light, temperature, hydrophobicity of the leaf surface if supplied via foliar application, leaf topography (cuticle and epidermal structures, leaf age, canopy development), mobility of the mineral nutrient in the plant and mobility of the mineral in the soil (if supplied via soil) (Zhu et al. 2007; Fernández et al. 2013). The physicochemical properties of the spray solution are also relevant if the element is applied via foliar solution. Critical factors are concentration, solubility, molecular weight, electric charge, solution pH and point of deliquescence (see explanation below) (Fernández and Eichert 2009; Fernández et al. 2013). Efficiency of fortification can be maximized by protected cropping or in a completely controlled production environment.

As mentioned above, the mobility of the element is decisive. For instance, I and Se are highly mobile in soil and in plants, and, thus, biofortification with these minerals has been particularly successful (Dai et al. 2004; Hartikainen 2005; Velu et al. 2014; Lawson et al. 2015). In contrast, Fe shows a low mobility in many soils, because Fe is rapidly and strongly immobilized by adsorption to soil particles and/or Fe is oxidized forming Fe-III complexes. Therefore, conventional Fe fertilizers have not been particularly successful in biofortification via soil application (Grusak and DellaPenna 1999).

Stabilized forms of Fe that are given together with a chelator (ethylenediaminetetraacetic acid or Sequestren®) can help to prevent immobilization. The same is true for Zn. Research indicates that soil application of Zn or Fe together with the chelator ethylenediaminetetraacetic acid provides higher soil Fe and Zn availability and therefore results in increased plant uptake (Wang et al. 2017). Alternatively, Fe and Zn can be applied directly onto the leaf via foliar application. This measure is far more effective compared with soil application. However, light radiation is critical, as several Fe(III)-chelates are known to be degraded by excessive exposure to sunlight (International Fertilizer Industry Association (IFA) 2013).

13.1 Penetration of Exogenously Sprayed Minerals into the Leaf

The plant surface is physicochemically optimized in a such way that it holds water within the plant. Only the smallest amounts of water exit the leaf through paths other than the stomata. The bidirectional exchange of water, solutes and gases between the plant and the surrounding environment is limited by a hydrophobic cuticle, which covers all aerial parts of the plant. This is the plant's first line of defence in terms of water loss and other biotic and abiotic factors (Baur 1998; Ahmad et al. 2015). The cuticular wax layer serves as a protective barrier, which consists mainly of long-chain hydrocarbon compounds, including alkanes, primary alcohols and other compounds (Shepherd and Griffiths 2006). Hydrocarbon chains co-align and form a hydrophobic water-repellent barrier, which makes the penetration of solutions difficult. This, however, means that the only notable entry path for an exogenously applied solution sprayed onto the leaf is via the open stomata. Stomata are leaf pores that allow gas exchange (mainly CO_2 and H_2O). Nevertheless, some environmental factors can alter the properties of the leaf surface, facilitating an unwanted bidirectional exchange of water and solutes. Leaf surface waxes can be removed by abrasion attributable to aerodynamic loading (e.g. windy conditions), impact of raindrops, dust and snow or leaf-to-leaf contact (Shepherd and Griffiths 2006). All of these types of abrasion make penetration easier. On the contrary, stresses such as UV radiation can lead to an increased thickness of the wax layer as protection against excessive light radiation, thereby hampering penetration.

The solubility of the fertilizer salt in the spraying solution is another factor that is critical for the efficiency of biofortification. $CaCl_2$, for example, has a medium to good solubility compared with other salts but can itself absorb the water of the surrounding air to form an aqueous solution. This property is referred as to 'deliquescence'; it can extend the retention time on the surface of leaves, depending on concentration, and promotes uptake. Moreover, the electric charge and pH of the spraying solution play a major role in uptake. Uncharged molecules tend to have low or no interaction with charged parts of the plant tissue and can cross the plasma membrane more easily than charged molecules, as the negatively charged external surface of the lipid bilayer would repel anions. This would hamper anion uptake (influx) across this cell membrane. The pH of the spraying solution is also critical because it can alter the surface charge of the plant cuticle. Cuticles have an isoelectric point at around 3. This means that when the pH exceeds a value of 3, the protons from the carboxyl groups will dissociate giving

rise to a negative charge (R-COOH become R-COO⁻) (Schönherr and Huber 1977) favouring the absorption of cations such as the divalent positive calcium. For instance, Lidster et al. (1977) have reported the highest calcium absorption by sweet cherry (*Prunus avium* L.) fruits when the applied $CaCl_2$ solution has a balanced pH of 7. The chloride, however, is repelled as it is a monovalent anion.

The ideal concentration of minerals for application depends not only on the physicochemical properties of the spraying solution (pH, electric charge) but also on factors such as plant species, leaf structure and size, age of the plant, nutritional status, plant metabolism and stomata opening (Kannan 2010; Wittwer and Teubner 1995; Wojcik 2004). For example, as a result of a highly active metabolism, cells can grow by means of expansion or cell division. This dilutes solute content, steepening the concentration gradient between the cell and the spray solution. The distribution of stomata within the ad- and abaxial surface of the leaves varies between plant species, a characteristic that should be taken into consideration for foliar application. Moreover, as mentioned above, abiotic factors such as light intensity, temperature and humidity should also be considered, as these factors influence the effectiveness of the treatment not the least by affecting (1) the size of the stomatal aperture, (2) the retention time of the applied solution on the leaves and (3) the degradation of the used minerals/complexes by light.

13.2 Improving Quality of Plant-Based Food by Mineral Fortification

Rice is poor in Zn, which is the reason that humans can become deficient in Zn if their nutritional intake is not balanced: this can occur in developing countries where rice is a primary staple food (Stein 2010; Cakmak 2008). Zn deficiency can lead to impairments in physical growth and problems with the immune system and brain function (Hambidge 2000; Hotz and Brown 2004). Wei et al. (2012) fortified, via foliar application, three rice varieties (*Oryza sativa* L.) with Zn, which was given to the plants as Zn-EDTA, Zn-Citrate, $ZnSO_4$ and Zn-amino acids. The Zn was exogenously applied three times in total, once at the panicle initiation stage and twice at 7 days after the flowering stage. The spray was applied after sunset when the stomata were assumed to be open. As a result, Wei et al. (2012) found that all grains contained more Zn after foliar application regardless of the Zn compound used in the solution or of the cultivar. Moreover, the bioavailability for human nutrition was increased. Based on the results of this experiment, the authors suggested that consumption of this rice should help to fight Zn deficiency.

In the human body, selenium (Se) can be found in the selenoproteins, iodothyronine deiodinases, thioredoxin reductases and glutathione peroxidases (Arita and Costa 2011). Pezzarossa et al. (2011) conducted a study to enrich peach (*Prunus persica* Batch.) and pear (*Pyrus communis* L.) with Se. The mineral was applied in two different concentrations (0.1 and 1.0 mg Se /l) to peach leaves. Foliar spraying of the Se solution resulted in a significant increase of Se concentration within the vegetative plant tissue and in the fruit. The more Se that was sprayed, the higher was the tissue concentration. Se leaf application also improved other quality-determining factors of the peach fruit: the shelf life of the fruit was prolonged because of the delay in fruit ripening (probably because of a reduction in cell wall softening). Similar results were obtained in the studied pear plants.

Li et al. (2017) performed an experiment on the iodine (I) fortification of pepper plants (*Capsicum annuum* L.). I deficiency is still a serious health issue worldwide and can lead to miscarriages, birth defects and reduced intelligence quotient (Mina et al. 2011; Andersson et al. 2012; Hetzel 1983, 2005; Laurberg et al. 2010). I is important for the production of thyroid hormones in the human body. Li et al. (2017) cultivated the pepper plants in a hydroponic system with Hoagland nutrient solution and added various concentrations (0, 0.25, 0.5, 1.0, 2.5 and 5 mg/l) of potassium iodide as the I source. I levels increased in all plant organs such as the roots, stems, leaves and fruit. Furthermore, ascorbic acid, total soluble sugar and acidity were also measured in all peppers: low to moderate dosages of I improved fruit quality by increasing the contents of ascorbic acid and soluble sugars while reducing the acidity of the peppers. The authors suggested that the consumption of these peppers should help to fight I deficiency.

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CO_2 Enrichment

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14.1 Introduction

Carbon dioxide (CO_2) is a colour- and odourless gas. It is a natural component of the air and an important indicator for its quality. During photosynthesis, energy from sunlight is used to reduce CO_2 to hydrocarbons (sugars). The electrons that are required for this reductive incorporation of inorganic carbon (C) into organic C-skeletons (so-called reductive assimilation) are released from water (H_2O) that decays into hydrogen and oxygen (O_2). Human (and animal) life would not be possible without O_2 derived from this photosynthetic light reaction. Therefore, photosynthesis is one of the most important biological processes ensuring life on earth. In a metabolic process that runs in reverse but complementary to photosynthesis, namely, cellular respiration, energy is gained by the degradation of these previously formed C-skeletons (photoassimilates). This process is referred to as oxidative dissimilation. Hereby, CO_2 is released, and O_2 functions as the final electron acceptor, forming H_2O.

Under conditions in which photosynthetic sugar production is limited because the precursor of the sugar molecules, namely, CO_2, is not available in sufficient amounts, an increase in atmospheric CO_2 concentration will increase photosynthetic sugar production. In other words, in the presence of enough sun-derived energy, an increase of CO_2 in the greenhouse atmosphere will increase the photosynthetic output of the plants. Thus, the enrichment of CO_2 in Controlled Environment Horticulture (CEH) is a measure for increasing the yield and amount of quality-determining sugars.

14.2 Improving Crop Yield and Quality by Preharvest CO_2 Exposure in Greenhouses

The term 'preharvest' denotes a process/treatment that is conducted before plant-based products are harvested. 'Postharvest', in contrast, refers to a process/treatment that is performed after harvesting. Especially during the winter in European and North American latitudes, the lack of natural light can limit crop growth in greenhouse horticulture (Hand 1984). Thus, the enrichment of the greenhouse atmosphere with CO_2 has become a common measure for enhancing photosynthesis and thereby biomass production (yield), providing that enough energy is available via artificial lightning (Chalabi et al. 2002; Hand 1984). Many examples demonstrate this effect, including tomato (*Lycopersicum esculentum* Mill cv. Vendor), cucumber (*Cucumis sativus* L.), lettuce (*Lactuca sativa* L. var. *crispa* L. cv. Eventai RZ and *L. sativa* L. var. *crispa* L. cv. Satine), melon (*Cucumis melo* L.), strawberry (*Fragaria x ananassa* Duch. cv. 'Elsanta'), ginger (*Zingiber officinale* Roscoe) and Labisia pumilia (*Labisia pumila* Benth.) (Hicklenton and Jolliffe 1978; Keutgen et al. 1997; Slack and Hand 1985; Mavrogianopoulos et al. 1999; Ghasemzadeh and Jaafar 2011; Ibrahim et al. 2010; Becker and Kläring 2016). Although Mortensen (1987) reported that the advantages of a CO_2 enrichment in the greenhouse atmosphere had been detected as early as the nineteenth century, the technique was not used commercially until the 1960s when both cheap sources of high-purity CO_2 and gas-tight greenhouse constructions became available (Hand 1984; Mortensen 1987). The horticulturist has to be aware that such a measure increases not only yield but also quality as sugars are relevant for both the taste (sweetness) and the caloric value of the food.

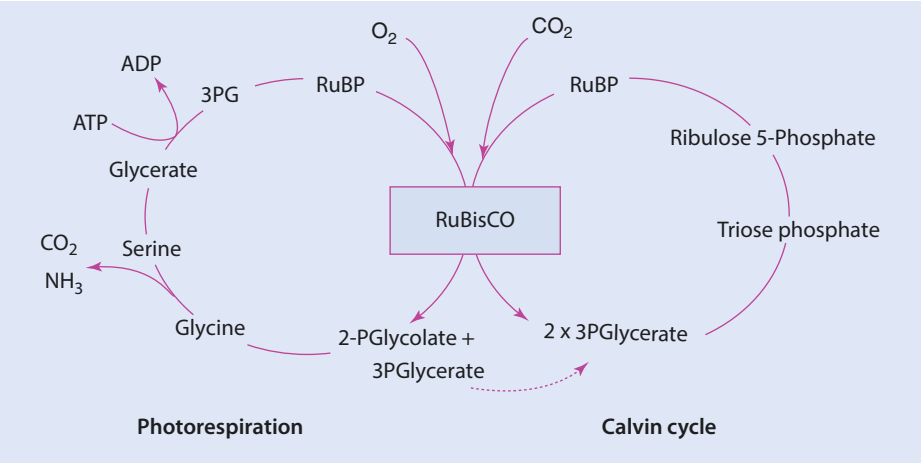

Fig. 14.1 The carbon fixation (Calvin cycle) and oxygenation reactions cycle (photorespiratory cycle) of RuBisCO. *Right* carbon fixation cycle. *Left* photorespiratory cycle. During the Calvin cycle, CO_2 is attached to ribulose-1,5-bisphosphate (RuBP). This gives rise to the formation of stable carbohydrates. During photorespiration, RuBP is oxygenized, giving rise to the production of 2-phosphoglycolate (2-PGlcyolate). The recycling of 2-PGlcyolate to RuBP requires the release of CO_2. RuBisCO, ribulose-1.5-bisphosphate carboxylase/oxygenase; PG, phosphoglycolate; ATP, adenosine triphosphate. (Image © Rachel Purdon, distributed under the terms and conditions of the Creative Commons Attribution license, license: CC BY-SA 3.0, license link: ► http://creativecommons.org/licenses/by-sa/3.0)

What is the physiological basis of the yield increases that result from CO_2 enrichment? In plants in which the 3-carbon-atom-containing molecule 3-phosphoglycerate is the first stable photosynthetic-derived hydrocarbon (these plants are called 'C_3 plants' because 3-phosphoglycerate contains three carbon atoms; examples are potato, spinach, tomato, apple, peach or grain cereals), the key enzyme that assimilates CO_2 into sugars during the photosynthetic Calvin cycle is the ribulose-1.5-bisphosphate carboxylase/oxygenase (RuBisCO). Of note, this enzyme shows an increased activity when CO_2 concentration increases (Lorimer et al. 1976) and is inhibited by increasing amounts of O_2 (Bowes 1991). Thus, a key factor that is attributable for the yield increase after CO_2 enrichments is an enhanced net assimilation rate (Nilsen et al. 1983). Another factor is the steepening of the CO_2 gradient between the atmosphere (high) and the inner leaf mesophyll (low). This drives the diffusion of CO_2 into the photosynthetically active mesophyll cells (Hicklenton and Jolliffe 1978). As result, more CO_2 enters the cells where it can be reduced by RuBisCO forming sugars. A third factor responsible for sugars being enriched in crops by increasing the CO_2 concentration in the greenhouse atmosphere is related to the substrate affinity of RuBisCO. Atmospheric oxygen competes with CO_2 as a substrate for RuBisCO, giving rise to photorespiration (Fig. 14.1). This is a problem in intensive plant production because photorespiration is a catabolic process that degrades the carbon that was previously fixed by photosynthesis. In this way, photosynthetic output can be reduced by 25% (Sharkey 1988). Hence, the horticulturist may increase the CO_2 concentration to counteract photorespiration. Nilsen et al. (1983) demonstrated that the enrichment of the atmospheric CO_2 concentrations

resulted in a reduction of photorespiration and thereby favoured CO_2 fixation via photosynthesis in tomato plants (*Lycopersicum esculentum* cultivar 'Virosa').

However, responses of plants to enriched atmospheric CO_2 concentrations seem to follow a saturation curve. Keutgen et al. (1997) showed that strawberry plants (*Fragaria x ananassa* Duch. cv. 'Elsanta') increase net photosynthesis up to an elevated CO_2 concentration of 600 ppm (atmospheric CO_2 concentration in Europe is currently ~ 400 ppm; however, this is anticipated to increase because of anthropogenic release of fossil-fuel-derived CO_2). Above 600 ppm, they observed a decline in net photosynthesis. Furthermore, after an initial stimulation of photosynthesis, the stimulatory effect decreases or even disappears (Stitt 1991). One main reason causing this acclimation of photosynthesis might be an accumulation of carbohydrates in source organs. This results in an imbalance between source and sink organs with regard to the allocation of sugars. Source organs are those that produce the sugars (e.g. photosynthetically active leaves), whereas sink organs are those that consume sugars (e.g. growing leaves, roots, fruits or tubers). An accumulation of carbohydrates in source organs may feedback-inhibit RuBisCO activity (Bowes 1991; Stitt 1991). However, this can differ greatly between plant species and is dependent upon factors such as plant age, habitus and the surrounding environment (Stitt 1991). For example, the yield of C_4 plants (the four-carbon-containing metabolite oxaloacetic acid is the first stable hydrocarbon in these plants; examples are the common purslane (*Portulaca oleracea*), the tropical leafy vegetable *Amaranthus tricolor*, maize, sorghum and sugarcane) cannot be sufficiently increased by enriching the CO_2 concentration (Bowes 1991). The vast majority of our horticultural plants belong to C_3 plants.

14.3 Changes of Quality by Postharvest CO_2 Exposure

In addition to a CO_2 exposure during plant growth, a CO_2 exposure after harvest is a measure for controlling the postharvest quality of horticultural crops. Alteration of the concentrations of O_2 and CO_2 in the storage atmosphere (decreasing O_2 and/or increasing CO_2 concentrations) can reduce the process of oxidative dissimilation of sugars and thus delay the decay of highly perishable fruits and vegetables (Chandra et al. 2015; El-Kazzaz et al. 1983; Mathooko 1996). Li and Kader (1989) report that respiration rates and the decay of strawberry fruits (*Fragaria x ananassa* Duch.) are lower when stored under lower O_2 (2.0%, 1.0% and 0.5% O_2) and/or increased CO_2 (10%, 15% and 20% CO_2). How can this be explained? If respiration is high, more sugars are degraded. This is also true for cell wall carbohydrates. If cell wall carbohydrates are degraded, the cell walls become softer (imagine a mushy fruit). As the perishability of harvested fruits or other plant products is mainly characterized by cell wall softening, the maintenance of cell wall firmness can be regarded as the main factor for improving postharvest shelf life by CO_2 exposure (Goto et al. 1996; Hwang et al. 2012). Hwang et al. (2012) suggest that a CO_2 postharvest treatment leads to an increased binding of calcium to water-soluble pectins, resulting in an increase of firmness of strawberry fruits. However, evidence for this relationship still needs to be established.

Lower respiration rates in fruit tissues, as induced for instance by CO_2 concentrations higher than 10% above ambient, can also impair the quality of fruits. This is because the activity of fermentative carbon metabolism can be induced when respiration is low, resulting in the accumulation of ethanol, which negatively affecting the odour and taste of strawberries. Other negative effects of CO_2 enrichment on quality have been reported. Shamaila et al. (1992) investigated the impact of various storage conditions (changing CO_2 and O_2 atmospheric concentrations) and times on the desirable and undesirable quality attributes of strawberries (*Fragaria x ananassa*, Duch., cv. 'Chandler'). They reported that strawberries stored under CO_2 enriched atmospheres (11% and 100% CO_2) developed undesirable quality attributes, such as an off-odour or bitterness, compared with strawberries stored under normal air conditions. Moreover, strawberries stored under normal air retained desirable attributes, such as a typical strawberry odour and sweetness, for longer compared with strawberries stored under atmospheres with increased CO_2 concentrations. A reason for the divergent outcomes of the studies by Li and Kader (1989) and Shamaila et al. (1992) with respect to CO_2 exposure at 10% or 11%, respectively, might be the differences in the CO_2 exposure times. Whereas Shamaila et al. (1992) exposed the fruits for 10 days, Li and Kader exposed the fruits for shorter time periods (1–8 days). Wang (1979) investigated the impact of CO_2 (20%, 30% and 40%) treatment for 3 and 6 days on quality traits of broccoli (*Brassica oleracea* var. *italic*). Broccoli treated with 40% CO_2 for 3 and 6 days and broccoli treated with 30% CO_2 for 6 days exhibited undesirable odour and flavour. This did not appear in broccoli treated with 30% CO_2 for 3 days and disappeared in broccoli treated with 30% CO_2 for 6 days after the broccoli had been transferred to normal air. However, CO_2 exposure delayed ethylene production and the loss of chlorophyll and ascorbic acid in all treatments. These are positive effects with regard to quality.

A further important quality attribute affected by CO_2 exposure is the colour of fruits and vegetables. Holcroft and Kader (1999) showed that strawberries (*Fragaria ananassa* Duch., cv. 'Selva') treated with CO_2 lose their typical red colour because of a loss of anthocyanin. This is most likely attributable to the decreased activity of important enzymes involved in the biosynthesis of anthocyanins such as phenylalanine ammonia lyase (Holcroft and Kader 1999). Likewise, Taşdelen and Bayindirli (1998) demonstrated that 3% CO_2 during storage affected the colour of tomato fruits (*Lycopersicon esculentum* cv. 144). The typical red colour of tomatoes originates from carotenoids, mainly lycopenes. The storage of tomatoes under a CO_2-enriched atmosphere leads to a delay in lycopene synthesis. The authors suggested that the delayed lycopene synthesis was related to an abducted ethylene production caused by CO_2 exposure, as lycopene production depends on ethylene synthesis.

In addition to the above-described effects, CO_2 postharvest treatment has been shown to reduce the astringency of persimmon fruits (*Diospyros kaki* L.), which is a welcome effect (Pesis and Ben-Arie 1984; Yamada et al. 2002). Astringency in persimmon fruits arises from soluble tannins accumulating in the fruits, especially in unripe fruits (Taira 1996). Moreover, clear differences in astringency occur between varieties. According to Nakamura (1973), naturally non-astringent fruits exhibit higher levels of

acetaldehyde in their fruit flesh. CO_2 exposure stimulates the production of acetaldehyde in the fruits (Pesis et al. 1988). Acetaldehyde is supposed to insolubilize tannins and thus to lead to a loss of the astringent effect (Matsuo and Itoo 1982).

Thus, a CO_2 postharvest treatment is a suitable tool for achieving a delay in the decay of stored fruits and vegetables and for controlling important postharvest quality traits. However, the success of a CO_2 postharvest treatment is highly dependent on the respective culture, the chosen CO_2 concentration and the CO_2 exposure time. For instance, the taste of fruits such as strawberry can be worsened by enriching with CO_2.

14.4 Effects of Climate Change-Driven Free-Air CO_2 Enrichment on Crop Growth and Quality

CO_2 is a so-called greenhouse gas. When solar radiation hits the earth, a part of this radiation is reflected by the earth and leaves the earth's atmosphere for outer space. If this did not happen, then the earth would heat up. However, some of these reflected electromagnetic waves cannot leave the atmosphere because they collide with gases in our atmosphere, i.e. water (= clouds) or natural occurring greenhouse gases such as CO_2. As a result, they are reflected towards the surface of the earth. Anthropogenic activities, such as burning fossil energy sources (e.g. coal), the tilling of grasslands or the utilization of moor soils that would otherwise act as carbon source, release increasing amounts of CO_2 into the atmosphere. The atmospheric CO_2 concentration thus rises. This drives global warming as more and more of the energy-rich radiation that is reflected from the earth into the atmosphere collides with CO_2 and is sent back towards the earth surface. The earth heats up. Whereas in 1955, the CO_2 concentration of the atmosphere was approximately 315 parts per million volume (ppmV), the concentration rose above 400 ppmV in 2017 (Federal Environment Agency 2017) and is anticipated to exceed 600 ppmV by the year 2100. The consequences of climate change are expected to be far-reaching. One of these consequences might be considerable changes in the yield and quality of horticultural products because of increased free-air CO_2 concentrations. The effects of elevated free-air CO_2 concentrations have been studied intensively for almost 40 years. However, most of the testbeds that have been used to simulate elevated free-air CO_2 concentrations, such as growth chambers, greenhouses, field tunnels and field chambers, cause too many problems giving rise to artefactual experimental setups (Ainsworth and McGrath 2010; Weigel and Manderscheid 2012). These include changes in microclimatic conditions, edge effects or limited pot sizes, all of which adversely affect the effects of elevated free-air CO_2 concentrations (Van Oijen et al. 1999; Weigel and Manderscheid 2012). In particular, limited pot sizes can reduce plant growth and horticultural production and thus represses any realistic effects of elevated CO_2 concentrations (Ainsworth and Long 2005). At the beginning of the 1990s, the first so-called FACE systems, standing for free-air CO_2 enrichment, were established. These systems allow the investigation of the impact of elevated free-air CO_2 enrichments on plant growth under open-air and field conditions (Ainsworth and Long 2005; Weigel and Manderscheid 2012). Horizontal or vertical vent pipes are placed in a circle around the investigated plot in order to pump CO_2-enriched air or pure CO_2 gas

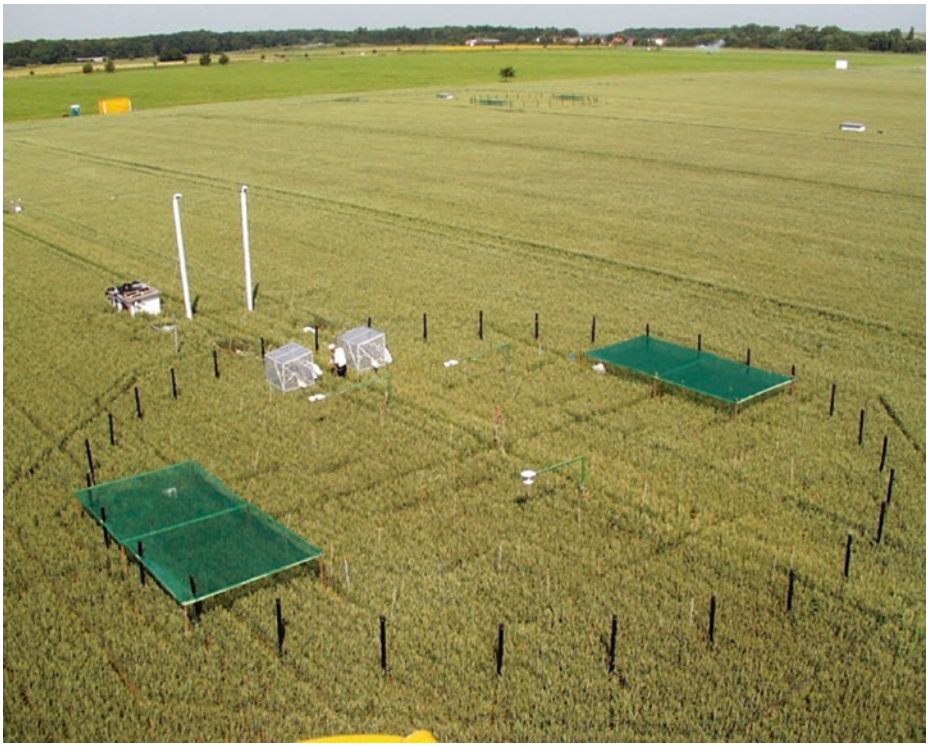

Fig. 14.2 A free-air carbon dioxide enrichment (FACE) experiment in Braunschweig, Germany. Winter wheat plants are exposed to elevated levels of CO_2 as they grow. These experiments allow the simulation of the effects of future atmospheric CO_2 concentrations on plant growth under field conditions. (Figure and figure legend taken and adapted from Weigel (2014). Open-access material distributed under the terms and conditions of the Creative Commons Attribution license (▶ http://creativecommons.org/licenses/by/4.0/))

into the experimental vegetation (Fig. 14.2) (Weigel 2014). Thereby, the free-air CO_2 concentrations are elevated up to levels of 475–600 ppmV. The size of the investigated plots ranges from 1–2 m in diameter (small-scale FACE rings) to 8–30 m in diameter (large-scale FACE rings) (Ainsworth and Long 2005).

Elevated free-air CO_2 concentrations have been shown to increase the growth and crop yields of many C_3 crops such as wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), soybean (*Glycine max* L.) and potato (*Solanum tuberosum* L.), because of the enhanced photosynthetic rates (Erbs et al. 2010; Nösberger and Long 2006). C_4 plants, such as sorghum (*Sorghum* spp.) and maize (*Zea mays* L.), do not show increases in yield under elevated CO_2 concentrations (Leakey et al. 2006). However, both crop types show optimized water use under elevated CO_2 concentrations as the plants open their stomata to a lower extent under elevated atmospheric CO_2 concentrations and, thus, transpire less (Katul et al. 2009; Manderscheid et al. 2014). Studies on the effects of elevated free-air CO_2 concentration on the yield and quality of horticultural crops are

limited. Bindi et al. (2001) investigated, with the help of a FACE system, the impact of elevated free-air CO_2 concentrations on the growth and quality of grapevines (*Vitis vinifera* L.). They found an increased fruit dry weight production and the stimulation of the production of acids and sugars, both of which are important quality determinants in grape production. However, during maturity stage, the effect of elevated CO_2 concentrations on the acid and sugar concentrations in grapes disappeared, and the final wine quality remained unaffected.

CO_2 enrichment in a controlled environment (without help of a FACE system) has revealed that flavonoid content in the medicinal plant *Scutellaria*, which belongs to the mint family, increases. In particular, the flavonoids scutellarein, baicalin and apigenin increase in concentration with increasing CO_2. Of note, the yield also increases (Stutte et al. 2008). Flavonoid content (kaempferol and fisetin), phenolic compound content (gallic acid and vanillic) and antioxidative capacity could be in increased in the rhizome of the medicinal vegetable plant Malaysian young ginger (*Zingiber officinale* Roscoe.) (Ghasemzadeh et al. 2010). The quality of *Hypericum perforatum*, known as perforate St. John's wort, can also be improved by CO_2 enrichment in a controlled environment. The amount of the principle medicinal components of St. John's wort, namely, hypericin and pseudohypericin, which are used in the treatment of neurological disorders, can be enriched under such conditions (Reddy et al. 2004).

In addition to the positive effects of elevated free-air CO_2 concentrations on C_3 crop growth, many studies indicate negative effects of elevated free-air CO_2 concentrations on crop quality. An increase of nonstructural carbohydrates, mainly sugars and starch, results in a decrease of nitrogen and protein concentrations (Loladze 2014). For instance, Taub et al. (2008) have shown a reduction of protein concentrations of about 14% in potato (*Solanum tuberosum* L.) tubers. This decrease in nitrogen and protein concentration is thought to be related to an impediment in nitrate assimilation (Dier et al. 2018). As described earlier, increased atmospheric CO_2 concentrations can lead to a reduction of photorespiration. During photorespiration, malate is transported from the chloroplasts into the cytoplasm. Here, it is oxidized to oxaloacetic acid in order to generate nicotinamide adenine dinucleotide (NADH). NADH empowers nitrate reduction. Thus, a reduction in photorespiration involves a lowering of nitrate reduction (Dier et al. 2018). In addition to decreases in nitrogen and protein concentrations, reductions in overall mineral concentrations such as those of potassium, calcium, iron and zinc have been documented in various plant species such as wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.) and rice (*Oryza sativa* L.) (Erbs et al. 2010; Wang et al. 2014). McGrath and Lobell (2013) have suggested that decreased crop mineral concentrations are related to decreased transpiration rates under elevated atmospheric CO_2 concentrations. The latter leads to a reduced mass flow of nutrients to the roots and thus to a lower nutrient uptake.

An overview of all previously discussed consequences of elevated atmospheric CO_2 concentrations on crop physiology, crop growth and crop quality is given in Table 14.1.

Table 14.1 Consequences (*positive* highlighted in green, *negative* highlighted in orange) of CO_2 enrichment in greenhouses, of CO_2 exposure after harvest and of climate change-driven free-air CO_2 enrichment of crop physiology, crop growth and quality

Consequences of …	Culture	References
… CO_2 enrichment in greenhouses		
Enhancement of photosynthesis, yield, flavonoid content (kaempferol and fisetin), phenolic compound content (gallic acid and vanillic) and antioxidative capacity	Ginger (*Zingiber officinale* Roscoe)	Ghasemzadeh et al. (2010); Ghasemzadeh and Jaafar (2011)
Enhancement in content of the flavonoids scutellarein, baicalin and apigenin	*Scutellaria*, belongs to the mint family	Stutte et al. (2008)
Enhancement in content of hypericin and pseudohypericin	St. John's wort (*Hypericum perforatum*)	Reddy et al. (2004)
Decline of net photosynthesis when CO_2 concentration of 600 ppm is exceeded	Strawberry (*Fragaria x ananassa* Duch. cv. 'Elsanta')	Keutgen et al. (1997)
… CO_2 postharvest treatment		
Delayed decay	Broccoli (*Brassica oleracea* var. *italic*)	Wang (1979)
Increased firmness	Strawberry (*Fragaria* × *ananassa* Duch., cv. Maehyang)	Hwang et al. (2012)
Reduced astringency	Persimmon (*Diospyros kaki* Thunb.)	Yamada et al. (2002)
Off-odour and off-taste	Strawberry (*Fragaria x ananassa* Duch. cv. 'Chandler')	Shamaila et al. (1992)
… climate change-driven free-air CO_2 enrichment		
Increase crop growth and yield	Wheat (*Triticum aestivum* L.)	Erbs et al. (2010)
Improved water economy	Maize (*Zea mays* L.)	Manderscheid et al. (2014)
Increase nonstructural carbohydrates	Grapevine (*Vitis vinifera L.*)	Bindi et al. (2001)
Decrease nitrogen and protein in plant-derived food products Decrease element concentrations in plant-derived food products	Potato (*Solanum tuberosum* L.) Rice (*Oryza sativa* L.)	Taub et al. (2008) Wang et al. (2014)

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Hormones

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15.1 Introduction

Plant hormones are molecules of widely different natures. They regulate plant growth and development either as part of the plant life cycle, namely, because of genetically programmed developmental changes, or in response to environmental stresses (Davies 2010). They play a fundamental role in the response of the plant to abiotic stress (Franklin 2008). These molecules can enhance or deter plant growth (Franklin 2008; Skirycz et al. 2009). They promote the production of other substances (e.g. proline, secondary metabolites, etc.) and enhance specific protective mechanisms (e.g. stomata closure) depending on the kind of environmental stress to which the plant is exposed (Wilkinson and Davies 2002). Numerous plant hormones are known. This chapter introduces five main plant hormones, namely, abscisic acid, auxin, cytokinin, ethylene and gibberellin, and describes the most important roles that they play within plant physiology.

15.2 Abscisic Acid

Abscisic acid (ABA) is generally known as an endogenous messenger for the regulation of diverse physiological processes (Kanchan Vishwakarma et al. 2017). ABA mainly controls seed dormancy, stomata closure and various signalling pathways for adjustments to drought stress, e.g. the activation of the anti-oxidative pathway for the synthesis of secondary metabolites such as flavonoids and anthocyanin (Tuteja 2007; Giribaldi et al. 2010). ABA is modulated by stress conditions, which activate the genes responsible for the ABA biosynthesis enzymes (Jiang and Zhang 2002). ABA induces stomata closure as one of the first responses to osmotic stress (such as induced water drought or high salinity). ABA also reduces plant growth, provokes senescence, induces ripening in fruits and is a potential candidate for communication between roots and shoots during osmotic stress. However, it also interacts with other plant signals during organ-to-organ communication (Vishwakarma et al. 2017).

External applications of ABA to plants cause responses that imitate those ensuing during stress and activate physiological pathways including the biosynthesis of secondary metabolites, such as flavonoids and anthocyanins. However, this can result in negative effects, e.g. stomata closure, reduction of nutrient uptake, deterrence of growth and development or osmotic imbalances, this last effect resulting in cell desiccation (Vishwakarma et al. 2017). Previous studies have demonstrated that external applications of ABA are useful in altering the quality of horticultural products. Villalobos et al. (2016) have shown an increase of red coloration in grape skin by the induction of flavonoid biosynthesis after ABA application. These results corroborate those of Macková et al. (2013) in cress (*Lepidium sativum* var. *capitata*). They applied 10 ml ABA solution at 10–1 mM to 3-day-old cress seedlings. After 7 days, the seedlings were harvested and examined. The results indicated a lower above-ground biomass, smaller leaf area, higher stomatal density (which increases water-use efficiency) and smaller stomatal aperture. Moreover, the polyphenol content of the cuticle wax was increased in treated plants.

15.3 Auxins

Auxins intervene in almost every characteristic of plant growth and development (Davies 2004). Auxins are transported cell to cell via specific protein carriers travelling from multiple cell sources (hundreds of cells) to small sinks, i.e. small groups or units of cells (Kramer 2015). Typical metabolic sinks for auxin are shoot apical meristems, lateral roots during growth, embryo cells during germination and growing cells above wounds (Kramer et al. 2007; Péret et al. 2013; Wabnik et al. 2013). Multiple kinds of auxins are known, with the most common form found in nature being indole-3-acetic acid (IAA). Auxin biosynthesis is highly complex, and different plant species can have different mechanisms, although they share some core pathways. Tryptophan (Trp) is the main source for auxin biosynthesis (Cheng et al. 2006; Stepanova et al. 2008; Zhao 2010). Moreover, auxins are reversibly converted to other chemical forms, called conjugates in several pathways. Conjugates are normally called 'stored forms' or 'bound auxins' and are considered to be the result of an auxin degradation mechanism (Andreae and Good 1955; Kramer 2015). The only conjugate that cannot be transformed back to its initial form is indole-3-acetyl-aspartate (IAASp). This is because IAASp cannot be hydrolysed by plants and therefore is considered as one of the auxin degradation products (Ostin et al. 1998). All these characteristics make the use of auxins attractive for enhancing plant growth, and similarly the use of amino acids, such as Trp, in order to facilitate the synthesis of these hormones in the plant.

Pramanik and Mohaphatra (2017) in their review concerning the roles of auxin in plants conclude that foliar applications of natural and/or synthetic auxins in tomato plants at various concentrations (10–50 ppm) at various developmental stages enhance early flowering, the number of flowers, fruit setting, biomass production and quality.

Sağlam et al. (2014) carried out an experiment in which various kinds of auxins (NAA, IBA and IAA) were applied to stem cuttings from the medicinal plant, the Antolina sage (*Salvia fruticosa* Mill). The cuttings were kept for 24 h in solutions of the three auxins at various concentrations and then transplanted into perlite medium under greenhouse conditions. After 1 month, those samples drenched in IAA were observed to have a statistically significant higher number of roots, whereas root weight and the number of roots were significantly higher at high concentrations of the three auxin types (240 ppm for NAA and IBA and 400 ppm for IAA).

In addition, the application of IAA at 1 μM can increase the content of volatile compounds, namely, aromatic compounds, in thyme plants (*Thymus vulgaris* L.) without changing qualitative characteristics (Affonso et al. 2009).

15.4 Cytokinins

Cytokinins play a crucial role in the regulation of environmental stress responses in cross talk with ABA (Wang et al. 2011). They regulate meristematic tissue development by enhancing cell division and morphogenesis. In addition, they delay senescence and induce flower and seed development, seed germination and the uptake and transport of

nutrients to sink organs (Zalabák et al. 2013). Moreover, changes in cytokinin levels modify plant stress tolerance (Albacete et al. 2008; Werner et al. 2010). Under stress situations, cytokinin levels and/or signalling can change depending on the stress intensity (Havlová et al. 2008). In other words, under short-term or mild stress, cytokinin levels might increase, whereas long-term or severe stress is associated with a reduction of growth and development activity and the relocation of energy sources and, therefore, the down-regulation of cytokinin signalling (Nishiyama et al. 2011; Albacete et al. 2008).

Chemically, cytokinins are low molecular weight compounds derived from adenine with bound isoprenoids or aromatic side chains (Werner and Schmülling 2009; Sukbong et al. 2012). Aromatic cytokinins are rarely found in plants, whereas isoprenoid cytokinins are more widespread (Strnad et al. 1997; Sakakibara 2006).

Every plant cell is capable of synthesizing cytokinins, although the strongest biosynthesis activity has been reported in roots, indicating a role for cytokinins in root growth and development (Miyawaki et al. 2004). Cytokinin biosynthesis is catalysed by isopentenyl transferases (IPTs), which use ADP, ATP and tRNA for the isopentenylation of adenine (Brugière et al. 2008). Cytokinin binds to receptors lying in the cell membrane and initiates a signal transduction cascade that leads to the activation of plant responses (Zalabák et al. 2013). Cytokinin degradation also plays an important role in plant physiological responses and is mediated by the cytokinin dehydrogenases, which are localized in the cytosol, vacuoles and apoplast (Smehilová et al. 2009; Kowalska et al. 2010). The overexpression of the enzyme cytokinin dehydrogenase decreases cytokinin levels and increases auxin content, inhibiting shoot development but enhancing root development (Werner et al. 2001, 2003), thereby increasing the capacity of the plant with regard to water and nutrient uptake.

Furthermore, cytokinins act as antagonists of ABA in various physiological responses (Sukbong et al. 2012). A reduction in cytokinin content is reported to lead to the up-regulation of ABA biosynthesis genes, whereas the opposite reaction, i.e. the down-regulation of ABA biosynthesis, promotes cytokinin production (Wang et al. 2011). This means that, for example, under drought stress, ABA levels would increase, as one of the main responses to osmotic stress, and that cytokinin levels would then be decreased.

An experiment performed by Sosnowski et al. (2017) is a good example of hormone application in controlled agricultural environments. In their experiment, alfalfa (*Medicago X varia T. Martyn*) seedlings were grown in pots under controlled environmental conditions and were sprayed with various plant hormones (auxin and cytokinin) at the six-leaf and at the first flower bud stages of development. The results showed a general increase in plant biomass for every plant organ, especially for stem length and root collar diameter, when auxins were applied at the six-leaf stage of development. The application of cytokinins, in general, increased the number of leaves and shoots but reduced inflorescence mass. On the one hand, carotenoid levels were increased by the application of cytokinins, whereas on the other hand, carotenoid levels were negatively affected by the application of auxins. These results lead to the conclusion that, in horticulture, hormone application at the correct moment and in suitable amounts can influence the product positively, because components such as carotenoids that promote human health can be enriched in plant-based foods.

Stancheva et al. (2010) recommend applications of the cytokinin 4PU-30 at 25 mg L^{-1} in combination with cytokinin DROPP at 100 mg L^{-1} to increase the production of 1,8-cineole and p-cymene in cineole-type spearmint plants (for use in essential oils, i.e. gums).

15.5 Ethylene

Ethylene is known as a multifunctional plant hormone controlling growth and senescence. It also controls the development of leaves, flowers and fruits and promotes the induction or inhibition of senescence depending on the ethylene level within the plant (Pierik et al. 2006; Nazar et al. 2014). The major precursor of ethylene is S-adenosyl methionine (S-AdoMet), which is a key intermediate in the reversible biosynthesis of the amino acid methionine (Met) (Kende 1993). Fiorani et al. (2002) showed that leaf growth was inhibited by the application of exogenous ethylene in slow-growing species of the Poaceae plant family (*Poa alpina* L. and *Poa compressa* L.), whereas at the same concentration of applied ethylene, leaf development was only slightly inhibited in fast-growing species of the Poaceae family (*Poa annua* L. and *Poa trivialis* L.). Moreover, reduced growth attributable to environmental stress has been related to the existence of reactive oxygen species (ROS) and nitric oxide (NO) in leaf tissue; these species are up-regulated by the presence of ethylene in plant tissue (Wilkinson and Davies 2010). However, ethylene is one of the most important hormones related to leaf senescence (Iqbal et al. 2017). Its biosynthesis rate is higher at the first stages of leaf formation, then declines until leaf maturity and finally increases again during senescence, a process that leads to the activation of nutrient recycling from old leaves to young tissues (Iqbal et al. 2017). Ethylene plays a similar role in flower senescence, fruit ripening and senescence, by interrupting the consumption of nutrients from dying tissue and redirecting these nutrients to organs with higher metabolic activity. Trivellini et al. (2011) have reported that the exogenous application of ethylene or of its biosynthetic precursor accelerates senescence in China rose flowers (*Hibiscus rosa-sinensis* L.).

During fruit ripening, ethylene regulates a cascade of biochemical processes that affect crop organoleptic characteristics (Barry and Giovannoni 2007) enhancing colour and taste by the production of carotenoids, anthocyanin contents, sugars and volatile organic compounds (VOCs) (Iqbal et al. 2017). However, in most cases, the reduction of ethylene production in crops is undertaken in order to increase the shelf-life of the harvested product. Ullah et al. (2016) have shown that the inhibition of ethylene production in the plant by the application of 1-MCP (1-methylcyclopropene), after harvest, reduces weight loss and softening and maintains sugars and organic compounds in the harvested fruit.

15.6 Gibberellins

The gibberellins (GAs) are a hormone group involved in the modulation of growth during environmental stress (Colebrook et al. 2014). They play a role in controlling cell elongation and division (Sponsel and Hedden 2010). They also promote plant develop-

ment in transition phases such as seed germination, stem elongation, leaf expansion, flowering, trichome development and pollen maturation (Achard et al. 2009). This means that plant genotypes incapable of producing GAs will exhibit limited development (Griffiths et al. 2006). Moreover, GAs play a major role in plant fertility, since they are relevant for the regulation of stamen elongation and the development of pollen tube growth (Hedden and Thomas 2012).

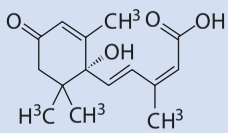
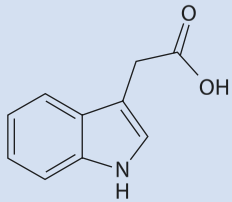
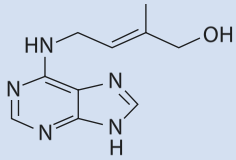
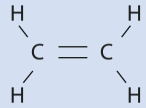
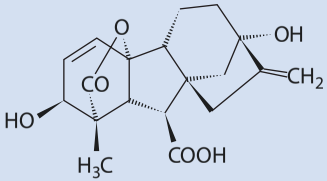
Chemically, GAs are tetracyclic diterpenoid carboxylic acids (Table 15.1), which are biosynthesized from trans-geranylgeranyl diphosphate in plastids via the methylerythritol phosphate pathway (Kasahara et al. 2002). Furthermore, GAs are synthesized at their site of action, namely, growing meristems (Kaneko et al. 2003). More than 130 different species of GAs have been identified in the plant, fungi and bacteria kingdoms, although only a few are biologically active (Yamaguchi 2000). Most of the non-bioactive GAs are precursors for bioactive species or are deactivated metabolites (Davière and Achard 2013). GAs are normally used in agriculture in order to manipulate flowering and fruit setting (Leite et al. 2003). Birnberg and Brenner (1987) and later King et al. (2001) have reported that the application of GA before flowering results in a decreased number of flowers and therefore fruits, whereas biomass is increased. Pal et al. (2018) recommend the combined application of gibberellic acid at 0.010 g/L with potassium at 5 g/L in foliar applications for cucumber (*Cucumis sativus* L.) in order to improve total soluble sugars and shelf-life.

In another example, a study by Leite et al. (2003) demonstrated the effects of using GA and cytokinin in soybean (*Glycine max* L.) crops at vegetative stage. Soybean seedlings were grown in a pot experiment under controlled conditions. The treatment comprised seed coating with 50 mg/l GA solution and two foliar applications of a solution composed of 100 ml /lf GA and/or 30 mg/lf cytokinin. The number of seedlings that emerged 15 days after GA treatment was lower than for the control plants (not treated with GA), suggesting a delay in seed germination under GA treatment. Foliar application of GA resulted in the enlargement of plant tissue regardless the presence of cytokinin. Leaf area was increased for all the treatments, but the number of leaves did not differ between the control and treatment. In addition, thickening of the roots was observed, although this might have been a symptom of the increase of ethylene production caused by an increase in the production of 1-aminocyclopropane-1-carboxylic acid (ACC) (Kaneta et al. 1997).

Thus, the application of GAs in horticulture can enhance biomass production and can be used in order to increase the size of root crops such as potatoes or celery root. Moreover, GAs can be applied in order to increase the amounts of essential oils of medicinal plants as has been demonstrated by Povh and Ono in 2007 in *Salvia officinalis* L. species after the application of 100 mgL^{-1} GA.

The main functions of plant hormones and their potential application in farming systems and horticulture are summarized in Table 15.1.

Table 15.1 Summary of concepts for the use of hormones in horticultural systems

Name	Abscisic acid	Auxins	Cytokinin	Ethylene	Gibberellin
Chemical formula					
Functions in plants	Stress regulation Stomata closure Induction of seed dormancy Activation of the antioxidant system Reduction of growth Senescence Ripening of fruits	Enhances plant growth and development Present in shoot apex, lateral roots and embryo cells	Stress regulator Cell division and morphogenesis Delayed senescence, flower induction and seed development Seed germination Transportation of nutrients to sink organs	Controls growth and senescence Up-regulation of ROS and NO Nutrient translocation to younger tissues	Growth regulator during stress Cell elongation and division Promotion of plant development in transition phases
Biosynthesis	In every plant tissue Capability of transport in the plant body Biosynthesis activated during environmental stress	Produced in multiple tissues and then translocated to sink organs	Derived from adenine Strongest biosynthesis activity in roots The presence of ABA down-regulates its biosynthesis	Biosynthesis rate is high at the beginning of tissue formation and during organ senescence	Its biosynthesis occurs in plastids Biosynthesis at the site of action (growing meristems)
Use in horticulture	Increase of secondary compounds in plant tissue Improved red coloration in grape	Enhances fruit setting and diameter Accelerates flowering Increases lateral root growth	Enhancement of vegetative growth	Enhancement of organoleptic characteristics in fruits	Manipulates flowering and fruit setting Increases plant biomass Increases oil content in medicinal plants

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Intercropping

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16.1 What Is Intercropping and Why Is It Done?

If two or more crops are cultivated together, then the plant communities will interact with each other. The outcome of these interactions can be neutral, antagonistic (competitive) or synergistic (facilitative; beneficial). Interactions that are advantageous for all interaction partners are of a facilitating nature. Competition, on the other hand, tends to limit the growth of the non-dominant species or of both interacting plants (Ehrmann and Ritz 2014). Intercropping refers to the simultaneous cultivation of two or more crops in the same (or slightly offset) place at the same time with the aim of taking advantage of any beneficial effects that the one crop species imposes on the other(s). Intercropping can be applied to any crop: vegetables, medicinal plants, field crops, pasture species, trees or a combination thereof[1] (Ehrmann and Ritz 2014).

Intercropping, viz. the combined cultivation of two or more different crops, can be considered as a controllable production system in both protected horticulture (e.g. under foil or glass) or under field conditions. Reasonably applied, intercropping has many benefits:

Improvement of quality, e.g. by increasing the content of titratable acids and vitamin C in tomato (*Solanum lycopersicum* L.) or glucosinolates in Ethiopian kale (*Brassica carinata*) (Liu et al. 2014; Ngwene et al. 2017). Titratable acids are relevant for taste, whereas vitamin C and glucosinolates can promote health benefits to consumers. Glucosinolates that are released into the soil can also decrease the pressure of soilborne pests (Hanschen et al. 2015).

Stronger plant growth and higher yield, e.g. by intercropping eggplant (*Solanum melongena* L.) with garlic (*Allium sativum* L.) (Wang et al. 2015).

Higher yield stability, based on compensation of, for example, drought- or pest-/pathogen-induced yield losses of crop A by the cocultivated crop B. Here, crop A usually has the higher yield potential under optimal growing conditions but is more sensitive to stress. Crop B cannot produce high yields under optimal conditions but can withstand stress better (Dodiya et al. 2018).

Higher net income, based on a lower requirement of fertilizers or a high (= better) land equivalent ratio (LER), e.g. in intercropping peppermint (*Mentha x piperita* L. CV. Mitcham) with soybean (*Glycine max* L. CV. Williams). This positive effect has been confirmed by a median LER of 1.17 in a meta-analysis based on 100 studies with various horticultural and medicinal crops (Martin-Guay et al. 2017; Amani Machiani et al. 2018).

16

The land equivalent ratio (LER) is the relative land area that is required under solo cropping to produce the yield that can be achieved under intercropping. For example, an LER of 1.3 means that you need 30% more land in solo cropping than in intercropping to achieve the same yield.

1 Tree-based intercropping systems are referred to as alley cropping or agroforestry (Ehrmann and Ritz 2014).

Improvement of soil fertility, e.g. lowering of the levels of soil acidification and salinization (Ehrmann and Ritz 2014; Wu et al. 2016). Both soil acidity and salinity hamper root growth and interfere with nutrient uptake. Salinity can also cause cellular toxicities (see ▶ Chap. 7).

Effective pest and diseases control, e.g. intercropping with marigold (*Tagetes patula* L.) can decrease damage from the carrot rust fly (*Psila rosae*) or nematodes by attracting predators and the release of nematicidal metabolites (Ehrmann and Ritz 2014; Jankowska et al. 2012).

Improvement of the utilization (use efficiencies) of resources, e.g. water, light and nutrients, because of the different plant architectures and resource demands (Ehrmann and Ritz 2014).

However, all these advantages are also associated with some drawbacks. First, harvest and weed control are more complex and more difficult to mechanize. Second, care must be taken that no incompatible plants are combined. The combination of incompatible crops species can reduce yield and quality (Tringovska et al. 2015). This usually occurs because of the competition for light, water or nutrients. Therefore, a knowledge of (in)compatible crop combinations is important (see ◘ Table 16.1).

16.2 Intercropping Patterns and Plant Cultivation Measures Affecting Plant-Plant Interactions

Intercropping can be achieved in horticulture by two approaches.

- The first and most common way is to plant one or more main crops that are intercropped with one or more secondary crops that provide at least one of the benefits mentioned above. This means that, for example, the second crop, which might be garlic (*Allium sativum* L.), improves either the quality attributes or growth of the main crop, which might be tomato (*Solanum lycopersicum* L.). This method can also improve soil fertility (Liu et al. 2014).
- The second way is to cultivate two or more main crops such as lettuce (*Lactuca sativa* L.) and onion (*Allium cepa* L.) that influence each other positively. This can result in the improved quality of all main crops and/or in improved soil fertility (Kapoulas et al. 2017).

Four main intercropping patterns are available: row, stripe, mixed or relay intercropping, as shown in ◘ Fig. 16.1 (Ouma and Jeruto 2010; Martin-Guay et al. 2017).

Additional factors to the spatial arrangements shown in ◘ Fig. 16.1 will affect the way in which intercropped plants interact with each other and their environment:

- Optimal crop density (i.e. the amount of space that the crop needs for optimal growth and the design that suits the crops best: replacement[2] or additive[3]; Vandermeer 1989). In general, adequate space must be provided for each crop in order to trigger any beneficial effects and to reduce competition (Ouma and Jeruto 2010).

2 Replacement design means that a certain amount (based on sole crop densities) of the one crop is replaced by the second crop.

3 Additive design means that at least one of the crops has the same density as in solo cropping.

Table 16.1 Examples of compatible and incompatible crops

Main crops	Secondary crops
Tomato (*Solanum lycopersicum* L.)	++Garlic (*Allium sativum* L.)[1], ++basil (*Ocimum basilicum* L.)[3], ++marigold (*Tagetes patula* L.)[3], §bean (*Phaseolus vulgaris*), §cabbage (*Brassica oleracea*)[9], §lettuce (*Lactuca sativa* L.)[8], —white mustard (*Sinapis alba* L.)[3]
Cucumber (*Cucumis sativus* L.)	+Garlic[2], +onion (*Allium cepa* L.), §lettuce
Garlic	++Tomato[1], +cucumber[2], +mustard (*Brassica napus*)[7], §cabagge[5], §broccoli (*Brassica oleracea* var. *italica*)[5], §cauliflower (*Brassica oleracea* var. *botrytis*), §eggplant (*Solanum melongena* L.)
Lettuce	§Cucumber, §eggplant, §cauliflower, §marigold, §tomato[8]
Onion	+Cucumber, +mustard[7], §cabbage[5], §cauliflower, §pepper (*Capsicum annuum*)[9], §broccoli[5]
Eggplant	+Onion, §bean, §cos lettuce (*Lactuca sativa* L. var. *longifolia*), §leaf lettuce (*Lactuca sativa* L. var. *crispa*), §cowpea (*Vigna unguiculata* Walp ssp. *sesquipedalis*), §pepper[9], §garlic
Cauliflower	++Legume (*Fabaceae s.l.*), §cos lettuce (*Lactuca sativa* L. var. *longifolia*)[6], §leaf lettuce (*Lactuca sativa* L. var. *crispa*)[6], §bean [6], §onion[5, 6], §beet (*Beta vulgaris*), §carrot (*Daucus carota* ssp. *sativus*), §coriander (*Coriandrum sativum*), §garlic[5], §leek (*Allium ampeloprasum*)[5]
Cabbage	+Bean, §tomato[9], §garlic[5], §onion[5], §pepper[9], §leek[5]
Pepper	+Ginger (*Zingiber officinale*)[9], §eggplant[9], §okra (*Abelmoschus esculentus*)[9], §marigold[9], §bean[9], §onion[9], §cabbage[9], §marigold
Peppermint (*Mentha piperita* L.)	++Soybean (*Glycine max*), ++faba bean (*Vicia faba* L.)[4]
Mustard	+Garlic (*Allium sativum* L.)[7], +onion (*Allium cepa* L.)[7], —tomato[3]
Soybean	++Peppermint
Faba bean	++Peppermint[4]
Bean	+Pepper[9], §tomato, §eggplant, +cabbage
Marigold	++Tomato[3], §pepper[9], §lettuce
Broccoli	§Garlic[5], §onion[5]

Sources: [1] Liu et al. (2014); [2] Xiao et al. (2013); [3] Tringovska et al. (2015); [4] Amani Machiani et al. (2018); [5] Ünlü et al. (2010); [6] Yildirim and Guvenc (2005); [7] Sarker et al. (2007); [8] Filho et al. (2011); [9] Kahn (2010)
§ compatible but not supportive, + supportive, ++ improves quality, — incompatible

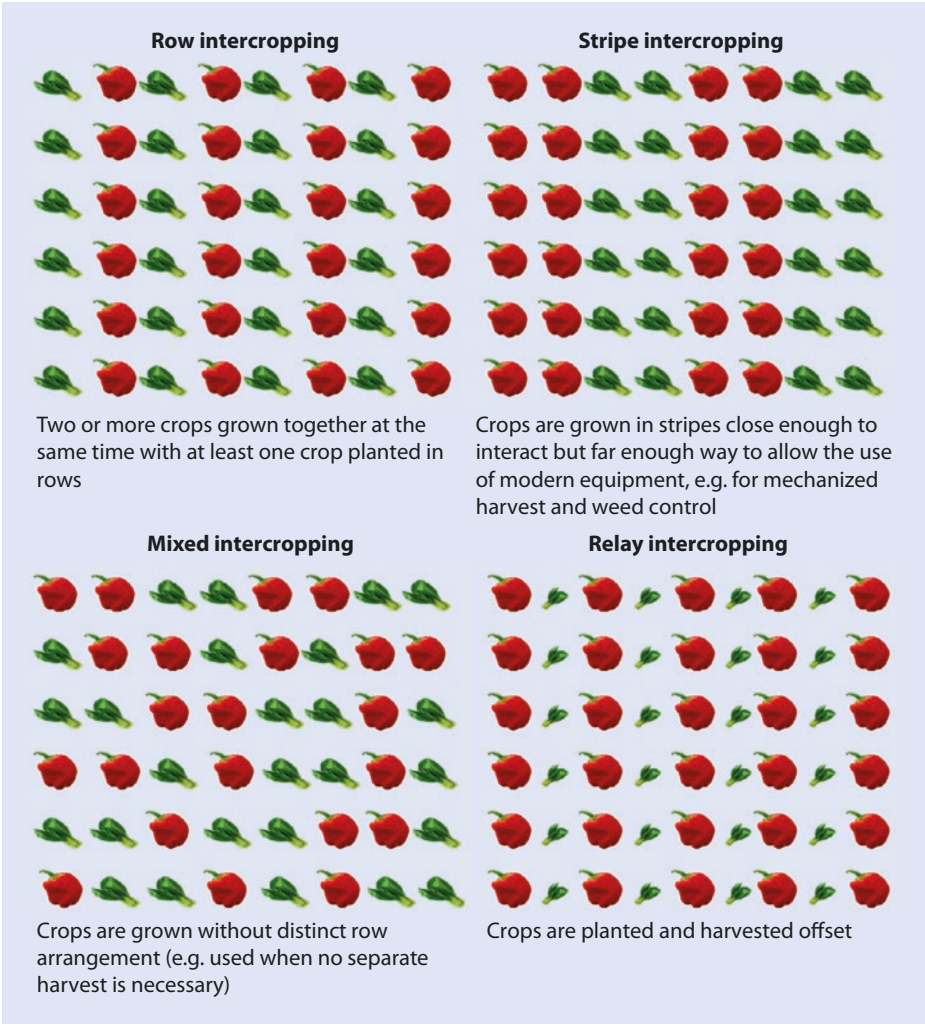

Fig. 16.1 Intercropping patterns as adopted from Ekanayake et al. (1997) (Hiddink et al. 2010)

- Maturity dates of the crops (e.g. the time at which crops are flowering or ready for harvest). This is important, for example, for planning intercrops with staggered maturity dates or developmental periods. Since the maximal requirements of the cocultivated crops do not coincide with regard to time, crops do not compete for resources at the same time and are therefore used more efficiently (Ouma and Jeruto 2010).
- Architecture/vigour of the crop (e.g. the height or width of the plant, whether the plant deep-rooting or shallow-rooting). For the ideal use of resources, different architectures of crops are beneficial, e.g. tomato (tall plant with deep roots) can be complemented by garlic (small plant with shallow roots) because the garlic plants use resources from the upper soil horizon, whereas the tomato plants also root in deeper horizons. This is referred to as niche complementarity (Brooker et al. 2015).

Table 16.2 Examples of physiological and morphological changes/reactions in intercropping

Physiological change/reaction	Morphological change/reaction
Release of allelochemicals (definition given below) (Cheng et al. 2016) Increasing activity of antioxidative enzymes (Ahmad et al. 2013) Accumulation of human health-promoting plant metabolites (Ngwene et al. 2017) Release of volatile organic compounds (VOCs) and root exudates (Pierik et al. 2013) Alteration in chlorophyll content (Ahmad et al. 2013) Alteration in uptake of plant mineral nutrients (Xiao et al. 2013)	Shade avoidance (Pierik et al. 2013) Adaptation of root architecture (Belter and Cahill Jr 2015) Increased or inhibited growth in certain organs of the plant, such as leaves or stem (Wang et al. 2015) Promotion/suppression of cell division (Cheng et al. 2016)

16.3 What Happens to the Plant When Plants Are Intercropped?

Intercropping can have markedly different effects on plant physiology and morphology (see Table 16.2).

Such effects emerge either indirectly by the change of abiotic or biotic environmental factors (e.g. soil attributes or biota) or directly by plant-plant interactions.

In intercropping, the direct effects of plant-plant interactions can be the result of a recognition process that involves the perception of signals that are emitted from a neighbouring plant. Such a signal can be a metabolite that is (1) segregated as a root exudate (Bais et al. 2006) or (2) released as a volatile organic compound (VOC) (Pierik et al. 2013). However, perception of a neighbouring plant can also be based on processes that are unrelated to molecules, e.g. by the recognition of changes in the light spectrum (shading; Pierik et al. 2013) or by mechanical factors (de Wit et al. 2012) possibly even including acoustic signals[4] (Gagliano et al. 2012). An overview is given in Fig. 16.2.

Root exudates are substances produced by roots and secreted into the rhizosphere. Root exudates can be of a diverse nature. They include proteins, soluble sugars, amino acids, organic acids, fatty acids, sterols, enzymes and phenols (Bais et al. 2006; Dennis et al. 2010).

16

4 Gagliano et al. (2012) have reported the reaction of young roots of maize (*Zea mays*) to a continuous 220 Hz tone, whereby the root tip clearly benty towards the sound source.

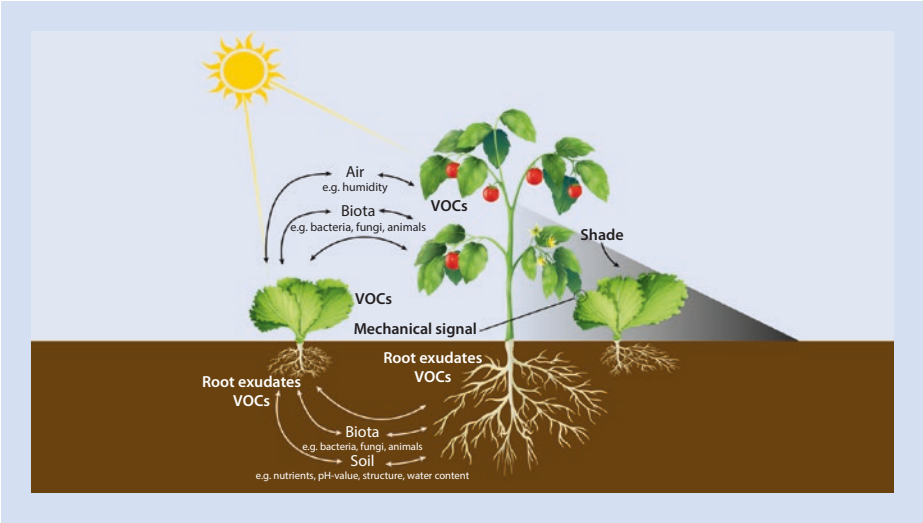

Fig. 16.2 Scheme showing direct interactions and indirect interactions (by influencing biota, air or soil) in intercropping. *VOCs* Volatile organic compounds. (©blueringmedia and ©shaitan1985 – ► stock.adobe.com (the license for the Adobe Stock Images is an "adobe stock standard license" and is part of an Adobe Stock subscription. Illustration created by Adrian Vollmer with modified Adobe stock images. ©Adrian Vollmer)

Volatile organic compounds (VOCs) are defined as any organic compound that can be vaporized into the atmosphere under normal air pressure (Dicke and Loreto 2010). Biogenic (plant produced) VOCs are released from above- and belowground tissues and range from small molecules, such as ethylene or methanol, to relatively large, carbon-rich VOCs such as mono- and sesquiterpenes (Pierik et al. 2013).

After the plant has perceived the presence of a neighbouring plant, the plant can react and take measures to grow. For instance, plants can induce defensive strategies such as positioning their own leaves at a different angle in order to avoid the shade from the other plant (shade avoidance). Offensive strategies are thought to inhibit the performance of proximate competitors (e.g. allelopathy) (Kegge and Pierik 2010).

Allelopathy is the release of secondary metabolites, also called allelochemicals, produced by living organisms such as plants (but also by fungi or bacteria) and secreted into the environment and includes their impact on other organisms. Effects can be negative or positive. A negative effect induced by allelochemicals is the inhibition of growth (usually at high concentration). A positive effect is any improvement of growth (usually at low concentration) (Ahmad et al. 2013). The underlying mechanisms are not

yet fully understood, but recent research indicates that allelochemicals increase, for instance, the activity of antioxidant enzymes and stimulate root growth and chlorophyll synthesis by so far unknown mechanisms (Ding et al. 2016; Ahmad et al. 2013). Some secondary metabolites are thought to act as both allelochemicals and plant hormones (Yamada et al. 2010).

One example of an allelochemical is diallyl disulphide (DADS). It is a volatile organosulfur compound derived from garlic and is known to be responsible for the strong allelopathic potential of garlic (Cheng et al. 2016). The influence of DADS on tomato root growth depends on its concentration: it stimulates at low concentrations but inhibits at higher concentrations, probably by altering the content of phytohormones (indole-3-acetic acid (IAA), zeatin riboside (ZR) and gibberellic acid (GA)) (see ► Chap. 15).

Another reaction that arises as a result of the detection of a neighbouring plant is the adaptation of root architecture (Kegge and Pierik 2010). For instance, plants that are cropped together with other plants are thought to be able to extend their roots away from the roots of neighbouring plants into nutrient-/water-rich areas that have not yet been occupied by their neighbours (interception) (Pierik et al. 2013). This is most probably one of the mechanisms that leads to a better acquisition and uptake of nutrients and water.

In addition to these direct effects, we have to consider indirect intercropping effects. These effects are caused when the one plant influences/changes abiotic and/or biotic environmental factors, which have consequences for the other plant. Such effects include the mobilization of phosphorus (P) from organic soil compounds through the activity of segregated plant enzymes (Dakora 2003), the change of the chemical attributes of the soil (e.g. pH value) or the support of soil biota, which promotes or impairs the companion plant. Other examples are the regulation of pest populations (through trap crops[5], disruptive crops[6] or the promotion of antagonists) (Vandermeer 1989) or a maize-legume intercropping system, in which the symbiosis between legumes and nitrogen-fixing bacteria reduces atmospheric nitrogen to mineral nitrogen that can be used by the maize (Yu et al. 2010).

At present, many aspects of the way that plants interact with each other or their surroundings remain to be elucidated. This is probably related to the large number of possible crop combinations multiplied by the large number of the ways that crops can influence one another and interact with each other. Further research is thus required.

16

16.4 How Can Intercropping Be Used to Improve the Quality of Horticultural Crops Without Decreasing Yield?

Successful experiments focused on quality improvements through intercropping have been conducted with promising results.

5 Trap crops attract pests and therefore entice them from the main crop.
6 Disruptive crops disrupt the ability of a pest to efficiently attack/find the main crop.

16.4.1 Improving Quality of Greenhouse Tomato Plants by Intercropping

Tringovska et al. (2015) carried out an experiment in which marigold (*Tagetes patula* L.), basil (*Ocimum basilicum* L.), lettuce (*Lactuca sativa* L.) or white mustard (*Sinapis alba* L.) was intercropped with greenhouse tomato plants (*Solanum lycopersicum* L. var. Dimerosa). During the experiment, two harvests were conducted. Without decreasing yield, the quality-related attributes of tomato fruits including antioxidant content were positively affected by the companion crops in most cases. However, the data were inconsistent between the two harvests. Only basil led to a significant increase of antioxidants at both times.

16.4.2 Improving Essential Oil Quality and Yield of Peppermint Intercropped with Soybean

According to the results of Amani Machiani et al. (2018), intercropping peppermint (*Mentha x piperita* L. CV. Mitcham) with soybean (*Glycine max* L. CV. Williams) can improve the quality of peppermint essential oil by increasing the content of menthol and decreasing the content of the undesirable menthofuran. In addition to the improvement of quality, an LER of 1.46 was achieved. This was attained by planting peppermint and soybean in rows of 3:2 with a distance of 45 cm between them. The optimum density was considered to be 45 plants m^{-2} for soybean and 12 plants m^{-2} for peppermint. Amani Machiani et al. (2018) postulated that the reason for the improvement was that the plants complemented each other leading to a better utilization of resources and therefore to an increase in the number of leaf gland cells. However, a lack of evidence and understanding of the underlying mechanisms remains. Further research is needed to elucidate this topic.

16.4.3 Improving Quality by Intercropping Ethiopian Kale and African Nightshade

The work of Ngwene et al. (2017) has revealed that intercropping with African nightshade (*Solanum scabrum*) increases the total content of glucosinolates in Ethiopian kale (*Brassica carinata*) while maintaining biomass production and the content of other human health-promoting minerals in both plants. When the primordial leaves of the plants were fully established, they were planted at a distance of 15 cm between them. After 6 weeks, they were harvested.

Glucosinolates are secondary metabolites that are produced by the plant and whose enzymatic degradation/breakdown is triggered by damage to the cells (e.g. by predators, cutting, boiling) to drive off/deter predators. They can be broken down into glucose, sulphate and *isothiocyanates*, thiocyanates or nitriles. *Isothiocyanates* possess anticancerogenic, antimicrobial and antihydroid effects.

The authors assumed that the overlap of the roots and the subsequent defence reaction were the reasons for the increase of aliphatic glucosinolates. However, further research needs to be conducted to establish this postulate.

In summary, little is known about the interrelations of intercropping (and the underlying mechanisms). However, intercropping is clearly an ingenious way to improve the quality of our food.

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Exercises

Turning theory into practice is of the utmost importance if lasting learning success is to be guaranteed. In ► Chap. 3, experiments and exercises are introduced that can be conducted by students or growers in order for them to enrich the nutritive value of their vegetables. The mechanistic knowledge that has been introduced in ► Part II concerning ways in which production factors (e.g. potassium) influence crop physiology is applied in experiments (e.g. the quality improvement of French fries by the optimized potassium fertilization of the potatoes from which they are made).

Contents

Acrylamide Concentrations of Deep-Fried Potatoes

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C.-M. Geilfus, *Controlled Environment Horticulture*,
https://doi.org/10.1007/978-3-030-23197-2_17

17.1 Introduction

A high consumption of acrylamide is assumed to increase the risk for various types of cancer such as breast or ovarian cancer (Pedreschi et al. 2013). Therefore, acrylamide concentrations are regulated in the European Union, beginning in 2017, for products such as wheat-based bread (50 µg kg^{-1}), roasted coffee (400 µg kg^{-1}) or ready-to-eat French fries (500 µg kg^{-1}) (CEC 2017). Gerendás et al. (2007) have described a positive correlation of nitrogen (N) fertilization and the concentration of asparagine and reducing sugars in potatoes, whereas increasing potassium (K) fertilization ameliorates the accumulation effects (see also ▶ Chap. 6). Both asparagine and reducing sugars are precursors for the formation of acrylamide, which is produced during the deep-frying of potatoes (Pedreschi et al. 2013). The following experiment was designed to demonstrate the effects of N and K fertilization on the acrylamide content of deep-fried potatoes, as based on the work described by Gerendás et al. (2007).

17.2 Materials

The materials used are presented in ◘ Table 17.1.

◘ **Table 17.1** Materials and tools

Materials	Chemicals
Potato tubers (planting material)	NH_4NO_3
Boxes or egg cartons	K_2SO_4
Mitscherlich pots – 10 l (12)	$Ca(H_2PO_4)_2$
Sand (grain size ≥0.2 mm)	
Perlite	$MgSO_4$
Knives	Fe-EDTA
Flasks/beakers	$MnSO_4$
Scale	$ZnSO_4$
Magnetic mixer	$CuSO_4$
Heating unit	H_3BO_3
Thermometer	$(NH_4)_6Mo_7O_{24}$
Pot suitable for frying	$CaCO_3$
Deionized or distilled water	
Fat or oil for deep-frying	

17.3 Methods

The experiment is motivated by the work of Gerendás et al. (2007). The experiment design is presented in Fig. 17.1.

1. Plant cultivation – preparation of potato tubers
   A minimum 12 tubers of the chosen potato variety should be prepared. The tubers should be of a similar size and weight. Only healthy planting material should be used. The tubers are placed in boxes or in egg cartons, which are then stored at an ambient room temperature of 15–20 °C under natural light conditions. After 2 weeks, the first green sprouts should appear. The tubers can be planted at this stage. The pre-sprouting of the tubers is not mandatory for the experiment's success, but it accelerates the experiment/growing time. As a result, the potatoes should be ready for harvest earlier than the untreated ones (Hagman 2012).
2. Preparation of fertilizer solutions
   In total, four different fertilizer solutions have to be prepared: the first basic fertilizer solution (BFS) contains all the nutrients but lacks N and K. The other three nutrient solutions each contain different rations of N:K, each. The BFS solution is the base for mixing the other ones. This is done by adding the respective amounts of N and K to the BFS solution.
   2.1. Preparation of BFS
   A beaker is filled with 300–500 ml distilled water and is placed on a magnetic mixer. The fertilizer salts are weighed (see Table 17.2, "Nutrient amount in g per pot") individually and are added successively to the water in the beaker. The

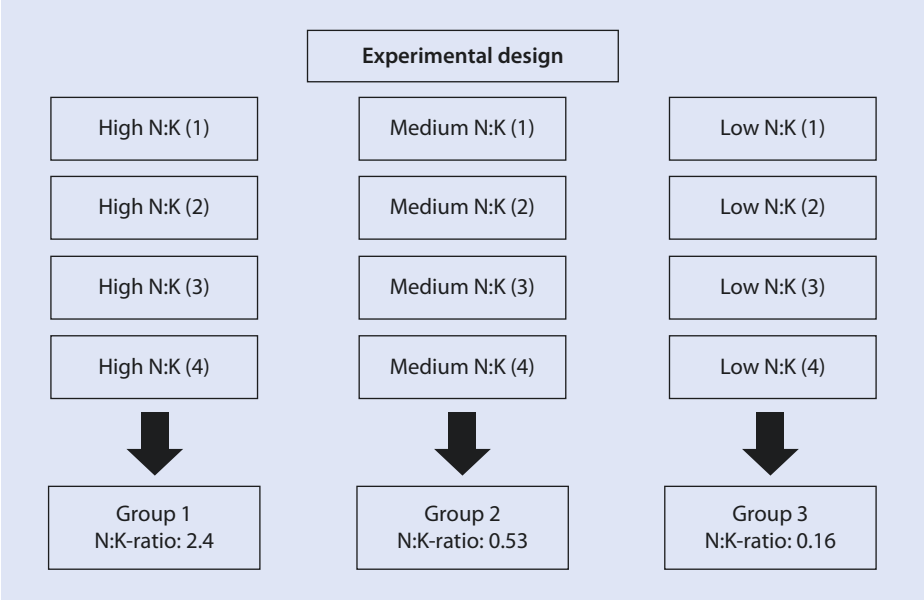

Fig. 17.1 Experiment design; N, nitrogen; K, potassium

Table 17.2 Fertilizer salts and the respective nutrients, fertilizer salt amounts (FA), nutrient amounts (NA) and conversion factors (CF) for the preparation of the basic nutrient solution that lack N and K

Salt	Amount of applied salt in g per pot	Nutrient	Nutrient amount in g per pot	Conversion factor
$Ca(H_2PO_4)_2$	2.267	P Ca	0.600 0.388	0.26467 0.17124
$MgSO_4$	2.971	Mg S	0.600 0,792	0.20192 0.26639
Fe-EDTA	Depends on product	Fe	0.025	Depends on product
$MnSO_4$	0.0412	Mn	0.015	0.36383
$ZnSO_4$	0.0123	Zn	0.005	0.40508
$CuSO_4$	0.0075	Cu	0.003	0.39814
H_3BO_3	0.0343	B	0.006	0.17484
$(NH_4)_6Mo_7O_{24}$	0.0035	Mo	0.002	0.57706

The CF is the calculated quotient of the nutrient's molar mass proportion of the salt and the salt's molar mass. Formula: FA = (NA ∗ 12)/CF

weights need to be multiplied by 12 to obtain the total weights for the basic nutrient solution for 12 pots. Salt residues should be washed down from the tools into the mixing container by using deionized or distilled water. Afterwards, the solution is topped up to 1200 ml with water. To simplify the process, an appropriate stocking solution can be prepared beforehand. To make the stock solution, the used salts are diluted in a smaller amount of water at a ratio respective to the final basic nutrient solution. Subsequently, the stock solution can be diluted with distilled water to obtain the basic nutrient solution. The amount of iron (Fe)-EDTA has to be calculated for the specifically used product, as the products on the market contain Fe at various concentrations. In Table 17.2, the Fe amount per pot is listed as 25 mg per pot. If the Fe-EDTA salt contains, for example, 13.3% Fe, the amount of applied fertilizer salt per pot (FA) would be 187.970 mg (FA = 25 mg/(13.3%/100)).

2.2. Preparation of the other three nutrients solution that differ in the N:K-ration
The principles for the preparation of the NKS are the same as those for the preparation of the BFS (be aware that you have to combine the BFS as given in Table 17.2 with the respective amount of N and K as given in Table 17.3). For each N-K solution, a beaker is filled with 300–500 ml distilled water and placed on the magnetic mixer. The fertilizer salts are weighed individually and added successively to the water in the mixing container. The respective weights for the used salts per pot for the NKS are given in Table 17.3 under

Table 17.3 Fertilizer salts and respective nutrients, fertilizer amounts (FA), nutrient mounts (NA) and conversion factors (CF) for the preparation of the N-K solutions

N-K ratio	Salt	Amount of applied salt in g per pot	Nutrient	Nutrient amount in g per pot	Conversion factor
High (2.4)	NH_4NO_3	6.858	N	2.400	0.34998
	K_2SO_4	2.228	K	1.000	0.44874
Medium (0.53)	NH_4NO_3	4.572	N	1.600	0.34998
	K_2SO_4	6.685	K	3.000	0.44874
Low (0.16)	NH_4NO_3	2.286	N	0.800	0.34998
	K_2SO_4	1.1142	K	5.000	0.44874

The CF is the calculated quotient of the nutrient's molar mass proportion of the salt and the salt's molar mass. Formula: FA = (NA ∗ 12)/CF

'Nutrient amount in g per pot'. The weights have to be multiplied by 4 to obtain the weights for the N-K solutions for four pots. Be aware that three NKS have to be prepared! Salt residues should be washed down from the tools into the mixing container by using deionized or distilled water. Subsequently, the solutions are topped up to 1200 ml with water.

3. Substrate mixing, pot filling, planting and nutrient application
   Nine parts of sand are mixed with one part of perlite and 1.0 g of $CaCO_3$. The Mitscherlich pots are filled with the obtained substrate leaving about 2 cm at the top edge of the pots. Afterwards, one tuber per pot is planted approximately 10 cm underneath the substrate surface. Every pot is watered with 100 ml BFS. Subsequently, 300 ml of the specific N-K solutions is applied to four pots each (as given in Fig. 17.1). The arrangement of the pots should be randomized to exclude experimental bias.
4. Plant watering
   Drainage of water through the pot base is undesirable in order to avoid nutrient losses. Therefore, irrigation should be based on the plants' demands. Any drainage water that arise should be collected in the pot saucer of the Mitscherlich pots and recirculated into the pots.
5. Preparation of potatoes for deep-frying
   Once the potato tubers have been harvested, they should be cleaned and peeled. Chips with a surface area of 9 × 9 mm and with the same length are cut from the potatoes.
6. Deep-frying of the potato chips and comparison
   Deep-frying fat or oil is heated to 125 °C, and portions of 115 g of potato chips per N-K solution are deep-fried for 2 minutes. Afterwards, all samples are once again deep-fried for 7 minutes at 175 °C. Finally, the colour of the chips from the different groups of N-K solutions can be compared. Higher acrylamide concentrations are expected to lead to browner chips (Fig. 17.2).

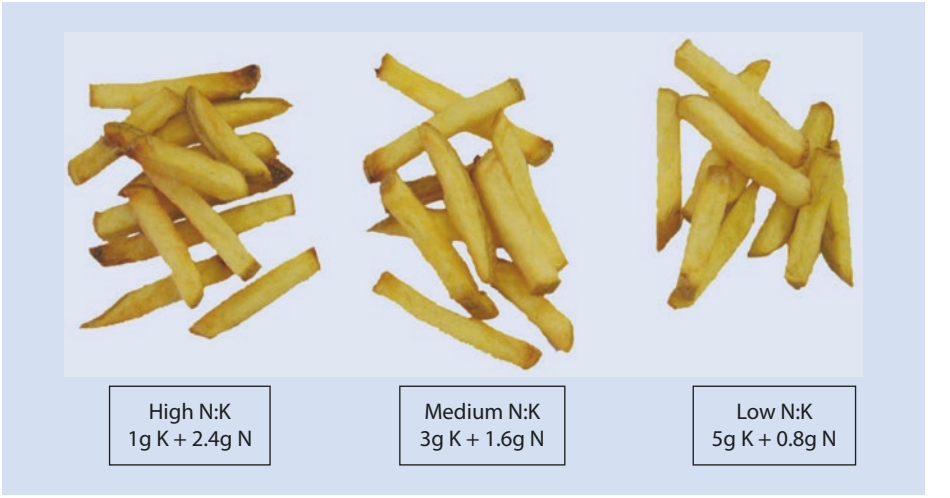

Fig. 17.2 Browning of fries from potatoes grown under various N-K ratios. The fries were deep-fried under identical conditions. (Picture from Jóska Gerendás)

17.4 Expected Results

The fries made from potatoes of the variants treated with higher N-K ratios should show a more intensive brown colour after deep-frying (Fig. 17.2), with the more intensive browning of the fries being linked to a higher acrylamide concentration. According to Gerendás et al. (2007), the production of acrylamide during deep-frying is attributable to the higher concentration of reducing sugars and asparagine under high N and low K fertilization (see ▶ Chap. 6). Both asparagine and reducing sugars are known to be precursors for the emergence of acrylamide.

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Enrichment of Anthocyanin in Pak Choi

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18.1 Introduction

Salt stress triggers the formation of reactive oxygen species in plants. This is because the salt ions (i.e. Na^+ and Cl^- in the case of NaCl-salinity) accumulate excessively in the chloroplast and mitochondria of the leaves where they damage proteins and membranes. In consequence, electron transport chains are disturbed, and electrons reduce O_2 to the superoxide radical ($O_2 \cdot^-$) (Dietz et al. 2016). Light is an important controllable factor for this experiment as the illumination of the plants with artificial light fosters the reduction of the photosynthetic electron flow, viz. it increases the reduction state of the redox components of the light reaction of photosynthesis. The more reduced the photosynthetic electron transport chain, the more radicals will be produced in response to salt stress. For the detoxification of these radicals and for the avoidance of excess radical production, plants produce and accumulate anthocyanins in, for example, epidermal cells. Anthocyanins are not only beneficial for the plant itself (Landi et al. 2015). As part of the human diet, they can have health-promoting effects (Miguel 2011) (see ► Chap. 7). Thus, the horticulturist is interested in enriching vegetables in anthocyanins. This can be achieved via the induction of a mild, short and tightly regulated salt stress in combination with high light exposure. However, stress intensity (NaCl dose and stress period) needs to be carefully adjusted in order to avoid biomass reduction.

18.2 Experimental Design

Pak Choi (*Brassica rapa chinensis*) plants are grown in a hydroponic culture system for 4 weeks. Starting at week 3, plants are stressed four times for 12 hours by the addition of 75 mM NaCl into the nutrient solution. This measure induces the biosynthesis of anthocyanins. After 12 hours of stress treatment, the nutrient solution is renewed, without the addition of NaCl. Light intensity is also increased during the stress period, as this promotes the over-reduction of the photosynthetic electron transport chain under conditions of salinity, thereby intensifying the biosynthesis of anthocyanins.

18.3 Materials and Methods

Plant Cultivation Pak Choi plants are grown hydroponically in climate chambers (14/10 h day/night; 20 °C/15 °C; 250 µmol m^{-2} s^{-1} light intensity; 60% relative humidity). Seedlings are germinated on moistened sterile quartz sand or perlite. After 8 days, the seedlings are transferred to 4 l plastic pots containing 25% of the full nutrient solution concentration. After 2 days of cultivation, the concentration of nutrients is increased to 50% of full concentration and, after 4 days of cultivation, to 100% of full nutrient concentration. The nutrient solution has the following composition at 100%: 0.1 mM KH_2PO_4, 1.0 mM K_2SO_4, 0.2 mM KCl, 2.0 mM $Ca(NO_3)_2$, 0.5 mM $MgSO_4$, 60 µM Fe-EDTA, 10 μM H_3BO_4, 2.0 μM $MnSO_4$, 0.5 μM $ZnSO_4$, 0.2 μM $CuSO_4$ and 0.05 μM $(NH_4)_6Mo_7O_{24}$. The solution is changed every 5 days to avoid nutrient depletion.

Stress Treatment Plants are cultivated for 4 weeks. Stress is imposed during the last 2 weeks. For temporarily stressing the plants with NaCl, a 5 M NaCl stock solution has to be prepared. An aliquot of this solution is added to the container with the nutrient solution to give a final NaCl concentration of 75 mM. In order to remove the NaCl after 12 hours, the nutrient solution is changed completely. This treatment is repeated three times. Additional lightning is necessary during the salt stress phases; light intensity should be increased from 250 to 400 μmol m^{-2} s^{-1}. This is repeated four times during plant cultivation. Control groups are not stressed by salts and high light.

Evaluation The accumulation of anthocyanins can be monitored both visually and analytically. Visual symptoms can be readily seen because the white leaf stalks of Pak Choi turn a reddish colour (see ▶ Chap. 7). This is because the anthocyanins are pigments that appear red or blue. For the analytical evaluation, plant material is extracted with weakly acidified alcohol-based solvents. Paper chromatography is an easy method for the separation and visualization of anthocyanins (for details, see Ishikura et al. 1978).

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Improving Flavour of Tomatoes

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19.1 Introduction

In human nutrition, tomato (*Lycopersicon esculentum* L.) fruits are an adequate source for important secondary metabolites such as lycopene, β-carotene, ascorbic acid or polyphenols that are antioxidatants (Lai et al. 2007). Consumers demand aromatic and tasty tomatoes, in addition to their health-promoting aspects. The sugar/acid ratio of fruits characterizes their flavour and is an indicator for their ripeness. Unripe fruits have a low sugar/acid ratio because of their high acid and low sugar content. During the ripening process, the acidity decreases, and the sugar content increases (OECD 2009). Environmental factors can influence plant growth, fruit development, plant health, the pattern and composition of secondary plant substances or flavour-active compounds. For instance, drought stress can decrease the yield of tomatoes on the one hand, while it has the ability to increase several fruit quality parameters such as lycopene content, on the other hand. Indeed, drought stress provokes a reduced uptake of N, Na, K, S, Ca and Mg in tomato fruits but can also increase the content of secondary metabolites (e.g. flavonoids and phenols), sugar content and the sugar/acid ratio leading to an improvement of the tomato flavour quality (Nahar and Gretzmacher 2002; Lahoz et al. 2016; Wang et al. 2011; Sánchez-Rodríguez et al. 2011). The challenge for the horticulturist is to induce a drought event in order to adjust the metabolism towards the production of the wanted secondary metabolites; however, stress must be gentle, as otherwise yield will be reduced.

19.2 Principle

This exercise gives practical guidance for implementing a 'drought stress treatment' experiment involving the use of perlite with a water content of 50% of the maximal water-holding capacity (WHC). A well-watered control is used for comparison; this is set to 85% of the maximal WHC. The exercise is conducted on tomato plants grown in a greenhouse (Fig. 19.1). The drought stress event is anticipated to increase total soluble solids, titratable acidity and the sugar/acid ratio in the tomato fruits, which is reflected as an improvement of the flavour of the tomato fruits.

Fig. 19.1 Tomato seedlings in rock wool cubes and mats with supporting laces and irrigation dippers

19.3 Materials

Plant material, 25 indeterminate tomato seeds; *for green house set-up*, 20 rock wool cubes (approx. 10∗10 cm); perlite (size 0–6 mm); 5 rock wool mats (100∗20∗8 cm) for 'standard irrigation'; 5 rock wool mats (100∗20∗8 cm, filled with perlite) for 'drought stress treatment'; tape; drain gutter/gullies each row; a dripping irrigation system (dripper, tubes, pump, tank; see ◘ Fig. 19.2); nutrient solution (see ◘ Table 22.1); additional lighting in case that it is too dark; and potassium hydroxide (KOH) and nitric acid 53 % (HNO_3) for pH value regulation; *for plant treatment*, hanging appliance; string and plastic clips for plant training; and pruning shears; *laboratory equipment*, EC/pH meter; homogenizer; a filter (muslin cloth or fine filter); refractometer (°Brix); distilled water; a 0.1 M NaOH solution for measurement of titratable acidity; gram scale; pH meter; burette and burette clamp (25 or 50 ml); magnetic stirrer and stir bar or automatic burette/titration console; pipettes (10 ml); and glass beaker (250 ml).

19.4 Plant Cultivation

Details for plant cultivation can be found in ▶ Sect. 22.2.3.

1. Cultivate 20 (+5 replacement plants) seedlings in germination soil.
2. For 'drought stress treatment', remove rock wool fibre from 5 of the 10 mats. Fill empty mats with Perlite (0–6 mm) and close mats with tape. Use untreated rock wool mats for 'standard irrigation'.
3. Place all 20 transplanted plants (in moistened cubes) on the mats (see ◘ Fig. 19.1). Attach plants to a hydroponic system, for example, as shown in ◘ Fig. 19.2 (e.g. row distance, 100 cm; plant distance, 50 cm). Randomize the position of both variants by changing positions of the mats ('standard irrigation' and 'drought stress treatment').

◘ **Fig. 19.2** Illustration of greenhouse set-up. Cultivation of indeterminate tomatoes

4. Provide optimal watering for rock wool-cultivated tomato plants, with the nutrient solution and explanation that you can find in ▶ Sect. 22.3.4, ◘ Table 22.1. Use two drippers per cube. The lack of water in the 'drought stress treatment' will be generated by lowering the water content of the perlite to a value of 50% of the maximal WHC.
5. *Pruning/nipping*: this measure is necessary for good plant handling, larger fruits and better light penetration and air circulation. Side shoots have to be pinched out twice a week. Only the main stem, their leaves and panicles remain (Naika et al. 2005). The pruning of the trusses to the same fruit number (e.g. seven) is important for achieving similar fruit size (Heuvelink 1996). *Trimming leaves:* remove yellow and older leaves for controlled development, better health control and larger fruits. Weekly cut or break out two to three leaves beneath the first developing panicle infructescence. Wait for the first trimming until there are enough leaves (15–16, fully developed) for photosynthesis. At the harvest point, the fruits should hang free (◘ Fig. 19.3). *Stem support*: a support by laces (easy wrapping of plants) or by clippers increases the yield and size of tomatoes. Hang down the plants about 20–30 cm, once a week, and position the plants that have become too long carefully to one side of the row. When the plants reach the edifice limit, head the stem above the last panicle and its first leaf. While treating the plants, vibrate them regularly for better pollination. Pay attention to hygienic care during treatment. Operate with clean hands, shears and gloves and with clean equipment and tools. The risk of a bacterial infection is present (Laber and Lattauschke 2014; Naika et al. 2005). Cultivate the plants until you are able to reap red fruits for at least 6 weeks in series to obtain sufficient data. In order to carry out this exercise, you need at least 10–12 fructified panicles per plant.

19.5 Sample Analysis

Preparation of Samples For 16 samples, harvest 8 tomatoes (same ripeness grade from the lowest truss) weekly from both variants ('standard irrigation' and 'drought stress treatment') in a randomized manner. Cut each tomato into pieces, homogenize and filter the mash separately. Additionally, harvest unripe fruits for a comparison with ripe fruits.

Measurement of Total Soluble Solids The refractometer measures the total soluble solids (TSS) in °Brix (equivalent to %) as a parameter for the sugar content. The detected sugars include mainly sucrose, fructose and glucose. Clean and calibrate the refractometer with distilled water before you measure a new sample. Use one drop of the juice from each sample for determination of °Brix values. Calculate the mean value for each variant, viz. drought versus well-watered plants (and optionally ripe versus unripe fruits) (OECD 2009).

Measurement of Titratable Acidity The titratable acidity represents the total amount of acids (malic acid, tartaric acid, citric acid) in fruit juices. By means of a chemical process, titration enables the determination of the proportion of acid in a sample, by using NaOH

Fig. 19.3 Greenhouse-cultivated tomatoes

as a counteractive reagent of the substance. For the potentiometric method, use 10 ml of tomato juice per sample. Transfer juice plus 50 ml distilled water with a pipette into a glass beaker. The calibrated pH meter is used to measure the pH of the juice-water-liquid, while the magnetic stirrer mixes the two solutions. The KOH in the burette will be slowly titrated into the glass beaker until the pH value is at 8.1. At this point, the titration will be stopped immediately. The titre (consumed NaOH in ml) will be recorded. The titratable acidity of the sample can be calculated with the value of titre and the milliequivalent factor of citric acid (0.0064) and the volume of tomato juice sample (10 ml) (OECD 2009; Suhl 2014) by using this equation:

$$\text{Titratable acid}(\%) = \frac{\text{NaOH Titer}(\text{ml}) \times 0.0064 \times 100}{10(\text{ml of juice sample})}$$

All devices should be cleaned with distilled water before starting a new measurement to avoid cross-contamination.

Calculation of Sugar/Acid Ratio The sugar/acid or °Brix/acid ratio classifies the degree of ripeness and flavour of the fruits. The value increases during the ripening process. It can be calculated by using this equation (OECD 2009):

$$\text{Sugar / acid ratio} = \frac{\text{total soluble solids}(\%)}{\text{titratable acid}(\%)}$$

Repeat the procedure weekly and discuss your results in the context of (1) the total soluble solids, titratable acidity and sugar/acid ratio induced by drought stress, (2) the drought stress-induced changes in the colouration of the fruit and (3) optionally the ripeness. In addition, plant growth and yield quantity of both variants can be recorded and compared. You can also evaluate the taste of the tomatoes.

Expected Results The drought stress variants should show both reduced vegetative growth and yield. Drought stress-induced differences in the total soluble solids, titratable acidity and the sugar/acid ratio are anticipated. Because of the higher TSS and sugar/acid ratio, fruit quality, flavour and taste (sweetness) might be improved as a result of the well-controlled lack of water. Drought stress is expected to result in the faster red colouration and ripeness of the tomato fruits.

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Biofortification of Carrots

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20

Many women and preschool children are deficient in one or more of the following minerals: calcium, iron, iodine (I), magnesium, selenium (Se) and zinc (Graham et al. 2007; White and Broadley 2005; Nestel et al. 2006). All these nutrients can be obtained by humans when they consume plant-based foods. Biofortification can be used to increase the amount of these nutrients in fruits and vegetables (see ▶ Chap. 13). The aim of the experiment in this chapter is to increase the content of Se and I in carrots (*Daucus carota*).

20.1 Principle

An aqueous Se or I solution is sprayed onto the leaves of carrot plants. The nutrients will enter the plant through the open stomata.

A wetting agent is used to reduce the surface tension of the sprayed solution, allowing efficient and maximized contact between the leaf surface and the spray solution. This leads to better access of the nutrients. After the nutrients have entered the cells, Se and I can be transported within the plant to the root. High air humidity is required during the spray applications to ensure open stomata and a longer retention time of the spray on the leaf surface because of reduced evaporation.

20.2 Materials

Container for growing; sandy or loamy soil; carrot seeds; spray bottles; iodine (I) fertilizer, e.g. KIO_3; selenium (Se) fertilizer, e.g. Na_2SeO_3; wetting agent/surfactant, e.g. Tween 80® or Silwet®.

20.3 Plant Cultivation

Fill the growing containers with suitable soil, namely, sandy or loamy soil, for good growth of the carrot storage root. Soil compaction is unwanted as it reduces the root growth of the carrot storage root. Carrots may take 2–4 months to mature. Make sure that the carrots do not experience stress. Many types of stress, especially heat and drought stress, lead to closed stomata, which hamper the uptake of the sprayed solution.

20.4 Preparation of the Spraying Solution

Prepare two separate solutions, namely, a 40 mM KIO_3 and a 6 mM Na_2SeO solution. Shake well to dissolve the salts. Add a wetting agent, e.g. 0.01% (v/v) Tween 80® or 0.1% (v/v) Silwet®. Shake gently after you have added the surfactant; otherwise excess foam is formed.

20.5 Conducting the Experiment

Divide the plants into three groups. One group will receive the iodine biofortification treatment, the second the selenium biofortification treatment, whereas the third is a control group. Spray the control group of carrots with water and surfactant only. Start the treatment when five to six leaves have emerged. Spray twice a week for at least 6 weeks. At each application, add approximately 2 g of the solution to each plant. Check this by weighing the container with the solution before and after spraying. Use the same distance between the sprayer and the plant leaves at each application. This is particularly important for achieving reliable results, viz. in order to apply the same amount of nutrients onto the leaf surfaces of plants derived from the different experimental groups. Treatments should take place in the morning, as the stomata are usually open at that time; however, low ambient humidity might thwart these plants, since it can result in closed stomata. Do not spray when the sunlight exposure is too high as the plants might be damaged. Spray both sides of the leaves, and prevent the solution from dripping into the soil by covering the soil surface. A temporary cover, for example, with paper is suitable. Make sure that, during spraying, the solution does not drift away from the plant because of windy conditions.

20.6 Evaluation of Results

I and Se content can be measured via various methods. We recommend the use of inductively coupled, plasma mass spectrometry (ICP-MS). ICP-MS is a type of mass spectrometry that is capable of detecting metals and several non-metals at extremely low concentrations.

20.7 Expected Results

The experimental groups that received a biofortification should have increased contents of either I or Se in the storage root when compared with the control group. This is because root vegetables such as carrots use the root as a storage organ. As a result, such treated carrots will provide increased benefits for human health.

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Enrichment of Flavonoids in Lettuce

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21.1 Introduction

21

Adaptation to stress involves the synthesis of plant hormones that induce metabolic responses to help plants to survive under that stress. Notably, the exogenous application of plant hormones on non-stressed plants can change plant growth, yield and the composition of plant metabolites (see ▶ Chap. 15) (Franklin 2008; Skirycz et al. 2009). This knowledge can be utilized to manipulate crops to accumulate favourable secondary plant compounds, even if no real stress event is present. A real stress event would be problematic as it might reduce biomass (Wilkinson and Davies 2010; Geilfus 2019). In this chapter, flavonoid production is induced in plants through the exogenous application of abscisic acid (ABA). For this, lettuce plants (*Lactuca sativa* L. var. Descartes) will be produced hydroponically in a nutrient film technique system (NFT) (see ▶ Chap. 4).

21.2 Plant Growth and Cultivation

Lettuce seeds should be allowed to germinate in perlite until the first two leaves are visible. In order to encourage germination, the perlite should remain moist. Later, the new plants are picked and deployed in rock wool cubes (7.5 cm × 7.5 cm; Cutilene®; Tilburg, the Netherlands). The lettuce plants should be grown in the greenhouse compartment of an experimental Venlo-type greenhouse by using NFT for 6 weeks at 10–17 °C. The greenhouse compartment should contain a total of two gullies for three different ABA treatments (three experimental groups), each gully having a length of 6 m and an inclination of 1% (◘ Fig. 21.1). The second gully is used as a replicate. Each gully should contain 15 lettuce plants at a distance of 0.4 m from each other, and the gullies should be wrapped with a white plastic film, in order to avoid the spread of algae and surface evaporation. A tank with a capacity of 300 l nutrient solution is placed at the end of each gully (◘ Fig. 21.1). The nutrient solution is based on the recipe of Hochmuth (2001):

200 mg nitrogen (N) l^{-1}(14.18 mM)
62 mg phosphorus (P) l^{-1}(2.00 mM)
150 mg potassium (K) l^{-1}(3.83 mM)
210 mg calcium (Ca) l^{-1}(5.14 mM)
50 mg magnesium (Mg) l^{-1}(2.05 mM)
70 mg sulphur (S) l^{-1}(0.002 mM)
2.5 mg iron (Fe) l^{-1}(0.0447 mM)
0.62 mg manganese (Mn) l^{-1} (0.011 mM)
0.03 mg molybdenum (Mo) l^{-1} (0.0003 mM)
0.09 mg zinc (Zn) l^{-1}(0.013 mM)
0.50 mg cooper (Cu) l^{-1} (0.007 mM)
0.44 mg boron (B) l^{-1}(0.040 mM)

Fig. 21.1 NFT system attached to water container (*left*); NFT system nutrient solution entry (*right*)

For optimal growth conditions, the nutrient solutions are adjusted with mineral fertilizer to an electrical conductivity of 2 dS m^{-1}.

The gullies are flushed with this nutrient solution at a flow rate of 600 l h^{-1} for 24 h each day, and the drainage solution is completely cycled. To achieve this kind of fertilization, a conventional pump (EHEIM universal 600, EHEIM GmbH & Co. KG; Deizisau, Germany) can be used.

21.3 Experimental Design and ABA Application

Plants should be grouped in order to set up three different treatments (T) (T0 = 0 mM ABA, this is the control; T1 = 0.01 mM ABA; T2 = 0.04 mM ABA). Each dosage of ABA is applied to five different lettuce plants. In detail, ABA is exogenously applied onto the leaves every 84 h, with a total of nine applications from the beginning to the end of the experiment. Each plant receives 0.8 ml of the ABA spray solution per treatment. To facilitate ABA plant uptake, the ABA solutions need to be prepared with 1‰ of a wetting agent (Silwet®L-77, PhytoTechnology Laboratories®; 14610 W. 106th St Lenexa, KS 66215). Harvest the plants 1 day after the last application to ensure high values of flavonoids in the plant tissue. If you wait any longer, the flavonoids might be degraded, as plants do not really need such high quantities because of to the absence of real stress.

21.4 Non-invasive Measurements During Experiment and Flavonoid Detection

Lettuce head diameter (cm), biomass (fresh and dry weight) and flavonoid content can be analysed after 6 weeks of growth. Lettuce heads should be weighed in order to obtain their fresh weight. Afterwards, they should be sliced into quarters. Three quarters should be immediately frozen at −80 °C with liquid nitrogen for the analysis of flavonoids. The remaining quarter is then weighed and subsequently dried in an oven at 105 °C for 24 h to calculate the fresh and dry mass. The frozen samples are freeze-dried in order to obtain dehydrated samples without the risk of degrading the flavonoid. After being dried, the samples should be ground. Flavonoids are extracted from the weighed samples and quantified via HPLC (High-Performance-Liquid-Chromatography) by using, for example, the methods of Förster et al. (2015).

21.5 Expected Results

Flavonoid content should increase in proportion to the amount of ABA applied, namely, the highest flavonoid content should be detected for the 0.04 mM ABA treatment. Since ABA has the function of slowing down growth under stress, the 0.04 mM ABA variant should show growth reduction. Experiment can also be done with reddish leafy vegetables. Since these plants have the genetic capacity to express more flavonoids, results are expected to be more prominent.

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Effect of Germination Substrates on Tomato Plants

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22.1 Introduction

Well-developed and strong plant seedlings are a prerequisite for high-quality yields. Weak seedlings form smaller plants produce lower crop quantity and lead to later harvest dates. Modern closed-system intensive horticulture allows the continuous production of high-quality tomatoes. However, care must be taken that a planned nutrient deficiency does not disturb plant development during germination and seedling emergence, finally endangering yield quantity and/or quality. Thus, not only the mature plant but also the seedling must be supplied with a sufficient amount of nutrients. Seedlings should therefore be produced under controlled conditions (Domínguez et al. 2014; Gruda 2005). In particular, growth-relevant properties of the growing substrate have to be optimized. Attributes such as water-holding capacity (WHC), nutrient content and nutrient availability exert crucial influences on germination and seedling development (Roldán and Soto 2005).

Here, we outline two practical exercises to illustrate both short-term and long-term effects of different propagation substrate compositions on the phenotype and yield of tomato plants (*Solanum lycopersicum* L.). For this experiment, perlite, potting soil and a perlite-potting soil mixture are used from sowing until the transplantation and cultivation of the plants in a closed rock wool-based hydroponic system. The impact of these different substrates on seedling development will be evaluated based on a comparison of several growth parameters and the quality-related attributes of the seedlings and fully developed crops.

22.2 Analysis of the Short-Term Effect of Various Germination Substrates on Seedling Development

22.2.1 Principle

This experiment aims to investigate the influence of various growing substrates on seedling vigour; this effect is attributable to the different nutrient availabilities of the substrates, while irrigation is carried out only with nutrient-low tap or rain water. The exercise takes about 3 weeks: from the date of sowing until the plants have reached the seedling stage. The protocol provides guidance for the greenhouse cultivation of 30 tomato seedlings on three different growing substrates ('perlite' 100%; 'potting soil' 100%; 50%:50% (v/v) 'substrate mixture' of perlite and potting soil). We will explain the way that the comparative growth parameters should be measured and describe a simplified procedure for testing the strength of both salinity and acidity (pH Value) in soil. The importance of salt content and pH for plant growth are explained in more detail in ▶ Chaps. 6 and 7. In brief, the electric conductivity (EC) reveals information about the sum of ions, viz. nutrients, in the rooting medium. The pH is critical for the mobility of the nutrients.

Fig. 22.1 1-week-old tomato seedlings, germinated in various substrates ('potting soil', 'perlite', 'substrate mixture')

22.2.2 Materials

For cultivation: 30 tomato seeds (e.g. indeterminate vine tomato); 30 small plant pots with a diameter of 6 cm; 'potting soil' as conventional propagation/gardening substrate (mixture of diverse components such as peat, clay, composted bark; see Gruda 2005); 'perlite' (size 0–6 mm); 'substrate mixture' of potting soil and perlite (50%: 50%, (v/v)); irrigation water (tap or rain water). *For measurements*: caliper, ruler, EC/pH metre, 4 beakers (>200 ml).

22.2.3 Seedling Cultivation

1. Prepare 10 pots per substrate type.
2. Sow one seed in each pot. Irrigate the substrate before and after sowing for easier moisturization (Naika et al. 2005).
3. Put the pots in a random order (Fig. 22.1), and place them in a bright setting without direct sunlight; at 14 °C min. to 30 °C max.; use artificial lightning if necessary (at least 50 µmol m^{-2} s^{-1} measured on the top of the plants and turned on for 18 h per day^{-1}; Laber and Lattauschke 2014; Krug et al. 2002). Optimal germination temperature: 25 °C for about 5 days. Cultivation temperature until start of harvest: day 19–20 °C/night 17–18 °C (Laber and Lattauschke 2014).
4. Water all pots regularly and uniformly for 3 weeks until the seedlings have developed the first three true leaves.

22.2.4 Measurements

Plant Growth Throughout the growth period, phenotypic appearance such as variation in the colour of the leaves (e.g. discoloration) or habitus of the seedling should be documented. Additionally, plant height (daily growth rates), the number of true leaves

(no cotyledons), plant diameter (from top view; leaf tip to leaf tip) and stem diameter (by caliper) of the 2- and 3-week-old plants should be measured and recorded (Roldán and Soto 2005).

Assessment of Substrate Effects on soil pH and EC Values An EC metre reveals information about the salt content of the nutrient solution (electric conductivity, 1 EC = 1 mS/cm), viz. allows an assessment of the ion concentration of the nutrient solutions. A pH metre enables measurements of the pH (acidity/alkalinity; pH range: 0–14). The pH is relevant for the availability of the nutrients. For tomato, an optimal pH range is 5.5–6.8 (Naika et al. 2005), and an optimal EC of the nutrient solution is 2.5–5.0 mS/cm, depending on development stage (Lattauschke 2004).

1. Fill four beakers with 150 ml irrigation water (tap water or rain water).
2. Add 5 g of each substrate into three of the cups.
3. Stir for 1 min measure the pH and EC values of all four cups.
4. Stir again and repeat the measurements after 30 min and 60 min.

22.2.5 Evaluation

As mentioned above, the EC value is indicative of the amount of nutrients in the substrate solution. The pH value is also critical for the supply with nutrients as both availability and uptake of nutrients depends on pH. The amount of available nutrients in the substrate solution is estimated by subtracting the EC of the pure tap or rain water from the EC of the tap water or rain water that was mixed with the substrates and is expressed in mS/cm. The higher the EC, the more nutrients are released from the substrates. The pH value is also anticipated to be affected by the substrates. Almost no salts or nutrients will be dissolved from the inert perlite substrate. Thus, neither salt content nor pH value will change. This is however thought to change with an increasing content of 'potting soil'.

What outcome can be expected from the experiment? Nutrient differences of the substrates will be reflected by differences in the development of the tomato seedlings (see ◘ Fig. 22.2).[1] The nutrient-free perlite may cause severe deficiency symptoms in young plants. One effect might be that perlite-grown plants show discoloured dark-green leaves with a purple-reddish coloration on the underside, as an indication of P deficiency (◘ Fig. 22.3) (see ▶ Sect. 6.2). Moreover, a bright and pale discoloration on the tops of the leaves might be indicative of a nitrogen (N) deficiency (▶ Sect. 6.1) (Laber and Lattauschke 2014; Sonneveld and Voogt 2009). In total, the plants with the highest nutrient supply ('potting soil'), as estimated by EC, should grow better than those with the lowest EC ('perlite'). Since P and N are critical for growth and biomass formation, the perlite-treated plants are anticipated to show the most reduced biomass.

1 A specific detailed analysis by the method "Inductively Coupled Plasma Emission Spectroscopy" (ICP-OES) offers valuable clues regarding individual nutrients and components/primary plant compounds (N, P, K, Ca, Mg, Fe, etc.) in the substrates (Bettinelli et al. 2000; Dannehl et al. 2015).

Fig. 22.2 Comparison of 3-week-old seedlings, grown in 'substrate mixture' (*left*), 'perlite' (*middle*) and 'potting soil' (*right*)

Fig. 22.3 Phosphorus (P) deficiency in tomato seedling leaves results in violet discoloration at the adaxial (under) side. This is based on an accumulation of anthocyanins (see ▶ Sect. 6.2) under P deficiency, as 'perlite' lacks P

22.3 Comparison of Long-Term Effects of Different Germination Substrates on Tomato Yield and Fruit Quality

22.3.1 Principle

To evaluate the long-term effects of the various germination substrates on the growth of tomato plants and fruits, an experimental period of 6 months is required. The tomato seedlings should be cultivated in the different germination substrates as described above in ▶ Sect. 22.2 ('potting soil', 'perlite', 'substrate mixture'). Afterwards, they should be transplanted into a closed rock wool-based hydroponic system and fertilized by a regular drip irrigation of nutrient solution. For an evaluation of long-term effects, the growth and quality-related parameters should be measured weekly until the end of the crop.

22.3.2 Materials

Plant material: 24 tomato seedlings raised on the different germination substrates (see ▶ Sect. 22.2). *For the greenhouse setup*: 24 rock wool cubes (ca. 10 × 10 × 8 cm); 12 rock wool mats (100 × 20 × 8 cm); perlite (0–6 mm); drain gutter/gullies in each row; a dripping irrigation system (dripper, tubes, pump, tanks); nutrient solution (■ Table 22.1); optional additional lighting; potassium hydroxide (KOH); nitric acid 53% (HNO_3). *For plant treatment*: Hanging appliance; string and plastic clips for plant training; pruning shears. *For measurement*: EC/pH metre, caliper, ruler, knife, cabinet dryer, nine ovenproof containers (approximately 200 ml).

22.3.3 Plant Cultivation

1. Cultivate 30 seedlings for about 2 weeks in the 3 different substrates (follow the procedure: *Seedling Cultivation* in ▶ Sect. 22.2). Use the best eight out of ten seedlings of each variant.
2. Transplant the chosen seedlings into moistened rock wool cubes, and fill cube holes with perlite.

■ **Table 22.1** Standard nutrient solution for tomatoes for closed rock wool cultivation and standard EC value of solutions (Lattauschke 2004, modified)

Nutrients	Concentration [mg/l]	Concentration [mmol/l]
NO_3-N	144.2	10.3
NH_4-N	7.0	0.5
P	37.2	1.2
K	234.6	6.0
Ca	128.3	3.2
Mg	24.3	1.0
SO_4	144.2	1.5
Fe	0.8	0.015
Mn	0.6	0.010
Zn	0.3	0.004
B	0.3	0.02
Cu	0.05	0.00075
Mo	0.05	0.00050
EC [mS/cm]: 2.5–5.0 at root area		

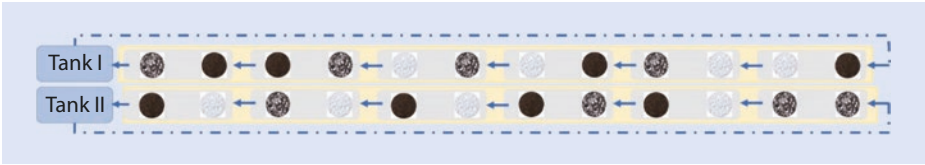

Fig. 22.4 Example for randomized cultivation setup for 24 tomato seedlings (once cultivated in the three different substrates): *black* 'potting soil', *light-grey* 'perlite', *dark grey* 'substrate mixture'. Experiment is repeated in two rows, each being supplied with nutrient solution via different tanks. Two transplanted seedlings are positioned per rock wool cube. Arrows indicate direction of flow of nutrient solution

3. Cut two 10 × 10 cm openings on the top of each mat for the cubes (distance to margin: 25 cm, distance between openings: 50 cm).
4. Arrange the greenhouse setup. For the closed rock wool hydroponic system, fertilization is achieved via the nutrient solution, which is applied with a drip irrigation system (see ▶ Sect. 22.3.4). Prepared mats are placed in gullies.[2] Consider randomized block designs for the distribution of mats for qualified statistical evaluation (row distance 100 cm, plant distance 50 cm).
5. Saturate rock wool mats with nutrient solution before cutting drain slots below the sides of the mats.
6. Position all 24 planted cubes in a random order into the openings in the mats. Label plants from 1 to 24 (Fig. 22.4). Place two drippers into each rock wool cube next to the plant.
7. Start the irrigation with the nutrient solution and pay particular attention to a tomato-convenient climate control system.[3]
8. Cultivate the plants until you can gather red fruits for at least 6 weeks in series to obtain sufficient data.

22.3.4 Details for Tomato Fertilization

For the irrigation and nutrient supply, use a standard solution for tomatoes (Table 22.1). In the recommended closed hydroponic system, the nutrient solution circulates as follows: tank-plants-drain-tank (Fig. 22.4). Note that a certain concentration of nutrients may be present in the irrigation water (tap water, rainwater, wash water) and should therefore be taken into consideration. Regulate daily the pH value to the optimal range of pH 5.5–6.8 with caustic potash (increases pH) or nitric acid (lowers pH). The composition of the nutrients can be customized according to the requirements of the plants during their various growth stages (Lattauschke 2004; Laber and Lattauschke 2014). Empty and refill the tank every second week to avoid nutrient depletion. Irrigate during the day between sunrise and sunset. The water requirement depends on the factor level

2 Planting distance for tall tomato types (indeterminate) between rows: 0.75 m; between plants: 0.5 m (Naika et al. 2005).

3 Cultivation temperature after start of harvest: day: 17–18 °C, night: 16–17 °C; airing temperature: 23–24 °C; relative humidity: day: 70–76%, night: 70–82% (Laber and Lattauschke 2014, modified).

of plant development of the plant, e.g. plant size, solar radiation and temperature. We recommend that the plants be watered at 100 ml/plant per hour until fruit growth begins. Afterwards, they can be watered three times per hour. A water content in the rock wool mats of 50–80% is recommended (Lattauschke 2004). The use of bumblebees for better pollination and of supplementary CO_2 is advantageous.

22.3.5 Measurements

Plant Appearance and Plant Growth Record all visually distinctive features (e.g. pest and disease pattern, general appearance). Start to measure growth parameters immediately after the plants have been transplanted into rock wool cubes. On a weekly basis, record the height, number of true leaves (no cotyledon), stem diameter (by caliper) and plant diameter (from top view). At the end of cultivation, record the total plant height (if not headed) (Roldán and Soto 2005; Dannehl et al. 2015).

Yield Quantity and Quality The first harvest can be expected at approximately 10–12 weeks after sowing (Laber and Lattauschke 2014). Document (1) the date of the first harvest for each plant, (2) the weekly mass of tomato fruits as marketable fruits (good size >50 g, ripe, healthy) and non-marketable fruits (small size, cracking, blossom-end rot). In addition, the fruits can be grouped into classes of <50 g, 50 g to ≤70 g and >70 g. At the end of cultivation, record blossomed trusses and the number of fructified trusses of each plant (Inden and Torres 2004; Dannehl et al. 2015).

Important: Tomato quality is influenced by fruit ripeness. In contrast to ripe tomatoes, unripe tomatoes show lower nutrient values. For this, select only similar red coloured and ripe fruits for the weekly harvest. Leave unripe, pale, and red-orange-green fruits on the plant. Where possible, only one person should perform the harvest and measurements each day in order to prevent subjective errors (Radzevičius et al. 2012; Ho et al. 1987).

Fruit Dry and Fresh Weight Measurements A high dry weight is one of the many quality attributes of tomato as it is an indication of a higher nutritional value (Heuvelink 1996). Here, it is used for a comparison of the fruit weights of all three cultivation methods (germinated in potting soil, perlite or substrate mixture).

1. Measure the empty weight of each of the nine oven-proof containers. These weights are needed for taring the balance. Mark the pots to avoid mixing them up.
2. Prepare three pooled samples from each treatment. For this, gather three tomatoes (randomized) from each treatment with nearly equal weight (e.g. 70 g) and equal red ripeness colour. Cut the tomatoes of each group into accurate quarters. Place one quarter of each tomato fruit in one container. Discard the remaining three quarters.
3. Weigh all nine containers with the fresh mass.
4. Dry the fresh material at 105 °C for 24 h in a cabinet dryer (Dannehl et al. 2015).
5. Reweigh the containers and subtract the empty container weight. Calculate fresh/dry weight ratio. The fresh and dry weight determinations of tomatoes should be repeated weekly to obtain a temporal course for these parameters. Other comparable quality attributes (e.g. sugar/acid ratio) of tomatoes can be recorded as mentioned and evaluated in ► Chap. 19.

22.3.6 Evaluation

All three treatments ('potting soil', 'perlite' and 'substrate mixture') should lead to differences in external plant appearance, growth parameters and yield. Overall, the plants germinated in 'potting soil' are anticipated to show the highest yield, the earliest date of harvest and the highest quantity of marketable fruits. Further, the mentioned parameters are expected to be adversely affected in plants grown in the 'perlite' variant, followed by plants germinated in 'substrate mixture'. No significant differences are assumed for the fruit dry matter between the three different treatments. However, former growth deficits that occurred during early seedling development might appear throughout the growth period (compare ▶ Sect. 22.2; short-term analysis of seedling cultivation in different germination substrates).

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Supplementary Information

C.-M. Geilfus, *Controlled Environment Horticulture*,
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Index

A

B

C

D

E

F

G

H

I

J

K

L

M

N

O

P

Q

R

S

T

U

V

W