



HUNGARIAN UNIVERSITY OF  
AGRICULTURE AND LIFE SCIENCES

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**MOLECULAR EFFECTS OF  
SILICATE APPLICATION  
ON CUCUMBER SEEDLINGS**

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## 1. LIST OF ABBREVIATIONS

<b>ANOVA</b>	Analysis of variances
<b>AOX</b>	Alternative oxidase
<b>APX</b>	Ascorbate Peroxidase
<b>AA</b>	Ascorbic acid
<b>BHT</b>	Butylated hydroxytoluene
<b>CAT</b>	Catalase
<b>cDNA</b>	Complementary Deoxyribonucleic Acid
<b>CSiT</b>	Cucumber Silicon Transporter
<b>dNTP</b>	Deoxy nucleoside triphosphate
<b>ER</b>	Endoplasmic reticulum
<b>ETC</b>	Electron transport chain
<b>GA</b>	Gibberellic acid
<b>GPX</b>	Guaiacol peroxidase
<b>GR</b>	Glutathione reductase
<b>HG</b>	Hoagland
<b>JA</b>	Jasmonic acid
<b>LOX</b>	Lipoxygenase
<b>LPO</b>	Lipid peroxidase
<b>Lsi</b>	Low silicon
<b>MDA</b>	Malondialdehyde
<b>mRNA</b>	Messenger Ribonucleic Acid
<b>NADPH</b>	Nicotinamide adenine dinucleotide phosphate
<b>NIP</b>	Nod 26-like major intrinsic protein
<b>PCD</b>	Programmed cell death
<b>PCR</b>	Polymerase Chain Reaction
<b>POD</b>	Peroxidase
<b>PUFA</b>	Polyunsaturated fatty acid
<b>qRT-PCR</b>	Quantitative reverse transcription polymerase chain reaction

<b>RBOH</b>	Respiratory burst oxidase homolog
<b>ROS</b>	Reactive Oxygen Species
<b>RT</b>	Room temperature
<b>RT-PCR</b>	Reverse transcription polymerase chain reaction
<b>SA</b>	Salicylic acid
<b>SD</b>	Standard deviation
<b>Si</b>	Silicon
<b>SOD</b>	Superoxide dismutase
<b>TBARS</b>	Thiobarbituric acid reactive substances
<b>TBE</b>	Tris-Borate-EDTA
<b>UV</b>	Ultraviolet

## 2. INTRODUCTION

Cucumber is a warm season plant that grows rapidly at 24-29°C temperatures. It is native to the tropical regions of South Asia. Its origin dates back to 3000 BC in Indian subcontinents. It is an herbaceous vine that grows, blooms and fructifies normally even on a short-day illumination while requiring high humidity. Greater light intensity and solar radiation however improve its fruit production. It performs reasonably well in a variety of soil with a loose structure. This species is sensitive to a range of environmental stresses, such as water shortage, extreme temperatures and excess salt.

Silicon (Si) fertilizers have been used to improve crop performance for decades. It improves the chemical characteristics of acidic soils, also increases cation exchange capacity. Silicon is absorbed by plants as silicic acid, it is carried by the transpiration stream and deposited in plant tissues as amorphous silica. Numerous positive effects are attributed to silicon on plant production, including reduction of the impact of some diseases, delaying senescence, improving photosynthesis, growth, abiotic stress tolerance and mechanical resistance. Cucumber actively absorbs silicon compared to other eudicots though it is considered as a moderate accumulator. Si transporters involved in Si uptake and distribution have been identified in several plant species, including cucumber. It is directly and/or indirectly involved in enzyme activities, that regulate plants' normal process as well as serve as protection during stress conditions.

Molecular breeding efforts of cucumber are conducted intensively worldwide. Availability of the full genomic sequence of this species by Huang et al., (2009) led to the discovery and characterization of various genes. Due to increasing demand for high quality products from the consumer's side, new hybrids of cucumber are released frequently. Therefore, the most current approaches of breeding are directed to improving relevant traits by properly understanding the molecular basis of the most important physiological processes.



### 3. OBJECTIVES

We will investigate how silicon application affects cucumber's normal growth and physiology as well as we wish to study how Si is involved in molecular mechanisms regulating certain genes which play key roles in protection and defense mechanisms. Cucumber plant cultivars will be grown in a semi-hydroponic cultivation system to keep control over the experiments, specifically to maintain a steady Si supply and to avoid interactions with other elements, which might come from the soil. In order to achieve the proposed results, the following objectives are set:

- Determine the fresh weight of the cultivars
- Photosynthetic pigment contents will be analysed
- Abiotic stress inducers such as lipid peroxide, hydrogen peroxide and Thiobarbituric Acid Reactive Substances amounts will be measured
- Putative silicon transporter genes will be analysed by RT-qPCR
- Lipoxygenase genes previously expressed in cucumber will be investigated in response to silicon supply and will be analysed by RT-qPCR.

## 4. OVERVIEW OF LITERATURE

### 4.1. Cucumber nomenclature

*Cucumis sativus*, known as cucumber, is an herbaceous plant from the *Cucurbitaceae* family or cucurbits. The family consists of 975 species categorized in 98 genera most commonly originating from tropical and subtropical regions (Baloglu, 2018). Evidently, cucurbits are counted among the earliest cultivated and most highly consumed crop plant families.

Cucurbits fruits bear ranging characteristics and fibers are used to make various products (Bisognin, 2002). Hence, its also considered as a model system for sex determination studies due to their rich diversity of sex expression within the family (Tanurdzic and Banks, 2004). Moreover, cucurbits are well suited for vascular biology and long-distance signaling studies, because of their readily obtainable nature of both xylem and phloem saps (Lough and Lucas, 2006)

This family includes some economically important species like melon (*Cucumis melo L.*), watermelon (*Citrullus lanatus*), squash and pumpkin (*Cucurbita spp.*) (Whitaker and Davis, 1962). According to FAO statistics, around 8 million hectares of land has been used to cultivate cucurbits and yielded more than 238 million tons of total production worldwide in 2019 (“FAOSTAT,” 2019). While, cucumber occupies the fourth place in the world vegetable production behind tomato, onion and watermelon (“FAOSTAT”).

### 4.2. Cucumber origin

Cucumber is considered the most important species amongst the *Cucurbitaceae* crops from Asia. According to Candolle (1884), India was the center of domestication for *C. sativus* and primarily spread to China in the east. Wild cucumbers have been found over much of the Indian subcontinent while cultivated ones are dispersed throughout China from 2000 years ago (Paris et al., 2012). There is great genetic diversity in Indian varieties compared to cucumbers in the rest of the world. Esteras

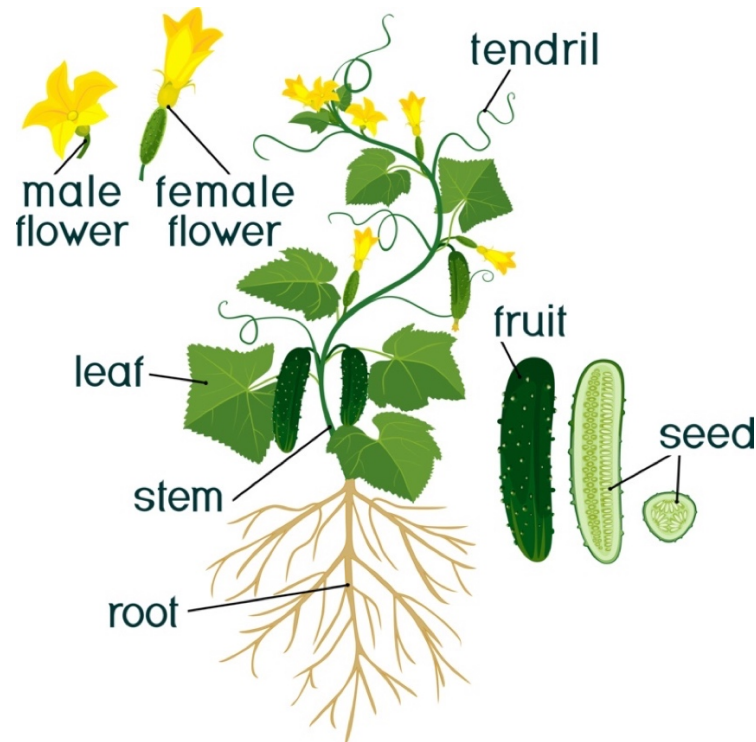
et al., (2011) concluded that cucumbers were domesticated on the Indian subcontinent by 3000 BC and China became the secondary center of genetic diversification of *C. sativus*. This explains the genetic difference of *C. sativus* between Chinese and heterogeneous Indian germplasms (Lv et al., 2012).

Phylogenetically, only *C. sativus* and *C. sativus* var. *hardwickii* have 7 ( $2n=14$ ) chromosomes, thus scientists believed *C. sativus* var. *hardwickii*, which is Chinese species first found in Himalayan foothills of Nepal, to be the wild ancestor of cucumber (Robinson and Decker-Walters, 1997). Based on the morphological similarity between *C. sativus* and *C. sativus* var. *hystrix*, this South-eastern Asian species was also believed to be the progenitor (Hancock, 2012). In fact, *C. sativus* var. *hystrix* is shown to be the closest relative to *C. sativus* based on DNA sequencing of plastid and nuclear markers for about a hundred *Cucumis* species. According to molecular clock data, a divergence of *C. sativus* var. *hystrix* was proven to be 8 million years old, which further suggests that cucumber is of Indian/Asian origin (Weng and Sun, 2011).

### **4.3. Cucumber botany**

Cucumbers (*Cucumis sativus* L.) are tender annual climbing plants with a vigorous root system. It consists of the main root, branched, and thin secondary roots having white elongated surface as well as adventitious roots above the neck (Robinson and Whitaker, 1974) Cucumbers are also considered prostrate and be able to root in soilless systems. They produce four or five main stems, that reach up to 2 m, with curling tendrils branching on the long vines. Dark green leaves are succulent with long petioles and possess 3-7 pointed lobes, covered with very fine hair. Lateral bud and flowers emerge from the armpit of each leaf (Figure 1).

Its fruit has ranging characteristics based on their geographical origins. According to the cultivated variety, fruits are rough or smooth pepo. Their development starts as light green fruitlets, turning to dark green, and become yellowish when fully ripened.



**Figure 1.** Schematic diagram of cucumber (Mariaflaya, 2018)

Cucumber seeds are distributed along the watery fruit pulp and are oval in shape and variable in numbers. Both seeded and seedless cultivars exist, the latter is also known as parthenocarpic. Parthenocarpic cucumbers are all female and develop seedless fruits without pollination. These cultivars are mainly grown in protected cultural systems such as a greenhouse. Although parthenocarpic varieties do not require bees, pollination and seed formation can take place when bees are present, which however can result in quality degradation (Le Deunff and Sauton, 1994). On the other hand, seeded cultivars are self-incompatible which requires pollen from a different plant for its seed and fruit development.

#### **4.4. Cucumber varieties**

Nowadays commercial varieties are all gynoecious, though the first known cultivars of cucumber were monoecious with several old cultivars of andromonoecious (Schaffer and Paris, 2003). Early cucumber cultivars were populations or inbred lines through open pollination of bulk increased or hand pollinated seeds. Nowadays, most hybrid cultivars are produced by crossing a gynoecious inbred line as the female with a monoecious inbred line as the male. Greenhouse cucumber hybrids are usually produced by two gynoecious inbreds with high gynoecious sex expression.

##### **4.4.1. Pickling varieties**

Pickling varieties have smaller sized fruits with thinner skin, which makes them suitable for pickling. Fruits have bumpy skin with less regular shapes and are usually lighter in color. The fruits of this cucumber grow up to 7 cm to 10 cm long and 2.5 cm wide and have a shorter growth cycle of 50 – 60 days. Most pickling cucumbers are harvested by machine due to their high plant population of 240,000/ha, though cucumber fruit injury incidents are higher. The pickling process degrades some of the nutrient values because of pickling in brine or vinegar with various spices.

##### **4.4.2. Slicing varieties**

Field-grown slicing cucumbers are known as fresh market cucumbers. These are larger and sweeter with thicker skin than pickling varieties. Fruits are generally longer and have smoother skin than those of pickling varieties. All fresh market cucumbers are harvested by hand, besides their thicker skin provides resistance to damage during handling and shipping.

##### **4.4.3. Greenhouse cucumber or burpless**

This type of cucumber is sweeter and easier to digest. Compared to other types of varieties, the skin is thinner, and fruits are normally 30-35 cm but can grow as long as 60 cm with rounded ends.

Typically grown in greenhouses, these cultivars differ from field types by being parthenocarpic, which requires no pollination. They are gynoecious, which produce female flowers exclusively.

#### 4.5. Nutritional value of cucumber

Cucumber contains mostly water (up to 96%) with only 4% dry mass (Zieliński et al., 2017). Fruits are low in calories and usually consumed fresh in salads or pickled. The seeds serve as a good source of protein, carbohydrates and crude fibers. Its oil is used for cooking, which increases high-density lipoprotein (HDL) and decreases low-density lipoprotein (LDL) and serum cholesterol (Mariod et al., 2017). Cucumber contains various vitamins, minerals, antioxidants as well as a biologically active compounds including alkaloids, flavonoids, etc., (Sarhan and Ismael, 2014).

**Table 1.** Nutritional value of cucumber per 100g

Energy content/Phytochemicals	Value
Energy	15 kcal
Protein	0.65 g
Total lipid	0.11 g
Carbohydrates	3.63 g
Dietary fiber	0.5 g
Minerals	
Calcium	16 mg
Iron	0.28 mg
Magnesium	13 mg
Phosphorus	24 mg
Potassium	147 mg
Sodium	2 mg
Zinc	0.20 mg
Vitamins	
Vitamin A	105 IU
Vitamin C	2.8 mg
Vitamin K	16.4 µg

Source: <http://ndb.nal.usda.gov/ndb/foods>

## 4.6. Cucumber production

Cucumber is a warm season plant that can utilize high temperature, humidity, light intensity and requires a continuous water supply and nutrients for optimum production. It prefers well drained, fertile soil with moderate to high organic matter. Also, performs reasonably well in a variety of soils with a loose structure and is able to grow within a pH range of 5.8 to 6.9. Cucumbers tend to grow fast and provide high yield if provided appropriate nutritional and environmental conditions.

### 4.6.1. Temperature

Due to its tropical origin, temperatures between 20°C to 30°C during the day hardly affect production. The ideal temperature for growth and fruiting is between 24°C to 27°C with maximum growth and germination temperature of 28 – 30°C (Grimstad and Frimanslund, 1993; Papadopoulos and Hao, 2000). Although several varieties tolerate temperatures up to 32°C for a short period, the quality and flavor of most cucumber varieties diminish at high temperatures (Challa et al., 1995).

Generally, cucumbers are cold sensitive especially during the growing season. Fruits become bitter if exposed to low temperature. Temperatures below 12°C will result in slower growth and cause malformation in leaves and fruits.

### 4.6.2. Light intensity and relative humidity

It is a crop that successfully grows under high humidity, high soil moisture and high light in greenhouses (El -Aidy, 2007). Cucumber crop can withstand high light intensities and solar radiation (Hao and Papadopoulos, 1999). Fruits become lighter and can turn easily yellow during shelf life if grown under low light (Vonk and Welles, 1995). According to Marcelis (1993), increasing irradiance has increased the dry matter percentage of fruit and vegetative plant parts.

Due to its large leaf surface, an optimum relative humidity of 75-80% is required for growth. Thus, it is recommended by Bakker et al., (1987) to keep the relative humidity higher during the day to keep

fruit quality high. However, humidity over 90% with saturated atmosphere steam can cause fungal disease on crops through condensation or dripping. Fruit color changes (Fricke and Krug, 1997) and calcium deficiency (Bakker et al., 1987) are observed under high humidity during the storage period.

#### 4.6.3. Carbon dioxide

Carbon dioxide supply of 500-900 ppm is an optimum concentration for cucumber production in the greenhouse (Akilli et al., 2000). The optimum for CO<sub>2</sub> enrichment of the greenhouse depends on the quality, productivity and precocity of the crops. Generally, carbon fertilization allows the stimulation of photosynthesis and accelerates plant growth. However, excess CO<sub>2</sub> causes damage due to stomatal closure, which ceases photosynthesis and causes burns. For cucumber crops, yield increase has been reported by Peet and Willits (1987) with extended periods of carbon dioxide enrichment, and reduction of non-essential amino acids.

#### 4.6.4. Water

Cucumber has a sparse root system with most of the root length concentrated in the upper soil layer of 0.3 m. Thus, water is an important limiting factor for the production and quality of cucumber fruits (Bauder et al., 2011). In order to reduce the leaf wetting and disease spread, drip or furrow irrigation is recommended instead of overhead irrigation. Also in greenhouses plastic covering, especially transparent ones, are recommended with drip irrigation for increased productivity with enhanced vegetative growth (Ahmed et al., 2019).



#### **4.7. Cucumber genome**

The cucumber genome is sequenced by Huang et al., (2009) and became the first genome to be sequenced from the Cucurbitaceae family and the seventh completed plant genome among model plants such as *Arabidopsis thaliana*, rice, sorghum, papaya, poplar and grapevine (Baloglu, 2018). The second and third completed genome sequencing in Cucurbitaceae belonged to melon (Garcia-Mas et al., 2012) and a watermelon (Guo et al., 2013) respectively. The sequence of the cucumber genome allows researchers to conduct more in-depth studies and development for the breeding of cucurbits.

Seven chromosomes of cucumber have been sequenced using a combination of Sanger and next-generation Illumina sequencing in *Cucumis sativus* L., Chinese long inbred line 9930 (Huang et al., 2009) As a result, high genome coverage of 72.2 fold was obtained with approximately, 26682 genes predicted in the assembled genome. Total length was 234.5 Mb which is 30% less than predicted according to flow cytometry analysis of isolated nuclei, actual size is calculated to be 367 Mb (Arumuganathan and Earle, 1991).

Nowadays, more cucumber cultivars and inbred lines have been sequenced including deep resequencing of 115 cucumber lines (Qi et al., 2013), American Gy14 (Cavagnaro et al., 2010) and North European lines B10V1, which is monoecious inbred field cultivar (Osipowski et al., 2020).

#### **4.8. Silicon**

Silicon is a nonmetallic chemical element in the carbon family, which rarely exists in its pure form. The oxidized forms of Si called silicon dioxide and silicates are common in Earth's crust and are particularly important components of Earth's mantle. Si is found practically in all rocks, sand, clays and soils. Its compounds also occur in all kinds of natural waters, in the atmosphere as siliceous dust, in many plants as well as in the skeleton, tissues and body fluids of some animals.

#### 4.8.1. Silicon in soil

Earth surface is covered with crust containing 27.7% of silicon, being the second most abundant element next to oxygen (46.6%) (Mitra, 2015). Generally, 50 – 400 g of Si are contained per kg of soil. There are several types of Si forms commonly present which include quartz and crystalline combined silicates, secondary or clay and Si-rich minerals, and amorphous silica that are present in most soils (Orlov, 1985). They constitute a major portion of the soil in the form of silicate or aluminum silicates, which account for 90% of the earth's crust by mass. Generally, all Si forms are sparingly soluble and biogeochemically inert. Principal soluble forms of silicon in soil are monosilicic and polysilicic acids (Mica, 1986).

Monosilicic acid occurs in a weakly adsorbed state in the soil (Matychenkov et al., 1995) and has a low capacity for migration down the soil profile (Khalid and Silva, 1980). It can interact with aluminum, iron, manganese and heavy metals to form slightly soluble silicates (Horiguchi, 1988; Lumsdon and Farmer, 1995). Unlike monosilicic acid, polysilicic acid acts as an adsorbent. It is an integral component of the soil solution and mainly affects soil physical properties and it is important for the formation of soil structure (Matychenkov et al., 1995).

#### 4.8.2. Silicon in plants

According to a tissue analysis study conducted on a wide variety of plants, Si concentration ranged between 0.01 to 100 g kg<sup>-1</sup> of dry weight (Farooq and Dietz, 2015). Therefore, higher values of silicon present in the plant are equivalent to macronutrients such as phosphorous, nitrogen and calcium.

Among plants, sugarcane (*Saccharum officinarum*) 300–700 kg ha<sup>-1</sup>, rice (*Oryza sativa*) 150–300kg ha<sup>-1</sup>, and wheat (*Triticum* spp.) 50–150 kg ha<sup>-1</sup> absorbed the largest amount of Si (Datnoff et al., 2001). Moreover, barley (*Hordeum vulgare* L.), ryegrass (*Lolium perenne*), maize (*Zea mays* subsp. *Mays*) along with rice and wheat are all gramineous plants with much higher Si uptake than other plant

species. Dicotyledonous plants including cucumbers (*Cucumis sativus*), sunflower (*Helianthus annuus* L.) and wax gourd (*Benincasa hispida* L.) absorb Si actively (Liang et al., 2006). Nonetheless, some plants like tomatoes (*Solanum lycopersicum*) bean and several others are not able to absorb Si from soil (Liang et al., 2006; Nikolic et al., 2007).

#### 4.8.3. Silicon uptake and accumulation in plants

Plants take up Si via the transpirational water stream (Elawad and Green, 1979) which may or may not be facilitated by active transport. The incorporation of Si by plants depends directly on the uptake ability and beneficial effects are correlated with it (Takahashi et al., 1990). Thus, based on their variability to take up silicon, plants are classified into three groups. These include high accumulators (>5%), intermediate accumulators (1%), and low accumulators (<0.1% of their dry weight basis), such as rice, cucumber and tomato respectively (Deshmukh and Bélanger, 2016). Monocot plants tend to accumulate higher (up to 10%) than those belonging to dicots.

Plant roots absorb Si in the form of monosilicic acid or orthosilicic acid ( $H_4SiO_4$ ) at a concentration of 0.1 – 2.0 mM, which crosses the root plasma membrane solely at certain physiological pH ranges (Ma and Yamaji, 2006). Due to significant differences among plant species to take up Si by the root, three possible types of water-based Si uptake mechanism are proposed by Takahashi et al., (1990). Plants having higher Si uptake levels than water are classified as active accumulators, plants with similar rate of Si and water uptake are classified as passive, whereas those with lower Si uptake than water are referred to as rejective.

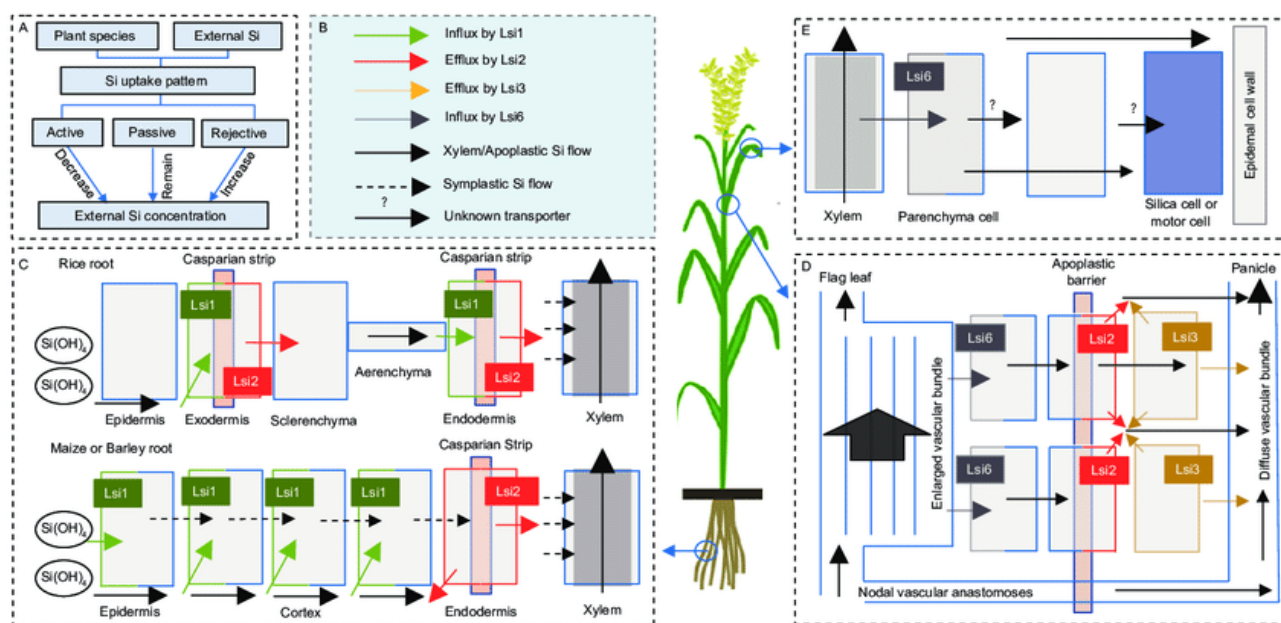
##### 4.8.3.1. Si transporters in plants

Si transporters are specific transmembrane proteins that mediate the whole uptake mechanism (Marron et al., 2016). The very first Si transporter was identified in rice. This discovery suggests that such a specific system for quite an active Si transport existed. This led to the identification and cloning

of *Lsi1* (*low silicon 1*) of rice, which transporter gene was localized on chromosome 2, using a mutant (Ma et al., 2004). According to Blast and ClustalW analysis, this transporter belongs to an aquaporin subfamily of the NIP3 (Nod 26-like major intrinsic protein3) major protein family (Gomes et al., 2009). It facilitates Si uptake into the root stele when present in the plasma membrane. In other ways, uptake can be prevented by the presence of Casparian strips, located at the exodermis and endodermis. *Lsi1* is expressed predominantly in the main and lateral roots but not in the root hairs, which is consistent with the a previous study finding lack of participation of root hairs in the Si uptake process (Ma et al., 2001). Thus, *Lsi1* is proven to be a major influx transporter of Si into rice roots (Figure 2).

*Lsi2*, belonging to the an anion transporter gene family, gene on the other hand is a possible Si transporter from the root to the vascular tissue in rice (Ma et al., 2011). It is located on the proximal side of the membrane and builds up in the mature root zone. Moreover, it is suggested to be an efflux transporter for silicic acid. Another influx transporter *Lsi6*, is expressed in the parenchyma cells of the leaves, moves Si across the vascular bundle.

Homologous genes for the rice *OsLsi1* influx transporter have been found in monocot plants such as wheat (*Triticum aestivum*) as *TaLsi1*, maize (*Zea mays* L.) as *ZmLsi1* and *ZmLsi6* and barley (*Hordeum vulgare* L.) as *HvLsi1* (Chiba et al., 2009; Mitani et al., 2009). *OsLsi2* efflux homologs have also been found in maize (*HvLsi2*) and barley (*ZmLsi2*). Whereas in dicot plants, soybean (*Glycine max*, *GmNIP2-1*, *GmNIP2-2*) (Deshmukh et al., 2013), pumpkin (*Cucurbita moschata*, *CmLsi1*) (Mitani et al., 2011) and cucumber (*Cucumis sativus* *CSiT1*, *CSiT2*, *CsLsi1* and *CsLsi2*) (Wang et al., 2014; Sun et al., 2017, 2018).



**Figure 2.** Schematic illustration of Si transport in plants (Yan et al., 2018)

#### 4.8.3.2. Si transporters in cucumber

Cucumber accumulates more Si than any other eudicot. Wang et al., (2014) first identified two influx transporters (CSi1 and CSi2) in cucumber. CSi1 belongs to NIP3 subfamily and is localized at the plasma membrane which was similar to OsLsi1. Both transporters are expressed mainly in roots and in mature leaves. The author concluded that these carriers accumulate Si in cucumber leaves rather than in roots when compared to other homologous influx transporters. They were also found to be circadian rhythm regulated genes.

More recently Sun et al., (2017, 2018) isolated influx transporter CsLsi1 and efflux transporter CsLsi2, which were mainly expressed in the roots. CsLsi1 protein was found to be localized at the distal side of the endodermis and in the cortical cells not only at the root tips but also at the root hairs. Whereas CsLsi2 was localized at the plasma membrane and mainly expressed in endodermal cells. Based on sequence analysis, CsLsi2 shares the highest, more than 97%, similarity to the respective pumpkin transporter, CmLsi2. Recent update has been made on CsLsi2 to be a CsLsi3 efflux transporter (Accession No XM\_004140721.3) (Genbank database).

#### **4.9. Effect of silicon**

Si plays a major role in plant life cycles and one of these roles is improving plant growth and yield especially in stress conditions. Some of the most important functions of Si include promoting plant photosynthesis by exposing leaves to the light, increasing resistance to disease and pathogens, and regulating metal toxicities as well as salinity and drought stresses. Others include protection against extreme temperatures, insufficient mineral nutrition etc., (Zhu and Gong, 2014). Generally, it is shown to have an important role in the physiological activity and structural makeup resulting in improved survival of higher plants that are exposed to various biotic and abiotic stresses (Liang et al., 2015).

Plant growth is dependent upon the existence and availability of several elements in the soil. These elements are categorized into three groups called beneficial, essential and toxic (Bienert et al., 2008). Elements categorized into a toxic group, unconstructively affect plant growth, while essential elements are critical for all plants in various growth conditions. Beneficial elements are vital for some plant species in certain growth conditions. The beneficial effects of Si are well documented on different plant species. Even though the essentiality of Si has always been a debate, regardless of its beneficial use and consideration by many authors as well as studies conducted on numerous plants. Nevertheless, Si is not only having positive effects on the plant itself but also benefits human health through food products derived from Si exposed plants by offering greater bone strength and improved nervous and immune systems (Farooq and Dietz, 2015).

##### **4.9.1. Effect of silicon in biotic stresses**

Biotic stress is caused by pathogens, insect pests and other living organisms. Plants have developed defense mechanisms to overcome those biotic stresses. Fortunately, the presence of Si increased the resistance of plants against stresses and correlated damages, which has been documented by various studies. Many scientists laid foundation for hypotheses regarding different defense mechanisms. The

first hypothesis is rooted from Wagner (1940), who suggested that Si acted as a mechanical barrier when deposited on the tissue surface, which prevented the penetration of pathogens into different host plants. Another hypothesis was that plants prevented the parasite propagation by accumulating phenolic compounds when Si is applied (Samuels et al., 1991). This hypothesis has been further analyzed and supported by Fawe et al., (1998) with the interaction of Si treated cucumber and powdery mildew infection. Interestingly, field and experimental data suggest that Si benefit, regarding biotic stress is limited to biotrophic (e.g., powdery mildew) and hemibiotrophic (e.g., rice blast) pathogens. In recent years, the importance of effector proteins especially in the case of biotrophs and hemi biotrophs for host–pathogen interactions have been highlighted. According to Giraldo and Valent (2013), fungal effectors are able to modify host cell structure, metabolism and function by interfering with signaling pathways. These fungal effectors are released into the apoplast and translocated into the cytoplasm through cell membrane or extrahaustorial matrix (Bozkurt et al., 2012). In plants, SiO<sub>2</sub> deposition is also located in the apoplast more specifically at the interface of the plasma membrane with the cell wall (Zhang et al., 2013). This makes the apoplast the interaction site for effectors and plant targets (Wang and Wang, 2018). Thus, many works presented in the literature support the evidence that Si interferes with effectors or receptors to contribute to plant resistance (Rasoolizadeh et al., 2018). Evidently, these findings are also true with plant–insect (particularly piercing–sucking type) interactions, which involve HAMP (herbivory associated molecular pattern) and effector triggered immunity that mediates plant defense mechanism (Hogenhout and Bos, 2011). Si deposited within the apoplastic space interferes with insects having piercing and sucking type feeding behaviour, which showed reduced probing time (Costa et al., 2010). Thus, insect receptors are trapped within the extracellular Si matrix, preventing insects from impeding the plant defense response or from recognizing the plant as a suitable host (Hogenhout and Bos, 2011).

#### 4.9.2. Effect of silicon in abiotic stresses

Abiotic stress covers a wide range of environmental adverse effects such as high or low temperature, drought, salinity, ultraviolet radiation and metal toxicity (Epstein, 1999). In order to increase plant derived food qualities and quantities, cultivated plants are required to utilize more and more efficient strategies to overcome those environmental adverse effects.

In respect to salinity and drought stress, Si is shown to improve root growth (Hameed et al., 2013) increase water uptake due to improved hydraulic conductance (Hattori et al., 2008) and root activity (Chen et al., 2011), which in turn increased root/shoot ratio in Si treated plants (Wang et al., 2015). Apart from morphological changes, modification of solute levels has been reported for proline (Yin et al., 2013), carbohydrates (Ming et al., 2012), glycine betaine (Torabi et al., 2015), total phenolics (Hashemi et al., 2010), total soluble sugars and total amino acids (Hajiboland et al., 2016) which minimize the osmotic shock and ion toxicity created by of salt stress. Moreover, Si supply can interfere with these stresses by regulating phytohormones (Kim et al., 2013) by decreasing Jasmonic acid (JA) (Hamayun et al., 2010), ethylene (Yin et al., 2016) and enhancing the level of endogenous Gibberellic acid (GA) (Lee et al., 2010) and salicylic acid (SA). In terms of polyamines, Si plays an important role by regulating the production of putrescine, spermidine and spermine (Yin et al., 2016) also affects ion transport, improving antioxidant ability and modifies osmotic potential (Alcázar et al., 2011).

Under drought condition, Si is deposited under the cuticle forming a double layer which prevents water loss via transpiration in shoots (Ma et al., 2001). In roots, Si accumulates around exodermis and endodermis cells, where Si transporters are located, blocking apoplastic bypass route where toxic ions such as  $\text{Na}^+$ ,  $\text{Cl}^-$  and  $\text{Cd}^{2+}$  enters via Casparian strip break or underdevelopment and accumulates in the shoot possibly to toxic levels (Flam-Shepherd et al., 2018). Si also contributes to the formation of the Casparian strip by stimulating suberin and lignin biosynthesis, which further protects plants against toxicants to bypass the apoplastic route (Fleck et al., 2015).



Under salt stress, Si decreased MDA concentration, the end product of lipid peroxidation, in barley (Liang et al., 2003), maize (Moussa, 2006) and grapevine rootstock (Soylemezoglu et al., 2009). Moreover, MDA concentration was positively correlated with Na<sup>+</sup> absorption in salt stressed cucumber and a negative correlation was found with Ca<sup>2+</sup> and K<sup>+</sup> absorption in Si treated cucumber (Khoshgoftarmanesh et al., 2014).

Ultraviolet radiation induces excess production of reactive oxygen species in plants (Beckmann et al., 2012). According to Li et al., (2008) and Fang et al., (2011), Si is shown to increase plant tolerance to UV-B radiation, which has more adverse effects than drought on plant growth. Shen et al., (2010) reported that Si application significantly reduced the membrane damage caused by a combination of drought and UV-B radiation in soybean seedlings.

High temperature is another limiting factor for plant germination, growth and propagation. When a plant is subjected to high temperature, cell membrane permeability increases with subsequent imbalance between ROS production and scavenging that can cause LPO intensification by resulting in an imbalance between production and scavenging (Zhu et al., 2010). Hu et al., (2020) reported that Si supply during high temperature stress kept the stomata open and Si also enhanced the resistance to cold stress through increased epicuticular wax deposition when poinsettia plants were exposed to high (40°C) and low (4°C) temperatures.

In the apoplast, H<sub>4</sub>SiO<sub>4</sub> polymerization results in an amorphous silica barrier (Exley, 2015), which can limit penetration of toxicants like Al, Cd, Mn and Zn into the symplast (Guerriero et al., 2016). A silicon based barrier for metal uptake can build up in the apoplasm of endodermal region, and in the xylem. Hence, Si deposition caused a decrease in free metal concentration in the apoplasm (Iwasaki et al., 2002) and enhanced the adsorption of metals in the cell wall (Ye et al., 2012) through apoplasmic transport. In the endoderm region, the porosity of the cell wall in the inner root tissue was reduced, thus decreasing metal concentration in the xylem and metal movement was restricted in the root

endodermis as a result of Si accumulation (Keller et al., 2015). Thus, limiting metal absorption by plant root and transfer of metals within plant tissues may be the main mechanism of Si to reduce metal toxicity stress (Etesami and Jeong, 2018).

#### **4.10. Oxidative stress**

Oxidative stress is basically the consequence of excessive accumulation of reactive oxygen species (ROS) triggered by certain circumstances especially during abnormal physiological states such as stress. This involves damage to the cell components leading to detrimental pathophysiological processes. Specifically, oxidative stress can be caused by external oxidizers such as extreme light, ozone, UV and gamma radiation and transition metals such as Cu, Fe, Hg, Mn etc., which produce hydroxyl radical. Moreover, it can be a response to biotic and abiotic stressors where ROS generation is induced through cellular programs. Lastly, ROS is produced as part of the normal physiology occasionally leading to programmed cell death and autophagy. However, the intensity and consequences of oxidative damage depend on a biological organism's ability to detoxify ROS and repair oxidative damage.

##### 4.10.1. Reactive oxygen species

Reactive oxygen species are regular products of plant cellular metabolism. Superoxide anion ( $O^{\bullet-}$ ), hydrogen peroxide ( $H_2O_2$ ), hydroxyl radicals ( $\cdot OH$ ) and singlet oxygen ( $^1O_2$ ) are the most frequently studied species of ROS, which are involved in the regulation of plant cell development and stress tolerance. High concentration of ROS is one of the oldest known stressors on Earth ever since the oxygen evolving photosynthetic organisms appeared (Mittler et al., 2011). All life forms have been exposed to ROS for at least 2.7 billion years since Cyanobacteria like organisms started producing oxygen from water molecules. Species and their biochemistry have been evolved with a constant rise of  $O_2$  level up to its present atmospheric concentration (Mittler et al., 2011). In the presence of ROS,

numerous redox active enzymes and biomolecules have evolved and oxidative stress recognition and antioxidative defense systems have become sophisticated. Remarkably, plants not only acquired pathways to protect from ROS toxicity but also started to use ROS as signaling molecules (Farooq and Dietz, 2015; Mignolet-Spruyt et al., 2016).

#### 4.10.1.1. Superoxide radical ( $O_2^{\cdot-}$ )

ROS are formed by the multistep reduction of oxygen through the addition of electrons. In the primary step, superoxide is formed during oxygen reduction. Triplet oxygen ( $^3O_2$ ) is the main form of atmospheric oxygen with two unpaired electrons, which have the same spin numbers, so it's called parallel spins. Spin restriction happens when parallel spins restrict the number of  $O_2$  targets to those that have two similar electrons with antiparallel spins, which results in decreased reactivity of  $O_2$ . In this state,  $O_2$  not toxic and it's not very chemically active. However, when there's an input of energy coming from various sources, it removes the spin restriction, and  $O_2$  acquires higher reactivity as a result. In plant cells,  $O_2$  loses its spin restriction by accepting a single electron, which produces more reactive  $O_2^{\cdot-}$  called superoxide anion (Demidchik, 2017).

Superoxide anion itself does not cause extensive damage, but instead, it undergoes transformation into more toxic and reactive ROS like hydroxyl radical which further causes membrane lipid peroxidation (Halliwell, 2006).

#### 4.10.1.2. Hydrogen peroxide ( $H_2O_2$ )

$H_2O_2$  is formed through the quick conversion of superoxide spontaneously, or by the action of superoxide dismutase (SOD) (Rodrigo-Moreno et al., 2013). It is considered the most stable among all ROS (Demidchik, 2015). Its lifetime (up to 0.1–1 s) is considered comparably long among other ROS (Halliwell and Gutteridge, 2015). Due to the antioxidant enriched alkaline nature of cytosol, lower enzymatic activities and acidic nature of extracellular space,  $H_2O_2$  may accumulate mostly in

the apoplast, which promotes cell wall oxidative burst (Mhamdi et al., 2012). In addition, it can also be enzymatically produced in the apoplast by ammine oxidases, NADPH oxidases and extracellular heme containing Class III peroxidases (Demidchik, 2015).

Hydrogen peroxide has two sides depending on its concentration. Specifically, at low concentration, it acts as a signal for senescence (Peng et al., 2005), photorespiration and photosynthesis (Noctor et al., 2002), stomatal movement (Bright et al., 2006), cell cycle and growth in development (Tanou et al., 2010). At higher concentrations, it can oxidize cysteine and methionine and inactivates several enzymes responsible for the Calvin cycle as well as may elicit PCD (Dat et al., 2000).

#### 4.10.1.3. Hydroxyl radical ( $\cdot\text{OH}$ )

Hydroxyl radical is an extremely short living ROS and is highly toxic in the presence of transition metals (Demidchik, 2017). Therefore, it can damage all biomembranes by triggering a chain reaction of lipid peroxidation (Chen and Schopfer, 1999) and it is also involved in redox signalling and PCD caused by oxidative stress (Demidchik et al., 2003). Fenton like reactions synthesizes hydroxyl radicals in plants (Fenton, 1984). It can also be produced by homolytic bond fission of water, which requires significant energy input by heat, freeze-drying, UV or ionizing radiation (Halliwell, 2006; Halliwell and Gutteridge, 2015). Besides, hydroxyl radical can probably be directly generated from  $\text{H}_2\text{O}_2$  and hydroperoxides under natural conditions e.g., by sunlight, which requires much less energy (Halliwell, 2006).

#### 4.10.1.4. Singlet oxygen ( $^1\text{O}_2$ )

Singlet oxygen is exceptionally reactive among other ROS and it is formed through the rearrangement of the electrons in oxygen molecules. In the chloroplast and mitochondria, activation of oxygen through light absorption can lead to the formation of two types of singlet oxygen namely non-radical and free radical. Lifetime and diffusion distance of singlet oxygen is comparably short according to

Asada (2006). Based on singlet oxygen sensor green measurement (SOSG), it can diffuse outside of the chloroplast and reach apoplastic space (Driever et al., 2009). Singlet oxygen can be responsible for causing severe damage to photosystems (PSI and PSII) which can lead to light induced loss of PSII activity. Even though the diffusion length is short, it still manages to reach proteins, pigments, nucleic acids and lipids (Krieger-Liszkay et al., 2008). Alternatively, singlet oxygen up regulates some genes responsible for photo-oxidative stress prevention (Krieger-Liszkay et al., 2008).

#### 4.10.2. Major sites for ROS production

ROS are formed in all aerobic organisms during electron transport as byproducts. They are produced under both normal and stressful conditions at various locations, like in the chloroplast, mitochondria, peroxisomes, apoplast, endoplasmic reticulum, plasma membrane and cell wall.

Chloroplast contains a well-organized thylakoid membrane system with efficient light capturing machinery (Pfannschmidt, 2003). The photosystems, PSI and PSII in the thylakoid membranes are the major ROS production sites. Abiotic stresses such as drought, salinity and extreme temperatures cause the formation of ROS especially superoxide via Mehler reaction at the photosystems (Figure 3). Chloroplastic ROS is mitigated by a range of ROS scavenging enzymes and pathways such as Fe and CuZn-SODs and also by the Asada-Foyer-Halliwell pathway as well as high concentration of antioxidants like ascorbic acid and GSH (Mittler et al., 2004). Controlling and scavenging ROS in the chloroplast is critical since chloroplast is the major source of ROS production (Tseng et al., 2007).

Mitochondria is another site for the production of H<sub>2</sub>O<sub>2</sub> and superoxide (Navrot et al., 2007). Since mitochondria have O<sub>2</sub> and carbohydrate rich environment (Rhoads et 2006) and is involved in photorespiration it contains sufficiently energized electrons to reduce oxygen for ROS formation. The electron transport chain of mitochondria has two major components known as Complex I and III (Noctor et al., 2007). Oxygen is directly reduced to superoxide in the flavoprotein region of Complex

I or NADPH Dehydrogenase (Figure 3). Whereas in Complex III, ubiquinone donates an electron to cytochrome and creates ubisemiquinone that favors leakage of electrons and generates superoxide (Murphy, 2009). Moreover, there are several enzymes present at mitochondria that are directly and indirectly involved in ROS production which includes aconitase and GAL respectively (Rasmusson et al., 2008). Mitochondrial ROS production is shown to be greatly increased during abiotic stress conditions as supposed to normal conditions (Pastore et al., 2007). Mitochondrial ROS production is mitigated by AOXs, type II NAD(P)H dehydrogenase and uncoupling proteins in the inner mitochondrial membrane.

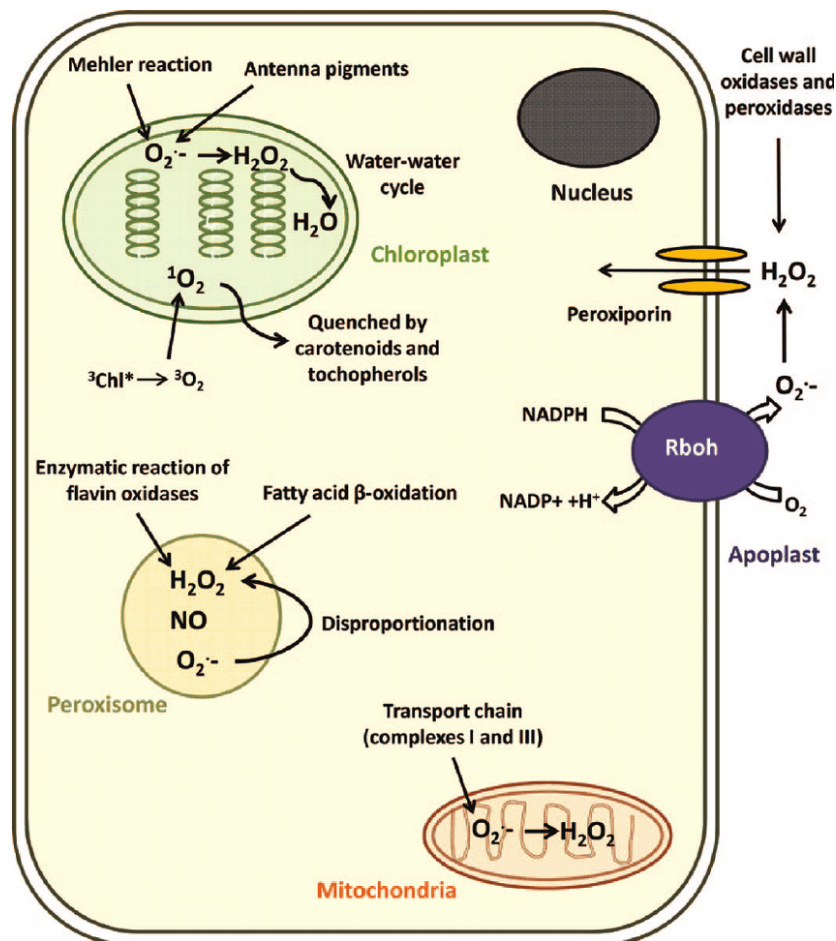


Figure 3. Major sites for ROS production. (Bose et al., 2013)

Peroxisomes are oxidative organelles responsible for intracellular  $\text{H}_2\text{O}_2$  production and scavenging (del Río et al., 2006).  $\text{H}_2\text{O}_2$  is produced by a number of metabolic processes in the peroxisome and is mostly generated at two different pathways. The first one is by xanthine oxidase in the peroxisomal matrix and the second one is by NADPH dependent small ETC, localized in the peroxisomal membrane, which releases  $\text{H}_2\text{O}_2$  into the cytosol (Figure 3). Peroxisome also generates large quantities of  $\text{H}_2\text{O}_2$  as a result of enhanced photorespiration by glycolate oxidase (Foyer and Noctor, 2009; Kerchev et al., 2016). Besides, fatty acid  $\beta$ -oxidation, flavin oxidase pathway and disproportionation of superoxide are additional metabolic processes producing ROS in the peroxisome.  $\text{H}_2\text{O}_2$  produced at the peroxisome can be mitigated by CAT (Kerchev et al., 2016).

In times of crisis, the space outside of the cell membrane known as apoplast becomes the site for  $\text{H}_2\text{O}_2$  production through the combination of stress signals and ABA (Hu et al., 2006). In particular, there are several apoplastic enzymes responsible for producing  $\text{H}_2\text{O}_2$ , which includes cell wall linked oxidases and polyamine oxidases etc., Apoplastic ROS during abiotic stress is alleviated by CuZn-SODs, APX, peroxidases that are bound to the cell wall and low level of ascorbate and GSH.

The NADPH mediated electron transport localized in the chloroplast thylakoids generates superoxide (Mittler, 2002). Organic substrate interaction followed by flavoprotein reductase produces free radical intermediate which further reacts with triplet oxygen to form complex, generating superoxide as a byproduct.

The plasma membrane surrounding a plant cell plays a key role by interacting with the outside environment and providing the cell with the necessary information for survival. Depending on the stress conditions, NADPH dependent oxidases localized at the plasma membrane are considered the most widely studied mechanism for ROS production (Apel and Hirt, 2004). Electrons transferred from cytosolic NADPH to oxygen yield superoxide anion which is dismutated into  $\text{H}_2\text{O}_2$  by SOD (Figure 3).

Other sources of ROS in the cell wall during stress conditions are cell wall localized lipoxygenases causing hydro peroxidation of polyunsaturated fatty acids (PUFA). According to Higuchi, (2006), lignin precursors undergo cross linking via hydrogen peroxide mediated pathways to reinforce the cell wall with lignin during pathogen attack.

#### 4.10.2.1. Lipoxygenases

Lipoxygenases (LOXs) are ubiquitous in the animal and plant kingdoms and are involved in various physiological processes (Brash, 1999). LOXs are a group of non-heme iron containing enzymes, which are responsible for the conversion of polyunsaturated fatty acids (PUFA) and lipids into hydroperoxyl fatty acids that may be degraded into oxylipins (Blée, 2002). In animals, LOXs are involved in the formation of leukotrienes and lipoxins, which are regulatory compounds mediating inflammatory responses (Samuelsson et al., 1987). Whereas in plants, LOXs are suggested to play a role in growth and development (Keereetaweep et al., 2015), synthesis of aroma compounds (Shen et al., 2014), ethylene synthesis (Griffiths et al., 1999), ripening and senescence (Hou et al., 2015) and most importantly, defense against biotic and abiotic stresses (Maschietto et al., 2015; Porta et al., 2008).

LOXs have been suggested to form biologically active compounds both during the plant's normal development and during environmental stress conditions (Savchenko et al., 2014). LOXs are classified as 9-LOX and 13-LOX, catalyzing the oxygenation of linoleic acid (LA) and linolenic acid (ALA), respectively (Feussner and Wasternack, 2002). 13-LOX pathway occurs in chloroplast while 9-LOX pathway occurs in cytosol (Porta and Rocha-Sosa, 2002).

There are primary and secondary pathways that are activated in response to physical e.g. wounding stress. These include hydroperoxide lyase (HPL) and allene oxide synthase (AOS) activities, which are responsible for green leaf volatiles (GLVs) and jasmonic acid production respectively (Birkett et



al., 2000). Volatile C6 and C9 aldehydes are products of unsaturated fatty acids metabolized by LOX and HPL and are important components of the aroma and flavor of fruits and vegetables such as tomato (Baldwin et al., 1991), cucumber (Chen et al., 2015), apple (Echeverría et al., 2004) and strawberry (Pérez et al., 1999) etc., These compounds are also released in response to plant tissue disintegration. The most important antimicrobial and fungicidal agents such as hexenals and hexenols are examples of C6 compounds which provide primary defense against pathogens (Hughes et al., 2009).

Various studies have been conducted on LOX gene expression and LOX activity regulated by biotic stress, wounding, low and high temperatures, hormones and signaling substances (Hu et al., 2015). In *Arabidopsis*, AOS expression was significantly induced along with JA level by wounding treatment (Park et al., 2002). The *LOX3* gene of *Arabidopsis* was induced by salt treatment (Ding et al., 2016), and overexpression of *DkLOX3* showed more tolerance to *Botrytis cinerea* (Hou et al., 2018). Upregulation of *LOX* genes was found in *A.thaliana* leaves due to cucumber mosaic virus (CMV) infection (La Camera et al., 2009). Currently, six *LOX* genes have been identified in *Arabidopsis thaliana* (Bannenberg et al., 2009) with proven enzymatic activity, each involved in various physiological processes and defense mechanisms.

Also, in tomato, six genes are identified, with two of them (*TomLoxA* and *B*) expressed in ripening fruits (Griffiths et al., 1999), *TomloxC* is found to be involved in production of flavor compounds, whereas *TomloxD* besides being expressed in fruits was also induced by wounding (Chen et al., 2004; Heitz et al., 1997). *TomloxE* is present in breaker stage fruits (Chen et al., 2004) while *TomloxF* encodes a 13-LOX protein (Mariutto et al., 2011) with expression stimulated by *Pseudomonas putida* BTP1 infection.

In maize, the *ZmLOX10* gene is up regulated by wounding and cold stress (Christensen et al., 2013). In brassica seedlings, drought stress increased LOX activity considerably (Alam et al., 2014). Salt stress significantly increased LOX activity in rice (Mostofa et al., 2015). In potato, PotLOX1 is found

to be responsible for tuber development (Kolomiets et al., 2001). According to Lim et al., (2015), the *CaLOXI* gene of pepper was shown to decrease the accumulation of hydrogen peroxide and downregulate lipid peroxidation through ABA and other gene regulation pathways. Moreover, in kiwi fruit, six *LOX* genes have been identified, which were found to be differently regulated during fruit ripening and senescence (Zhang et al., 2006).

Matsui et al., (1998) isolated cucumber root lipoxygenase, *CsLOXI*, which was shown to be able to act on acyl groups in phosphatidylcholine. In cucumber, the *LOXI* gene showed early expression during seed germination stage and it was shown to be localized in the lipid body (Matsui et al., 1999). More recently, fungicidal activity caused by mechanical wounding on cucumber was shown by aldehydes derived from fatty acid metabolism (Matsui et al., 2006). Genome wide analysis of the cucumber *LOX* genes was conducted by Liu et al., (2011) resulting 23 candidate genes. Yang et al., (2012) reported 13 detectable *CsLOX* transcript of cucumber out of 23 candidate genes and analyzed their expression during fruit development.

#### 4.10.3. ROS scavenging

Higher plants evolved to protect themselves from ROS toxicity and to use ROS as signalling molecules by acquiring various pathways (Mignolet-Spruyt et al., 2016). Accumulation and overproduction of ROS cause oxidative damage to plant cell membranes, proteins, RNA and DNA molecules, which can lead to oxidative stress (Mittler, 2002). Therefore, ROS detoxification systems must be present in all plants, which include enzymatic antioxidant components such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), glutathione reductase (GR) enzymes and non-enzymatic antioxidants like phenolic compounds, non-protein amino acids and carotenoids (Mittler et al., 2004). Tolerance of stress is directionally proportional to these antioxidant components because they serve as the first line of

defense (Demidchik, 2017). Si treatment is shown to increase the activity of key antioxidant components (Kim et al., 2017).

During abiotic stress conditions, two types of ROS may be produced which are metabolic ROS and signaling ROS. Metabolic ROS are responsible for flux control and alter different metabolic reactions (Miller et al., 2010). Whereas, signaling ROS, produced by various enzymes, e.g. by NADPH oxidase at the plasma membrane (Suzuki et al., 2012) are considered as stress sensors (Miller et al., 2010). ROS scavenging occurs in all cell compartments and each organelle compartment establishes and controls its own ROS homeostasis which can generate stress specific signals creating tailored acclimation response to specific stresses or their combination affecting the plant (Choudhury et al., 2017).

#### 4.10.3.1. Enzymatic antioxidants

At times of environmental adverse effects, SOD serves as the first line of defense against damage caused by ROS. It catalyzes the dismutation of the superoxide radical into oxygen and hydrogen peroxide. This further prevents the formation of hydroxyl radicals by the Haber-Weiss reaction. Superoxide dismutases have been classified into three families of isozymes according to the metal they bind. The first isozyme class is Mn-SOD localized in mitochondria, the second is Fe-SOD localized in chloroplasts and the last is Cu/Zn-SOD which is localized in cytosol, peroxisomes and chloroplasts (Mittler, 2002). SODs are upregulated by several abiotic stresses (Boguszewska et al., 2010) such as salinity stress in chickpea (Kukreja et al., 2005) and tomato (Gapińska et al., 2007). Whereas in grafted cucumber leaves, Mn-SOD and Cu/Zn-SOD gene expression and activities were higher during low temperature stress (Gao et al., 2009b). Hence, increased SOD activity and gene expression with elevated Mn, Cu and Zn amounts were reported under low temperature by Gao et al., (2009) in a cucumber cultivar.

CAT is present in almost all living organisms exposed to oxygen and it is responsible for catalyzing  $H_2O_2$  into water molecules and oxygen in the cells exposed to environmental stresses. It is considered the most energy efficient antioxidant enzyme not requiring reducing equivalent. Catalase is located in all major sites of  $H_2O_2$  production such as peroxisome, mitochondria, cytosol and chloroplast (Mhamdi et al., 2010). Catalase isozymes are encoded by *Cat* genes in angiosperms, which include *Cat1* expressed in pollen and seed, *Cat2* expressed in photosynthetic tissues in addition to roots and seeds, whereas *Cat3* is expressed in leaves and vascular tissues. CAT activity was found to be increased by water stress in drought-sensitive varieties of wheat (Simova-Stoilova et al., 2010). *Cicer arietinum* under salt stress also has increased CAT activity in both leaves (Eyidogan and Öz, 2007) and roots (Kukreja et al., 2005).

Ascorbate peroxidase (APX) is an essential component of the Ascorbate-Glutathione (Halliwell-Asada) cycle. It belongs to class I of peroxidase enzymes and predominantly scavenges hydrogen peroxide in the cytosol and chloroplast. Specifically, it catalyzes  $H_2O_2$  decomposition with the help of ascorbic acid into water and dehydroascorbate (DHA). APX isoenzymes with different characteristics are found in different cell compartments (Shigeoka et al., 2002). These isoenzymes are cytosolic, thylakoid membrane bound, stromal and microbody membrane bound, which are all differently respond to metabolic and environmental signals (Yoshimura et al., 2000). APX reduced the toxic effects of  $H_2O_2$  and generated drought tolerance in *Nicotiana tabacum* when overexpressed in the chloroplasts (Badawi et al., 2004). UV-B radiation increased APX activity in *Arabidopsis thaliana* (Rao et al., 1996). The activity of APX positively correlated with Pb treatment in *Eichhornia crassipes* seedlings (Malar et al., 2016).

GPX is another key enzyme responsible for removing  $H_2O_2$  during both normal and stressful conditions. It is also important for lignin biosynthesis and is involved in defense against biotic stresses. Electron donors of GPX are preferably aromatic compounds like guaiacol and pyrogallol (Asada,

1999). Hence, it is intracellularly active in the cytosol and vacuole and extracellularly active in the cell wall. According to Kandziora-Ciupa et al., (2013), GPX activity is responsive to and well correlated with heavy metal stress. The activity of GPX increased both salt-sensitive and salt-tolerant rice varieties during CdCl<sub>2</sub> stress (Roychoudhury et al., 2012a) and in drought stress (Basu et al., 2009).

GR is an integral enzyme of the Ascorbate Glutathione cycle that converts GSH to its more oxidized form GSSG using NADPH as reductant. Reduced GSH is further used up to regenerate ascorbic acid from MDHA and DHA. GR belongs to flavoprotein oxidoreductases and catalyzes the formation of reduced GSH from glutathione disulfide to maintain a high cellular GSH/GSSG ratio. GR is mainly found in chloroplasts and it also occurs in mitochondria and cytosol at lower amounts. Stressed rice seedlings displayed an increased activity of GR enzymes, which are involved in the regeneration of AA (Sharma and Dubey, 2005). Under salt stress, APX and GR activities were found to be higher in salt-tolerant cultivars of potato but found lower in salt-sensitive varieties. Salt sensitivity was attributed to the reduction of APX and GR activity during saline conditions (Aghaei et al., 2009).

#### 4.10.3.2. Non-enzymatic antioxidants

Non-enzymatic antioxidants are ascorbic acid, reduced glutathione,  $\alpha$ -tocopherol, carotenoids, phenolic compounds, flavonoids and proline. They function to orchestrate a plant's regular growth and development processes through cell elongation, timely senescence and cell death while protecting against cell damage (de Pinto and De Gara, 2004).

Ascorbic acid (AA) is undoubtedly at the first line of defense against ROS elicited damage (Barnes et al., 2002). It is an extensively studied antioxidant that donates electrons to various enzymatic and non-enzymatic reactions. AA is mainly formed by the Smirnoff-Wheeler pathway through a reaction catalyzed by of L-galactano- $\gamma$ -lactone dehydrogenase and a relatively small amount is also generated

from D-galacturonic acid. It is mainly concentrated in the cytosol and a considerable amount is present in the apoplast. AA protects the membranes from oxidative damage by reacting with hydrogen peroxide, hydroxyl and superoxide radicals. It can also regenerate  $\alpha$ -tocopherol from tocopheroxyl radical (Shao et al., 2005). It also prevents photo-oxidation of PSII by pH mediated modulation and downregulation of its activity. During drought stress, exogenous application of AA to wheat cultivars resulted in higher growth, chlorophyll content and, net photosynthesis compared to non-treated plants (Malik and Ashraf, 2012). In another example, exogenous application of AA before and during salt stress accelerated the recovery process and ensured long-term survival of tomato seedlings (Shalata and Neumann, 2001). AA also helped to relieve oxidative damage in wheat, by improving photosynthetic capacity and sustaining ion homeostasis (Athar et al., 2008).

Glutathione (GSH) is a low molecular weight thiol tripeptide abundant in nearly all cellular compartments which is involved in a wide range of plant cellular processes (Mullineaux and Rausch, 2005). As mentioned in the section dealing with GR, GSH scavenges ROS and generates GSSG as a byproduct. It also regenerates AA to yield GSSG. It is also involved in the formation of phytochelatins that are able to chelate heavy metal ions (Roychoudhury et al., 2012b). Arsenic (III) significantly decreased the GSH content in rice roots, due to its conversion to phytochelatins. GSH supplementation resulted in partial protection against arsenic stress, reducing the MDA content and restoring the growth of arsenic (V) exposed seedlings (Roychoudhury and Basu, 2012). GSH was also found to lessen the oxidative damage in rice chloroplasts caused by salinity stress.

$\alpha$ -tocopherol belongs to a family of lipophilic antioxidants that efficiently scavenges ROS (Holländer-Czytko et al., 2005). It has higher antioxidant capability than the other three tocopherol isomers. Tocopherols are synthesized and are present only in green plant tissues. They have the ability to react with oxygen to protect lipids and chloroplast membrane constituents and also protect PSII structurally and functionally by quenching excess light energy. Moreover, it is considered an effective free radical

trap by halting the chain propagation step of the LPO cycle. Acute exposure of UV-B leads to a decrease in  $\alpha$ -tocopherol levels in cucumber, possibly reflecting reactions with lipid radicals (Jain et al., 2003).

Carotenoids form another group of the lipophilic antioxidant family that is localized not only in photosynthetic but also in non-photosynthetic plant tissues. There are four ways in which carotenoids protect photosynthetic machinery. Primarily, they react with LPO products to end the chain reaction. Secondly, they scavenge singlet oxygen and generates heat as a by-product. Third, they react with triplet Ch ( $^3\text{Ch}^*$ ) and excited chlorophyll ( $\text{Ch}^*$ ) to prevent the formation of singlet oxygen. Lastly, they dissipate excess excitation energy via the xanthophyll cycle. In drought-resistant plants, the number of carotenoid molecules per chlorophyll unit increased under drought stress, thus their providing photo-protection from oxidative damages was concluded (Munné-Bosch and Alegre, 2000).

Flavonoids commonly occur in the leaves, floral organs and pollen grains of a number of species in the plant kingdom. They are divided into four classes based on their structures namely flavonols, flavones, isoflavones and anthocyanins. They are also diverse in terms of their role in pigmentation, germination and defense against plant pathogens and abiotic stresses. Flavonoids are known as a secondary ROS scavenging system for plants with photosynthetic apparatus damage due to excess excitation energy (Fini et al., 2011). According to Agati et al., (2012), flavonoids also has the ability to alleviate damages caused to the thylakoid membrane of the chloroplast and scavenge singlet oxygen. In water deficit affected rice cultivars, tissue concentrations of antioxidants like flavonoids and phenolics were found to be several folds higher in the tolerant cultivar than in sensitive varieties (Basu et al., 2009).

Proline is a powerful osmolyte also bearing antioxidant activity. It is synthesized from glutamic acid as a substrate via pyrroline 5 carboxylate (P5C) intermediate. Its biosynthesis is catalyzed by two enzymes P5C synthase and reductase. Proline is an efficient scavenger of hydroxyl radical and singlet

oxygen and also inhibits damages caused by LPO. During stress, it accumulates in large quantities due to enhanced synthesis or reduced degradation (Verbruggen and Hermans, 2008). In the case of rice seedlings exposed to high salt stress, proline showed higher level in a salt-tolerant cultivar as compared to salt-sensitive cultivars (Roychoudhury et al., 2008). The content of flavonoids and proline were also found to be enhanced in salt-tolerant cultivars of indica rice, which probably contributed to reduced membrane damage caused by LPO (Chutipajit et al., 2008). Proline, an osmoprotectant as well as a sink for energy to regulate redox potential, was found to have increased accumulation in drought-tolerant cultivars of chickpea under both control and drought stress conditions (Mafakheri et al., 2010).



## 5. MATERIALS AND METHODS

### 5.1. Plant material, growth conditions and Si treatment

Cucumber (*Cucumis sativus* L.) F1 hybrid ‘Dirigent’ cultivar was chosen as plant material. Seeds were germinated overnight at 24°C in 100 ml distilled water. Seeds were then transferred to pots (7.5 × 7.5 × 6.5 cm) filled with perlite medium (4 seeds in each pot) and grown in a controlled environment chamber. Growth conditions of 14/10 hours’ day/night illumination cycle (140  $\mu\text{mol m}^{-2} \text{s}^{-1}$  photosynthetic photon flux density) with a relative humidity of 55-60% and temperature of 26°C were applied throughout the experiments. Four pots were placed in a reservoir, with approximately 400 ml culture medium covering the bottom of the pots up to ~2 cm high. The plants were irrigated with distilled water for a week (until cotyledons appear) and then treated with half strength Hoagland solution (pH 5.8) (Millner and Kitt, 1992).

**Table 2.** Composition of Hoagland solution

0.5 x Hoagland solution (pH 5.8)	
Macroelements	Concentration (mM)
Ca(NO <sub>3</sub> ) <sub>2</sub>	2
MgSO <sub>4</sub>	1
KCl	0,5
KH <sub>2</sub> PO <sub>4</sub>	0,5
Na <sub>2</sub> HPO <sub>4</sub>	0,5
Microelements	Concentration ( $\mu\text{M}$ )
MnSO <sub>4</sub>	10
ZnSO <sub>4</sub>	0.5
CuSO <sub>4</sub>	1
(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub>	1
H <sub>3</sub> BO <sub>4</sub>	100
Fe-EDTA	200

Cucumber plants were treated with and without silicate supplementation (1.67 mM Si in the form of Na silicate, pH 5.8) (Flam-Shephard, 2016).

The fertigation solution in the reservoir was discarded every other day, and pots were flushed with a new solution from above. In the days between changing the media, the pH of the reservoir was adjusted to 5.8 with 1M citric acid. Samples were taken between 1-2 pm on day 31 after germination, then frozen immediately in liquid nitrogen and stored at -80°C for further use.

## **5.2. Element analysis**

Leaves were cut individually and cleaned by soaking in tap water for several minutes. The leaf dry weight (DW, g) was measured after the leaves were dried in the 40°C oven for 48 hours.

For nitrogen content determination,  $3 \pm 0,001$  g of dried plant samples were placed in a crucible inside an incandescent oven. The annealing furnace temperature was then raised to 500°C and the samples were incinerated. After cooling, 5 cm<sup>3</sup> of 1 mol/l HCl solution is added to the crucible. The contents of the crucible were transferred to a filtered funnel and washed with distilled water. The filtrates are collected in a 50 cm<sup>3</sup> volumetric flask. Quantification of the nitrogen content is performed according to the standard MSZ-08 1783/6.

Sodium and silicon contents were determined according to the standard MSZ-08 1783/5 where  $0,5 \pm 0,01$  g of plant samples were weighed and incinerated. Then hydrochloric acid is added to the ash and the whole is washed with the distilled water into a 100cm<sup>3</sup> volumetric flask. The elements measurements were performed using an ICP-OES Ultima 2 (Jobin Yvon Horiba) instrument.

## **5.3. Chlorophyll and carotenoid contents**

Chlorophyll and carotenoid contents were estimated according to the method of Arnon, (1949). Leaf tissue (0.1g fresh weight, FW) was ground in 3ml 80% acetone with a pinch of Sodium carbonate using pre-cooled mortar and pestle. Homogenized samples were transferred to polyethylene conical

tubes and the final volume was made up to 10ml with the addition of 80% acetone. The slurry was centrifuged at 1000 rpm for 5min at 4°C. The cleared solution was transferred to cuvettes and the optical density was measured at wavelengths of 663 nm, 644 nm and 480 nm against a blank (80% acetone) in a spectrophotometer. Levels of chlorophyll 'a' and 'b' as well as carotenoid contents were calculated (mg pigment/g FW) as:

$$\text{Chl a} = 12.7 \times A_{663} - 2.69 \times A_{644}$$

$$\text{Chl b} = 22.9 \times A_{644} - 4.68 \times A_{663}$$

$$\text{Cc} = 5.01 \times A_{480}$$

where Chl a and Chl b are chlorophyll 'a' and chlorophyll 'b', Cc is carotenoid content and A is absorbance.

#### **5.4. Thiobarbituric acid reactive substances**

To assay lipid peroxidation, the method of Heath and Packer (1968) was adapted with slight modification. The concentration of thiobarbituric acid reactive substances (TBARS) was measured. Leaf samples (0.1g FW each) were taken and ground in 1mL 0,1% trichloroacetic acid (TCA) for extraction. 20µl of 20% butylated hydroxytoluene (BHT) was added to the solution and mixed by vortexing. Homogenized samples were centrifuged at 13000 rpm for 10 min at 4°C to remove insoluble material. 0.25mL supernatant was transferred into a 2mL Eppendorf tube containing 1mL of 0.5% TBA in 20% TCA solution and gently mixed. The samples were kept at 96°C in a heating block for half an hour. Following incubation, tubes were placed on ice for 10 min and centrifuged again at 13000 rpm for 10 min at 4°C. Finally, absorption was measured at 532 nm and 600 nm, data were analyzed with Gen5 software (Powerwave XS2, Biotek, USA). TBARS concentration was calculated using an extinction coefficient  $\epsilon=155\text{mM}^{-1} \text{ cm}^{-1}$ .

### **5.5. Hydrogen peroxide and lipid peroxide assay**

For hydrogen peroxide and lipid peroxide assay, 0.2g leaf samples were homogenized in 1mL 10% H<sub>3</sub>PO<sub>4</sub>. The supernatant was used for the determination of H<sub>2</sub>O<sub>2</sub> and lipid peroxide according to Wolff's (1994) assay. The reaction mixture for determination of H<sub>2</sub>O<sub>2</sub> assay contained 100 μM xylenol orange, 250 μM ammonium ferrous sulfate, 100 mM sorbitol, 25 mM H<sub>2</sub>SO<sub>4</sub> plus 50μl of sample extract in a total volume of 1mL. Lipid peroxide assay solution was: 100 μM xylenol orange, 250 μM ammonium ferrous sulfate, 4 mM butylated hydroxytoluene, 25 mM H<sub>2</sub>SO<sub>4</sub> dissolved in 90% methanol and 50 μl of sample extract in a total volume of 1mL. Both assays had the same incubation time and absorbance was read at wavelength 560 nm. H<sub>2</sub>O<sub>2</sub> was used for calibration.

### **5.6. RNA isolation**

Tissue samples (100mg each) were homogenized in 1mL TRIZOL reagent using liquid nitrogen in a precooled mortar and pestle. Then samples are vortexed vigorously and kept at RT for 5 minutes. Then centrifuged at 13,000 rpm for 15min at 4°C. The supernatant is then transferred to a new 1.5mL Eppendorf tube. Phase separation is carried out using 0.2mL chloroform with centrifugation speed of 12,000rpm for another 15 min at 4°C. After the separation of two phases, the upper aqueous phase (60% of the total reagent used) is transferred carefully to a new tube. RNA is precipitated by mixing it with 0.5mL isopropyl alcohol and incubated at RT for 10 min until centrifugation. After the precipitation RNA is washed with 75% cold ethanol twice and centrifuged for 5 min in between. Finally, the RNA pellet is air dried for 5-10 minutes and dissolved in 50μl MQ water. RNA concentration was measured with an ND-1000 instrument from NanoDrop®.

## 5.7. mRNA analysis

### 5.7.1. cDNA synthesis

cDNA synthesis was performed on quantitatively normalized total RNA samples with Thermo Scientific First strand cDNA Synthesis kit, used with oligo(dT)<sub>18</sub> primers.

**Table 3.** Reagents for cDNA synthesis

Component	Amount
Template RNA	1 µg
oligo(dT) <sub>18</sub> primer	1 µl
5X Reaction buffer	4 µl
RiboLock RNase inhibitor (20U/µl)	1 µl
10mM dNTP mix	2 µl
M-MuLV reverse transcriptase (200U/µl)	0.2 µl
Nuclease free water	to 20 µl

All the ingredients in Table 3 were gently mixed and briefly centrifuged then incubated at 37° for an hour. Then the gDNA contamination of the synthesized cDNA was removed by using DNase I, RNase free kit by Thermo Fisher Scientific according to manufacturer's instruction. Each 1 µg of RNA was mixed with 1 µl of 10 × reaction buffer containing MgCl<sub>2</sub> and another 1 µl of DNase I reagent (RNase-free/1U) and finally, Nuclease free water was added up to 10 µl in volume. Hence, the prepared mixture was incubated at 37°C for half an hour. Then 1µl 50 mM EDTA was added to each tube and further incubated at 65°C for 10 min.

### 5.7.2. Primers

Actin7 gene was chosen as the endogenous control (reference gene) in order to normalize the signal value of each sample. A housekeeping gene is required to accomplish the determination of the actual

biological difference between control and treated samples. Efforts were also made to minimize mechanical errors such as inconsistent loading. Actin gene and respective primers were selected according to Wan et al., (2010), which showed stable expression on all the tested cucumber samples compared to other housekeeping genes. Other genes and specific primers were selected based on literature data and showed distinctive expression patterns in cucumber cultivars.

Silicon influx and efflux transporter genes are chosen accordingly (Sun et al., 2018; Wang et al., 2014). Moreover, 12 lipoxygenases were selected based on their detectable expression described by Yang et al., (2012). (Table 4).

**Table 4.** Primers used for PCR and qPCR

<b>Gene name</b>	<b>Cucurbit genomic database</b>	<b>Gene description</b>	<b>Forward primer (5'-3')</b>	<b>Reverse primer (5'-3')</b>	<b>Amplicon size (bp)</b>
Actin-7	CsGy2G015500	Reference gene	TCGTGCTTGACTCTGGTGATGG	TTCCCGTTCGGCAGTGGTTGT	171
CsLsi2	CsGy3G017160	Si transporter	TCTCCAACCGAAGAAAACG	TGGCAGCTGACCCTAACAAT	88
CSiT1	CsGy3G036580	Si transporter	CCGGTTGTTGGA ACTCAACT	GTGCACAGGTTTG TCACTGG	69
CSiT2	CsGy3G036600	Si transporter	AGCCATTGCAAGTTCACGTT	AATGATGCAAGCAAGGTTCC	78
LOX1	Cucsa.091300.2	lipxygenase	AGGACTTCCTGTTCCATTGGA	TGGAGGAAATTCCTTAAGACGA	227
LOX2	Cucsa.091340.1	lipxygenase	TCCAATTCATAAGCTACTTGTTCCT	ACCTTTCTCTCTCTTGATGAGATCA	208
LOX4	Cucsa.091290.1	lipxygenase	GCAACTCATGGGTTTACCC	CTTGACGCTCTCCTTTTCC	156
LOX8	Cucsa.091380.1	lipxygenase	TCCTGCTGACAAATACAAAACC	CTTTTGGATCTTTTTTGGAAAG	297
LOX9	Cucsa.185530.1	lipxygenase	TCGTCTCCTAGAGTTCCTCC	AATCATTACGGCGACATAA	395
LOX10	Cucsa.091390.2	lipxygenase	TGGGAGAAGAAGCATACTTAGAGG	GGAAGATAGCTCTGGTTGAGAAAG	286
LOX16	Csa4G286960.1	lipxygenase	TATAGCGCAAAGAAATTGTTCG	TGGGCTTTAGCAAGCCTC	255
LOX17	CsaV3_7G027160	lipxygenase	CCTCGTCGAATAAGAACTCTTC	GTGGAAGGTTGATAGTTTAGGG	188
LOX19	Cucsa.065250.1	lipxygenase	CTCATTGGCTGAGAACTCATTG	TGAATTAAGTCAGCAGGTAAAGCT	271
LOX20	Csa4G288610.1	lipxygenase	TTGCTTCCAATAAGATGGGAC	TCCCGTTACAGGATCAACCT	264
LOX22	Csa7G449420.1	lipxygenase	TCTCTAATAAGCCTTATCTTCCAGGT	TGCAGTCATATCAGTCTCGGA	260
LOX23	Cucsa.075520.1	lipxygenase	TAAATCATTGGTTAAGGACTCACG	CCATACCCCTTCGAATAAGATC	285

### 5.7.3. RT-PCR

PCR was performed using Dream Taq polymerase (Thermo Scientific®, USA) with silicon transporter genes (Sun et al., 2018; Wang et al., 2014) and primers specific to LOX genes (Yang et al., 2012). The thermal cycling parameters were as follows: 30 cycles at 95°C for 30 sec, 58°C for 30 sec, and 72°C for 1min, with the pre-denaturation cycle at 95°C for 2 min and 30 sec, and a final extension of 72°C for 5 min in Mastercycler (Eppendorf®, Germany). The amplified PCR products were analyzed by electrophoresis on 1.5% agarose gels. Control Actin7 gene (CsGy2G015500) sequences were amplified with the gene specific primers listed in Table 4.

**Table 5.** RT-PCR composition

Component	Amount per sample
10 x Dream Taq buffer	5 µl
10 mM dNTPs	1 µl
10 pmol Forward primer	1 µl
10 pmol Reverse primer	1 µl
Dream Taq polymerase (200U/µl)	0.25 µl
Template cDNA	1 µl
Nuclease free water	15.75 µl
Total volume	25 µl

### 5.7.4. Agarose gel electrophoresis

DNA fragments were separated by 1.5% agarose electrophoresis gel. The gels were prepared by dissolving the LE Agarose (SeaKem®) in the 1 × TBE buffer. To visualize the DNA, Eco Safe nucleic acid staining solution (Pacific image Co) is used. To load the samples, 12 µl PCR product was mixed with 2 µl 6 × DNA loading dye by Thermo Fisher Scientific (In case of Green 10 × Dream Taq buffer, no loading dye is required). Electrophoresis was performed at 80 – 100 V for 30 min to an hour.



Amplifications for gene specific primers were visualized under a BIO-RAD gel documentation system and the size of the fragments was determined using GeneRuler 1kb and 100 bp (Thermo Fisher Scientific).

#### 5.7.5. RT-qPCR

Silicon transporter (Holz et al., 2019) and a control actin gene (XM\_004147305) sequences were amplified with the gene specific primers listed in Table 4. RT-qPCR was done by using HOT FIREPol EvaGreen qPCR supermix (Solis Biodyne, Estonia) in StepOnePlus Real-Time PCR system (Applied Biosystems, USA).

**Table 6.** RT-qPCR composition

Component	Amount per sample
5x HOT FIREPol EvaGreen qPCR Supermix	2 $\mu$ l
Primer Forward (10 pmol/ $\mu$ l)	0.2 $\mu$ l
Primer Reverse (10 pmol/ $\mu$ l)	0.2 $\mu$ l
DNA template	1 $\mu$ l
RNase free water	6,6 $\mu$ l
Total volume	10 $\mu$ l

The qPCR cycles were set according to the manufacturer's instruction by Solis BioDyne Super mix dye. PCR amplification was initiated with DNA denaturation at 95 °C for 12 min in order to activate the polymerase. As the product amplicon of the reaction was assumed to have a length shorter than 150 bp, 20 seconds of annealing and elongation were adjusted. The annealing temperature of the reference gene was different than the other gene specific primers. A melting curve analysis was performed (65–95 °C) at the end of the run and the PCR product specificity was confirmed. The scheme of the temperature, time and cycles of the qPCR were in accordance with Table 7.

**Table 7.** Scheme for RT-qPCR

Cycle step	Temperature			Time	Cycles
	Actin7	Si transporters	LOX		
Initial activation		95 °C		12 min	1
Denaturation		95 °C		15 s	
Annealing	60 °C	58 °C	60°C	20 s	40
Elongation		72 °C		20 s	

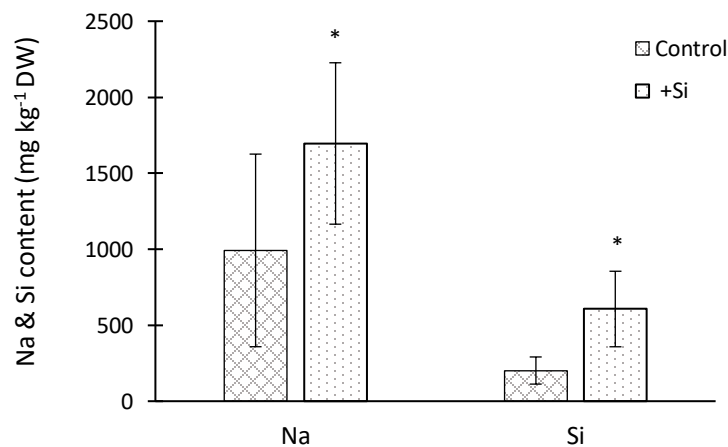
### 5.8. Statistical analysis

All data were presented as mean  $\pm$  standard deviation (SD). The comparison of means and the correlation analysis were performed using R (project for statistical computing) software. Significant differences ( $p < 0.05$ ) of means were determined by one-way ANOVA with Duncan's post-hoc test. Fold changes of induction were calculated according to the  $2^{-\Delta\Delta Ct}$  method by Bookout and Mangelsdorf (2003).

## 6. RESULTS AND DISCUSSION

### 6.1. Element analysis

To estimate the efficiency of silicon supplementation in our experiments, relevant elements of control and treated plants were measured. Treated plants had higher Si levels ( $681.68 \pm 190.33 \text{ mg kg}^{-1}$ ) in leaves when compared with control ( $212.561 \pm 96.104 \text{ mg kg}^{-1}$ ) plants (Figure 4), approving uptake of this element under the conditions applied. Si content of treated leaves in our system was lower than some values reported earlier in other studies (Sun et al., 2017). Differences may be due to dissimilar treatment conditions, i.e. continuous exposure from an early stage of development vs. sudden exposure in hydroponics.



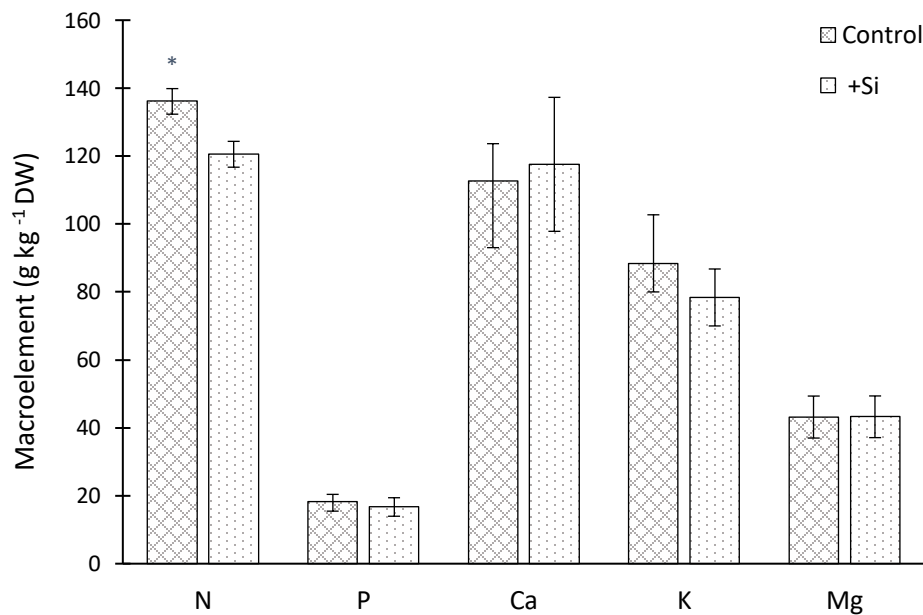
**Figure 4.** Sodium and Silicon contents in cucumber plants grown in perlite medium. Bars represent mean values per kg (DW)  $\pm$  SD from at least two biological replicates. Asterisks indicate a statistically significant difference at  $P < 0.05$

The sodium content of leaves was also analyzed in treated and control plants (Figure 4). Interestingly, the sodium content of treated leaves was higher ( $1725.098 \pm 243.48 \text{ mg kg}^{-1}$ ) than that of control ( $743.73 \pm 257.18 \text{ mg kg}^{-1}$ ), which is probably due to supplementation of this element in sodium silicate. Regardless of supplementation of Na, most crops including cucumber translocate very little Na to reproductive structures such as seeds, fruits or storage roots, which are the edible portions of many

crops, since translocation of such element is highly restricted in phloem. Besides, plant cells have various transport proteins including antiporters for reducing the internal concentration of this toxic ion (Pessarakli, 2001).

Although beneficial effects of the treatment could still be observed, this effect should be routinely considered when Si is supplemented in the form of sodium salt. Especially in irrigated lands since irrigation continuously delivers some Na salts, although at low concentrations. Besides, build-up of Na salt in soil is evident due to the plants' restrictive nature against Na uptake and favoring of K instead (Taiz et al., 2015).

Based on the element analysis conducted on major macro elements, no significant differences have been shown between control and treated plants except for nitrogen (Figure 5). Nitrogen content has decreased significantly with Si supply (Control:  $136.133 \pm 3.729$ ; Si:  $120.528 \pm 3.804$  g kg<sup>-1</sup>).



**Figure 5.** Macro element contents in cucumber plants grown in perlite medium. Bars represent mean values per dry weight (DW)  $\pm$  SD (n = 6). The asterisk indicates a significant difference between control and silicate treatment at  $P < 0.05$ .

Connection between nitrogen metabolism and silicon has been studied recently under stress conditions and also in non-stressed plants with variable results and conclusions. In case of 16 wetland plant species silicon and nitrogen contents were negatively correlated (Schaller et al., 2016). In 4 grassland species however no such correlation was found (Hao et al., 2020). Positive effect of silicon in non-stressed rice plants was attributed to changes in primary metabolism and source-sink relations. In this case a nominal decrease of nitrogen content was detected upon silicon nutrition in wild type (degrained) plants (Detmann et al., 2012). Improved nitrogen use efficiency was suggested as underlying the positive effect of silicium in several cases (Detmann et al., 2012). Effects of silicon in case of excess nitrate stress was attributed to enhanced nitrogen assimilation by Gou and co-workers (2020). Similar findings have been reported by Singh et al., (2006) in rice leaves, where Si-containing nutrient solution alleviated excess of N application. Our results however indicate silicon effects on nitrogen uptake and/or transport processes under normal nitrogen supply. This result well justifies further research to elaborate our understanding on the complex effects of this fertigation supplement on nitrogen homeostasis of plants.

Potassium concentration also decreased with Si application ( $78.372 \pm 8.384 \text{ g kg}^{-1}$ ) compared with control ( $88.389 \pm 14.32 \text{ g kg}^{-1}$ ), this effect however was not significant. Similar results were obtained for potassium with foliar and soil Si treatments, where K concentration was decreased in cucumber leaves (Emad et al., 2017). This might be due to a competition between two cations ( $\text{Na}^+$  and  $\text{K}^+$ ) since the family of transporters called HAK/KT/KUP are specializes in  $\text{K}^+$  transport across plasma membrane and some of which also mediate  $\text{Na}^+$  influx at high external Na concentrations (Taiz et al., 2015). Since, in our case Si is supplemented as a sodium salt whereas study by Emad et al., (2017) supplemented the Si through soil drenching. Hence, when other cations are abundant in the plant tissue, critical K concentrations kept low but when the cations are low, K concentrations increases depending on the plant species (Taiz et al., 2015).

In respect to Ca content, a modest increase was evident though not significant with Si treatment (Control:  $112.759 \pm 10.885$ ; Si:  $117.564 \pm 19.720$  g kg<sup>-1</sup>). A similar finding on cucumber seedlings was observed with exogenous silicon (Jafari et al., 2015). However, according to a study conducted by Khoshgoftarmanesh et al., (2014), a significant increase in Ca content was recorded only in cucumber cultivar exposed to salinity stress. In contrast to different cultivars, increased Ca content was reported in cowpea (Mali and Aery, 2008a), wheat (Mali and Aery, 2008b), aloe (Xu et al., 2015) and tomato (Li et al., 2015) with the application of silicon. Differences in the studies could be due to Si involvement in uptake and transport of different macronutrients also depending on any stresses applied.

No change was shown in case of P and Mg elements in both control and Si treatment, which were similar to the study conducted by Kamenidou et al., (2008) that have found no differences in the leaf macronutrients including phosphorous and magnesium as well as micronutrients between Si treatments and the control in ornamental sunflower (*Helianthus annuus* L.). Though no change has been associated with the additional Si supply, no deficiency was observed neither on the plant leaves nor in the recorded data. Since phosphorous is an important component of sugar-phosphate metabolites and phospholipids in plant cells, deficient plants show symptoms such as stunted growth and malformation. Leaves also become slightly purple due to excess anthocyanins (Taiz et al., 2015). Mg deficiency is characterized by chlorosis between the leaf veins especially on the older leaves due to its high mobility (Taiz et al., 2015). Similarly, no visible deficiency of Mg was observed on the seedlings.

## **6.2. Plant material and growth condition**

The appearance of plants was monitored visually throughout the growth period, with no signs of any malformation or deterioration. The appearance was the same in terms of color and shape but in case for silicate treated plants, width and leaf extension seemed slightly higher (Figure 6).

Application of external silicon as a fertilizer supplement was found beneficial for cucumber yield and stress tolerance (Voogt and Soneveld 2001). The impact of silicon in plants is often tested in hydroponic conditions by transferring seedlings into liquid media containing silicate (Etesami and Jeong 2018, Wang et al, 2015). Although aeration is normally used to decrease hypoxia, these conditions are far from physiological and differ substantially from cultivation practice.

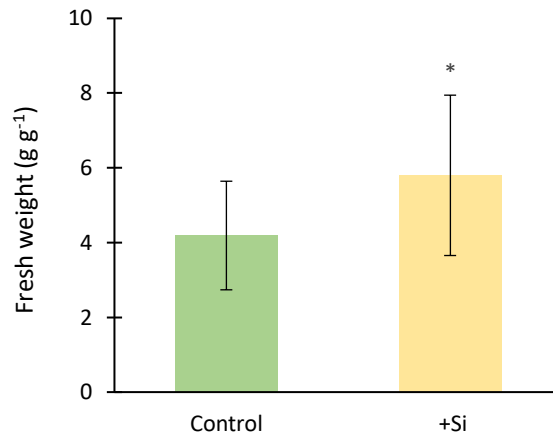
Perlite based semi-hydroponic cultivation system was applied for cucumber plants to minimize the gap between experimental and commercial growth conditions. Since cucumber is sensitive to various stresses, even if no stress condition is applied, minor environmental constraints may create basic, low level strain in plants. It is especially true in case of soilless growth systems even so under hydroponic physiological conditions (Morard and Silvestre, 1996). Soilless and sand cultures have been occasionally used to cultivate cucumber and other vegetables for experimental silicon treatments (Vercelli et al., 2017). Semi-hydroponic cultivation allows better aeration to the roots and it allows quick changing of media composition if needed. It also provides a low level of available silicate (Voogt and Sonneveld, 2001) allowing clearer distinction of the effects of external silicate provision.



**Figure 6.** Cucumber plant appearance after 31 days. Control plants on the left and Si treated plants on the right showing 3 biological replications.

In this medium plants can be subject to a long term, steady supply of Si, which is a more realistic approximation of potential field conditions. This is especially striking in the view of the recent “apoplastic obstruction hypothesis” of Si action (Coskun et al., 2019), which postulates interactions of Si in the apoplast as a major factor leading to its diverse physiological effects. These interactions may not be properly simulated by fast increasing Si level in liquid media, but more so with a steady Si supply for a longer growth period.

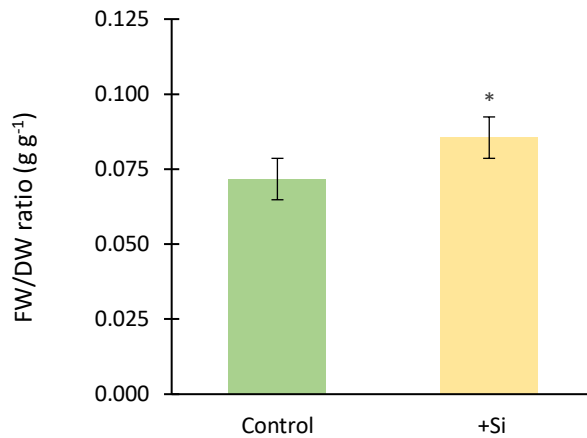




**Figure 7.** Total fresh weight of the *Cucumis sativus* L. leaves. Data shown are mean  $\pm$  SD ( $n = 16$ ) of three biological replicates. Asterisks indicate a statistically significant difference between control and Si treated plants at  $P < 0.05$

Total fresh weight of cucumber shoot was measured. Si treated ( $5.797 \pm 2.145$ ) plants were significantly higher when compared to control ( $4.193 \pm 1.452$ ) on all biological replications as shown in Figure 7. Under non-stressed conditions, Si treated plants may perform similarly or improve growth. In accordance with other studies, Si was effective to increase total dry weight (Zhu et al., 2004) and shoot dry weight (Maksimović et al., 2012) of *Cucumis sativus* cultivars without any stressor. In comparison with other cultivars, a similar trend was evident on rice cultivar where shoot dry weight was higher with silicon supply (Flam-Shepherd et al., 2018). Moreover, improved performance of plant growth with Si in soybean (Hamayun et al., 2010) under non-stress conditions has also been reported.

Pot studies generally measure the dry weight of young plants rather than the fresh weight due to a more precise estimation of biomass. Zhao et al., (2010) conducted a study on biomass estimation on sugarcane and found the same significance correlated on both FW and DW measurements in a single case and concluded that treatment effect on dry weight had the same effect on FW in most cases.



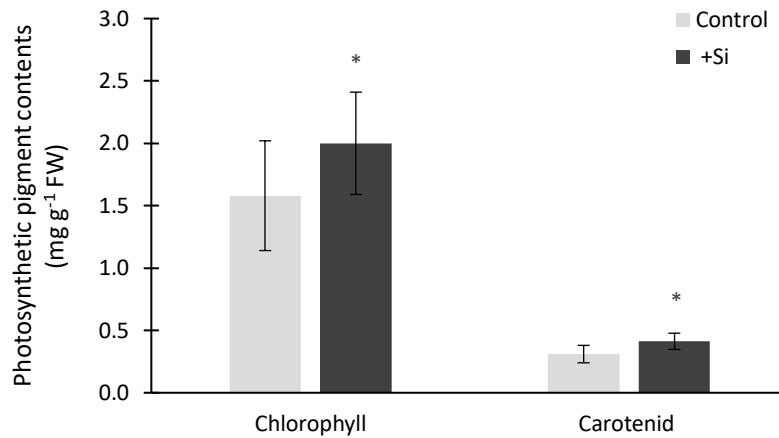
**Figure 8.** Shoot FW/DW ratio. Bars represent mean  $\pm$  SD ( $n = 6$ ). Asterisks indicate a significance difference between Si treated (+Si) and control plants at  $P < 0.05$

Thus, in order to evaluate the difference between treated and control samples we have measured the fresh weight and dry weight ratio. FW/DW ratio is calculated by dividing a constant weight of 1 g of fresh leaves to the final dried weight ( $1g/n_g = \text{dry weight}$ ). As a result, Si supplied plant was higher ( $0.0855 \pm 0.007$ ) than that of control ( $0.0717 \pm 0.009$ ), which was similar in total FW values shown in Figure 8.

### 6.3. Chlorophyll and carotenoid contents

Photosynthetic pigments like chlorophyll and carotenoids are important indicators determining photosynthetic capacity and hence plant growth. Positive effects of Si on photosynthetic machinery have been reported extensively. Thus, we have measured these photosynthetic pigments in correlation with silicon supplemented fertigation.

Based on our findings, addition of silicon resulted in increase of photosynthetic pigments (total chlorophyll and carotenoids) as shown in Figure 9.

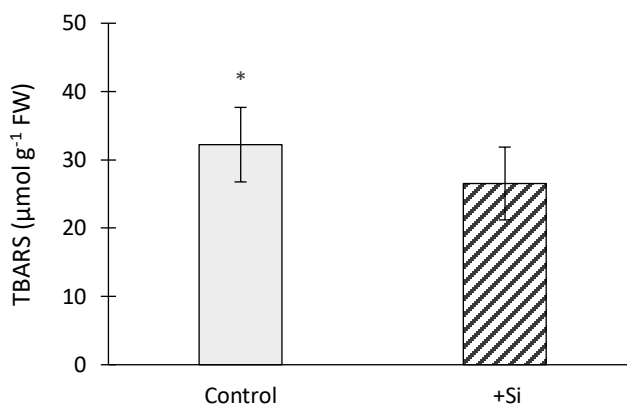


**Figure 9.** Effect of Si treatment on cucumber leaves' chlorophyll and carotenoid content. Data shown are values of mean  $\pm$  SD of at least two biological replicates. The asterisk indicates a significant difference between control and silicate treatment at  $P < 0.05$ .

Total chlorophyll content of leaves of silicate treated plants ( $2.00 \pm 0.4 \text{ mg g}^{-1}$ ) exceeded that of control plants ( $1.58 \pm 0.44 \text{ mg g}^{-1}$ ). The same trend was found in the case of carotenoids content (Control:  $0.31 \pm 0.07$ , Si:  $0.41 \pm 0.07 \text{ mg g}^{-1}$ ). Carotenoids are major components to absorb light during photosynthesis, which are also responsible to protect the plants from photo-oxidative damage (Pessaraki, 2001). Our findings are in line with a number of studies reporting increased contents of chlorophyll a, chlorophyll b, and carotenoids in leaves e.g. of wheat (Hussain et al., 2015; Rizwan et al., 2012; Tripathi et al., 2015) and cotton (Farooq et al., 2013) under Si supplemented fertigation. Stress conditions have often been shown to decrease leaf stomatal conductance which can lead to poor gas exchange and low transpiration rate (Pessaraki, 2001). Low  $\text{CO}_2$  availability can lead to overloaded electron transport chain and subsequent ROS production. However, supply of Si has reversed these deleterious effects as was approved by photosynthetic measurements. Thus, Si prevented oxidative damage inside the chloroplast, which could be often observed otherwise in plants under stress conditions (Pessaraki, 2001). Nonetheless, our results indicated that Si supply can ameliorate photosynthetic pigments content even when no apparent stress conditions were applied.

#### 6.4. Thiobarbituric Acid Reactive Substances

The tissue concentration of TBARS is frequently correlated with malondialdehyde (MDA) level, assumed to be indicative to the extent of lipid peroxidation suffered (Zhu and Gong, 2014). Therefore, the level of MDA, produced during the peroxidation of membrane lipids, is often used as an indicator of oxidative damages. Data showed significant changes of TBARS between control:  $41.05 \pm 7.05$  and Si treatment:  $30.80 \pm 5.72$   $\mu\text{mol g}^{-1}$  (Figure 10).



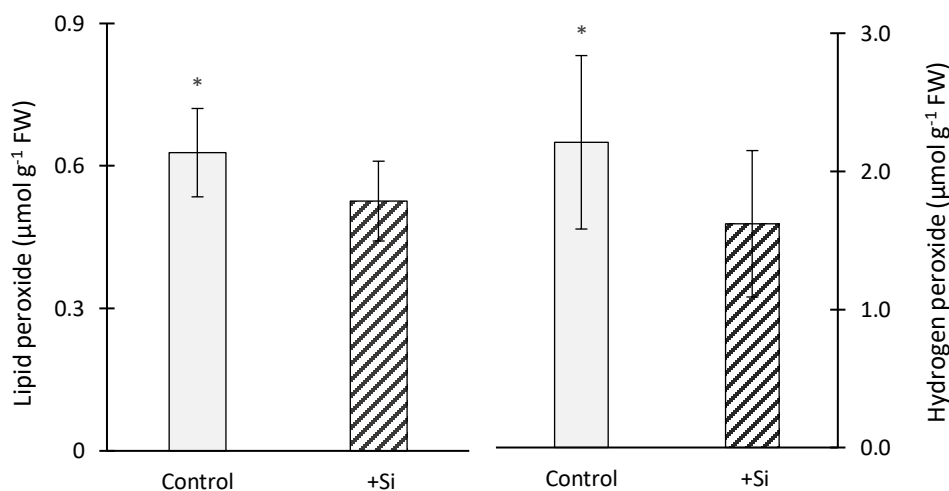
**Figure 10.** Effect of Si treatment on cucumber leaves' TBARS content. Data shown are values of mean  $\pm$  SD of 3 biological replicates ( $n=8$ ). The asterisk indicates significance between control and Si treatment at  $P < 0.05$

Our results correlated with the study conducted by Liu et al., (2009), where cucumber plants treated with exogenous silicon showed a significant decrease in MDA level during both normal temperature and chilling stress.

It is also widely accepted that Si application can ameliorate oxidative stress through regulating antioxidant enzyme activities and non-enzymatic antioxidant substance levels in plants (Liang et al., 2003; Moussa, 2006; Soylemezoglu et al., 2009). Khoshgoftarmanesh et al., (2014) reported that Si supply reduced the malondialdehyde level under salt stress by enhancing antioxidant enzyme activities.

## 6.5. Lipid peroxide and hydrogen peroxide content

Even under normal growth conditions several metabolic pathways produce reactive oxygen species (ROS) as by-products of their primary processes. Some ROS, particularly free radicals, may initiate chain reactions in membrane lipids leading to accumulation of lipid peroxides (LPOs) with subsequent structural and functional alterations in the membranes (Pessaraki, 2001).



**Figure 11.** Effect of Si treatment on cucumber leaves' lipid peroxide and hydrogen peroxide content. Data shown are values of mean  $\pm$  SD of 8 technical replicates. The asterisk indicates a significant difference between control and silicate treatment at  $P < 0.05$

Therefore, lipid peroxidation products were measured and were found lower upon Si treatment (Control:  $0.628 \pm 0.093$ , Si:  $0.530 \pm 0.084$   $\mu\text{mol g}^{-1}$ ) as shown in Figure 11. The presented result was in line with the findings of Zhu et al., (2004) which showed decreased lipid peroxidation in cucumber as well as Liang et al., (2003) showed decreased permeability of the plasma membrane of leaf cells and decreased LPO level in barley.

Lower hydrogen peroxide concentration was also demonstrated in treated samples (Control  $2.2 \pm 0.45$ , Si:  $1.62 \pm 0.1$ )  $\mu\text{mol g}^{-1}$ ) (Figure 11). Based on the studies regarding Silicon application, Song et al.,

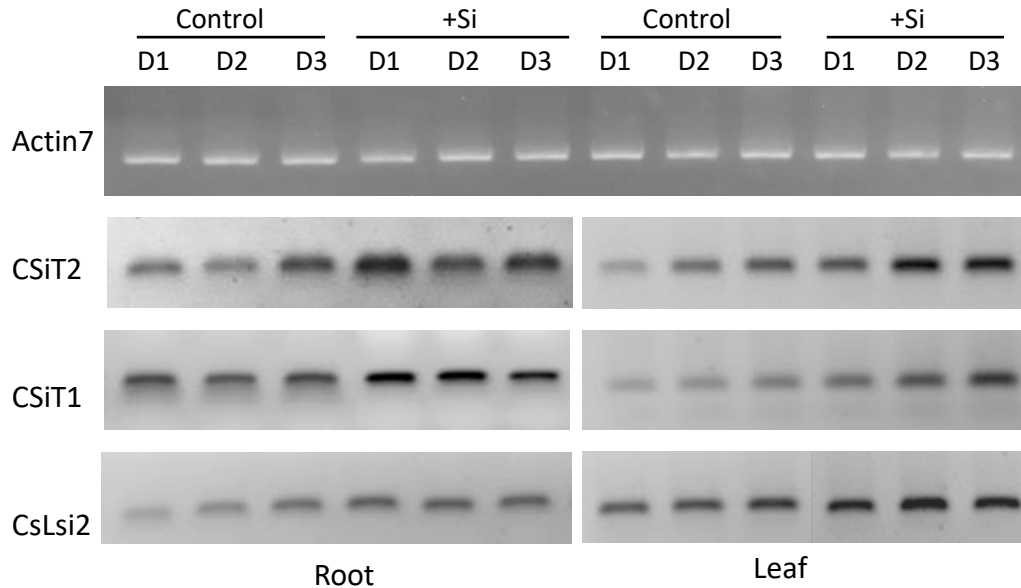
(2009) reported reduced H<sub>2</sub>O<sub>2</sub> contents by enhancing antioxidant enzyme activities and non-enzymatic antioxidant contents under Cd stress.

It is well documented that Si addition decreases LPO and H<sub>2</sub>O<sub>2</sub> content through regulating antioxidant activities, especially in stress conditions. According to Soylemezoglu et al., (2009) Si decreased H<sub>2</sub>O<sub>2</sub> concentration and lipid peroxidation in grapevine rootstocks through increased catalase (CAT) and superoxide-dismutase (SOD) activity. Furthermore, similar findings have been reported in various species like tomato (Shalata and Tal, 1998), wheat (Meneguzzo et al., 1999), cotton (Gossett et al., 1994) and barley (Liang, 1999).

Zhu et al., (2004) suggest that Si decreases the permeability of plasma membranes and the extent of membrane lipid peroxidation. Thereby Si may maintain membrane integrity and functions in salt-stressed cucumber, thus mitigating against salt toxicity and improving the growth of plants. Therefore, the above findings illustrate, in relation with our present study, that Si treatment can prevent the increase of hydrogen peroxide and lipid peroxide contents in diverse species exposed to various stressors. Our findings therefore demonstrate lower oxidative stress levels in Si treated plants.

## 6.6. Si transporter genes

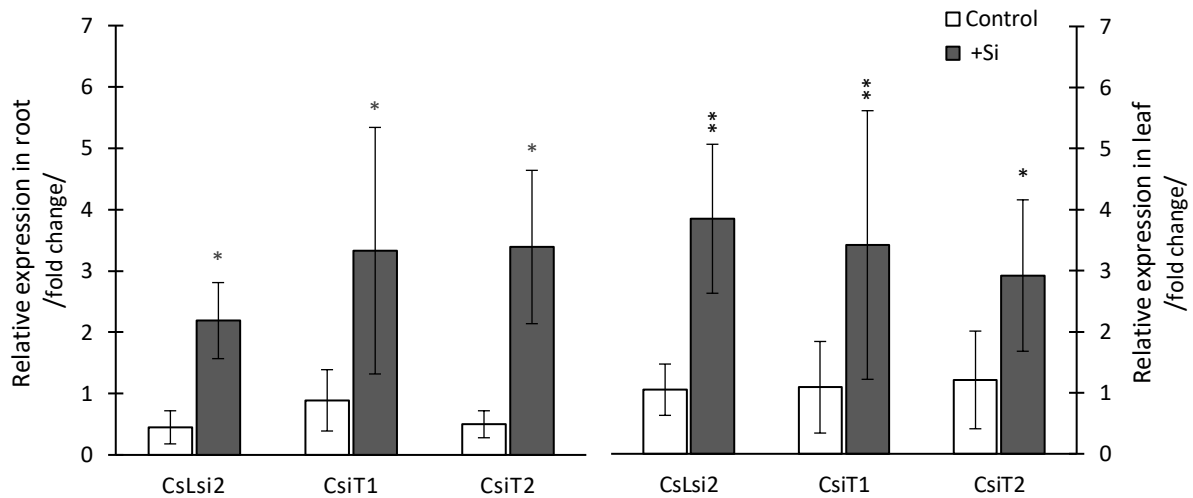
Extensive studies testified on beneficial effects of Si on plant growth and stress tolerance in several species, however Si accumulation was found variable among plant species. Cucumber is a widely used dicot model for silicon accumulation thus, number of transporters have been identified including efflux transporter CsLsi2 and two influx transporters CSiT1 and CSiT2.



**Figure 12.** Amplification products of RT-PCR on Si transporter genes of cucumber root (on the left) and leaf (on the right). PCR products were run on 1.5% gel electrophoresis with a 100bp marker. Actin7 is used as a reference gene. (D1-3: 'Dirigent' biological replicates)

In connection with silicon accumulation, the expression of known silicate transporters was also investigated in both root and shoot tissues. According to Wang et al., (2015), transcript level of both influx transporters was detected by RT-PCR examination in root, leaves, stems, and flowers of cucumber plants with most abundance in roots and mature leaves. Whereas expression of rice *Lsi1* and *Lsi2* (Ma and Yamaji, 2006), and barley *HvLsi1* (Chiba et al., 2009), the Gramineae counterpart genes, was mainly restricted to roots. Based on our RT-PCR examination of cucumber, both known

influx transporters were expressed in roots and mature leaves (2<sup>nd</sup> and 3<sup>rd</sup> leaves). The efflux transporter *CsLsi2* was expressed in both roots and leaves which is in line with the findings of Sun et al., (2018) where the transcripts were detected in roots, stems, laminae and petioles.



**Figure 13.** Effect of Si treatment on the relative expression of silicon transporter genes in cucumber root and leaf. Values are expressed as fold change. Bars represent mean values  $\pm$  SD of three biological replicates. The asterisk indicates significance difference between control and silicate treatment (\*  $P < 0.05$ ; \*\*  $P < 0.01$ ).

Quantitative RT-PCR results approved upregulation of *CsLsi2*, a silicon efflux transporter (Sun et al., 2018) as well as two further transporters (*CsiT1* and *CsiT2*) (Wang et al., 2014) in response to 1.67 mM of exogenous silicon supply both in leaves and roots. Influx transporters expression was examined with 1.0 mM Si supply (Wang et al., 2014) whereas efflux transporter was examined with 1.7 mM of exogenous Si (Sun et al., 2018). Thus, these data indicate that the appropriate amount of Si taken up by the cucumber plants may be transported by the putative Si transporters. This is in agreement with the approved role of these genes in Si transport.



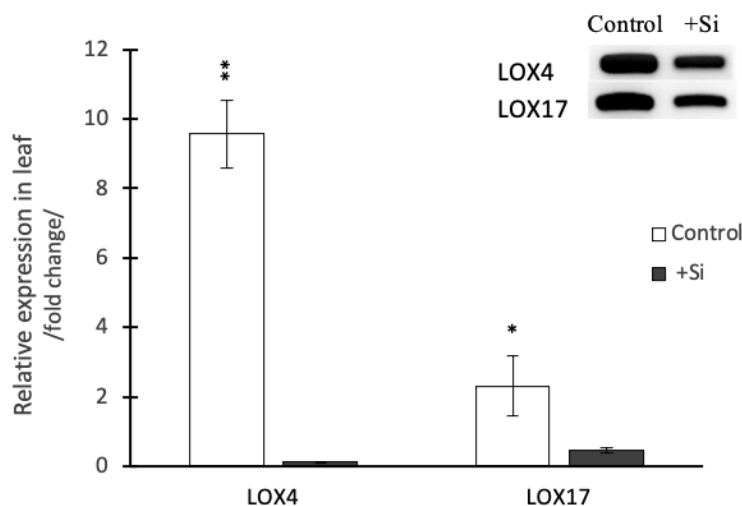
### 6.7. The effect of Si treatment on the expression of LOX genes

Numerous studies have suggested that LOXs play a vital role in plants normal growth and development, synthesis of aroma compounds, ripening and senescence as well as defense against biotic and abiotic stresses (Hou et al., 2015; Keeretaweep et al., 2015; Maschietto et al., 2015; Shen et al., 2014). Thus, we have conducted RT-PCR to study the expression of LOX genes in cucumber leaf tissues with and without Si supplementation. We have chosen twelve genes with transcripts previously detected in cucumber tissues by Yang et al., (2012). Our results from RT-PCR have shown that all chosen genes were expressed in the *Cucumis sativus* leaf tissues studied (Figure 14).



**Figure 14.** Comparison of the expression level of different LOX genes in Si treated cucumber leaves with control. Actin7 is used as a reference gene.

As for the molecular mechanism behind the protection Si treatment may offer in oxidative stress, upregulation of antioxidant systems has been frequently evoked. Here we tested whether regulation of lipoxygenase genes may represent an additional possibility contributing to the mitigating effect. As we expected, downregulation of all *LOX* genes was evident on Si treated leaves. For further evaluation, quantitative RT-PCR was done on two representative genes *LOX4* and *LOX17*, selected based on their expression pattern. According to RT-qPCR results, the presented  $\Delta\Delta C_t$  data approved that both *LOX4* and *LOX17* genes were found significantly downregulated by Si treatment (Figure 15). This validates the value of the semi-quantitative data obtained by RT-PCR (Figure 14).



**Figure 15.** RT-qPCR relative expression of *LOX4* and *LOX17* genes in cucumber leaf. Values are expressed as fold change. Bars represent mean values  $\pm$  SD of at least two biological replicates. The asterisks indicate significance difference between control and silicate treatment (\*  $P < 0.05$ ; \*\* $P < 0.01$ ).

Sequence analysis of the cucumber genome by Liu et al., (2011) have identified 23 *LOX* genes, which belong to either 9-LOX or the 13-LOX pathways based on their localization to cytosol or chloroplast respectively. Thus, Liu et al., (2011) first selected 12 expressed *LOX* genes then divided them into two types, with half of them (*LOX1*, 2, 4, 8, 9, 10) belonging to type 1 (9-LOX) and the other half

(*LOX16, 17, 19, 20, 22, 23*) belonging to type 2 (13-LOX). Therefore, protein sequences of all twelve genes were run with DeepLoc-1.0 software to predict their subcellular localization (Supplementary Figure 2). Based on our findings, *LOX16, LOX17* and *LOX19* genes also belonged to type 1 due to their protein products predicted localization in the cytoplasm. Thus, only *LOX20, LOX22* and *LOX23* remained in the type 2 category due to their protein products predicted localization in the chloroplast. Interestingly, *LOX2* and *LOX9* proteins were found to be putatively localized in the mitochondria and associated to the cell membrane, respectively.

Among the complex role of LOXs play in plant growth and homeostasis, participation in stress responses has been described (Viswanath et al., 2020). In our experiments, coordinated downregulation of LOX genes paralleled decreased levels of lipid peroxidation and hydrogen peroxide content in silicated plants. This corresponds with results presented by Gunes et al., (2007) who described alleviating effect of Si on abiotic stress induced lipoxygenases and hydrogen peroxide accumulation. Additionally, our recent research conducted on different cucumber F1 hybrid ('Joker') has shown downregulation of several redox related genes with presumed pro-oxidative effects, including LOXs, due to silicon treatment (Szegő et al., 2021). Results further approved the redox protective potential of downregulating pro-oxidant genes and enzyme activities, resulting in reduced hydrogen peroxide contents as marker of decreased oxidative burden.

Lipoxygenases are involved in the synthesis of fatty acid hydroperoxides and reactive oxygen species, especially in the context of ethylene and jasmonic acid mediated responses (Kreslavskii et al., 2012; Prasad et al., 2017). These studies also highlighted the signaling roles as to aid in plants' adaptive and tolerance mechanisms. Whether the link between redox protection and downregulation of lipoxygenases is direct through pro-oxidant activity or more complex through e.g., oxylipin biosynthesis and hormonal actions remain to be determined.

## 7. CONCLUSION

The presented experiments focused on the effects of Si supplementation on cucumber physiology and molecular mechanisms. In these studies, a fertigation solution of half strength HG was applied as control with 1.67mM of Si treatment on an F1 hybrid cultivar of cucumber. All the experiments were conducted using semi hydroponic cultivation system with perlite.

Experimental data allow several conclusions to be drawn. In the soilless perlite medium plants can be subject to a long term, steady supply of Si. This is a more realistic approximation of potential field conditions than changing media for hydroponic – which is a frequent practice in this type of research. Results approved growth promoting potential of Si treatment. This was accompanied by higher chlorophyll and carotenoid content of leaf tissues.

With respect to molecular mechanisms putative silicon transporter genes were upregulated with Si application in both roots and shoot. Therefore, the appropriate expression of these transporters allowed the increased accumulation of Si by uptake and transport in cucumber, which further favors the positive effects correlated with it.

Moreover, our results indicated that Si supply can ameliorate oxidative stress by decreasing important indicators such as hydrogen peroxide and lipid peroxidation levels even when no stress conditions were applied. The background of Si mitigating effect on redox balance was further tested. *LOX* gene expression was investigated since it has been frequently found induced in response to biotic and abiotic stress. Our findings showed coordinately downregulated expression of *LOX* genes in response to silicon supplemented fertigation. This observation draws attention to this class of enzymes as potential players in the context of redox protection offered by Si.

## 8. NEW SCIENTIFIC RESULTS

The conducted research on cucumber Dirigent cultivar revealed significant differences in both growth parameters and stress related gene induction in response to Si treatment in perlite medium. The obtained results are of high importance with potential application in current cucumber production:

- Our data revealed that a perlite based, semi-hydroponic growth system was suitable to conduct silicon supplemented fertigation experiments on young cucumber plants. Results confirmed several known physiological and molecular effects associated with silicon in cucumber, and also revealed new data with more insight into Si action.
- Genes specific to putative and approved silicon transporters were expressed at significantly higher level in silicon treated plants in both roots and shoots.
- Increased silicon and sodium tissue concentrations were approved in leaves of sodium silicate treated plants. This effect correlated with several physiological changes: Chlorophyll and carotenoid contents of silicon treated cucumber were increased compared to control. Significantly higher plant fresh weight of silicon supplied plants was found.
- Higher level of silicon in leaves also correlated with less oxidative damage, illustrated by redox markers, such as malondialdehyde, lipid peroxide and hydrogen peroxide contents.
- Downregulation of all expressed lipoxygenase genes was found in response to Si treatment

## 9. REFERENCES

### 9.1. List of publications providing basis of thesis

#### Published papers in referred journals

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## 10. APPENDICES

### Housekeeping gene and Si transporter genes sequence

Cucumis sativus actin-7 (LOC101215469), mRNA

Accession: XM\_004147305.3

CsGy2G015500

Actin-7 F

Actin-7 R

171

tcattttcccttcccttcccttctccattccttcccttctctccattttcccatctcaccttctcgcttccgcaaccgaga  
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tttccagccatccctcattggaatggaagctgctggaatccacgagactacctacaactcaatcatgaagtgtgatgtg  
gatcagaaaagatttgatggcaacattggtctcagtggtggttctaccatggtccctggatcgctgacctgatgag  
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tccatcgtccacaggaagtgttctaagttcaatatgtggctttgaaatggtagtattttccttttagttggctttttttg  
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cgtatgcccattgctttccccccatacaccctatgagtttgagattcgtaataactactgctgattttacgggtaagaa  
gtaattgatccctctggatgtttatggtggtgggcaaaaacaactttccttccaaaatctttcccttgcccttgcccta  
cctttgccaaatctgtgctgcaaccatgtctttttcttttagtaggagatgattgtgatgtttttttccctttttccct  
ttatccttctcttattctctcctatttccctatttccctatttccctatttccctatttccctatttccctatttccct  
tttgaaaacattaatgttaatagttattgtaagacaaatattagtcagtttgctccttgagggtcataaacttagctatgc  
aaaatgatatttttattattggcttggagctttatagtcattcatattta

**Cucumis sativus silicon efflux transporter LSI3 (LOC101214719), transcript variant X4, mRNA**

NCBI Reference Sequence: XM\_004140721.3

CsGy3G017160 cucurbitgenomics.org/feature/gene/ CsGy3G017160

Putative transporter arsB

CsLsi2 F

CsLsi2 R

88

```
ccagcctagaagcttcataaggggccccctctttttagggcctttgactcattaatgagaaagtcgagatttcttcgac
gacggagcaaaaaacatcgatggctgctccggcgacgattgggagcccgcctgactgtgatagattggataacaatg
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actttttgtttcctctcaattactgtgagaacctccataaatccatttttttttatttgattcctattcgttttcgaac
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atctatatatatatatataatgaagaatgtttccttgaagagctgttacaatttaagtatgaacaaatgttcaat
ataggttgagttgaaatggaaatgaaccaataaatagccattttttgttcga
```

## Cucumis sativus silicon transporter Lsi1 mRNA, complete cds

GenBank: KX434755.1

Cucurbitgenomics.org/feature/gene/CsGy3G036580

Aquaporin NIP2-1-like

CsiT-1 F

CsiT-1 R

69

```
atgagttctattcaaaatcctcagctctccaaccaagaagctggttggtgatgtaacgaattcgtatctggtgaaaacc
agattctaacgctccaagtttgatctttctcaaaaacccttaccctcctgggtttcccgaagctggtggcggagg
tgatagcaacctatgtctagtggttgatgacatgtggggcggcagcattgaacgcgagcgatgcacggagagtgtcgag
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cccggctgtcaccatggcttttgctgcaactcgacactttccatggaaacaggttccattgtatggagcagcacaactga
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tctgatttacaagctttagttatggagattggtgttacattctctatgatggttggactcttgccgttgcaactgacac
caaagcagtaggagagctggcaggtatcgcagttggctcagctggttgatcacttccatcttagctggacctgtatcag
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gggcccggttggttggaaactcaactgggggcatgggtcatataagttcatacgtgcccagtgacaaacctgtgcaac
ttaatctc
tccacattcttttctcactcaaaatgcgaagaatgtcagatcagatggttagcgaagcaatcattaa
```

## Cucumis sativus Si transport-like protein 2 mRNA, complete cds

GenBank: FJ595948.1

Cucurbitgenomics.org/feature/gene/CsGy3G036600

Aquaporin NIP2-1-like

CsiT-2 F

CsiT-2 R

78

```
atggcgagacgcagcgacgatgaagaagcctttattgccttgggaaacgagtgctctgatcctcaaccacccttgttccg
cgaccgatttgatgaactttaccgcccagattctcacgaaagcttgtggcggagggtgatcgctacgtatttgcctgtgt
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cactacgcatattaatggatccaattcaagatttaggtactacttaccacatggaccggcttgaagcacttgttatg
gagattggttctcattttgtatgatggttgcacttctcgggttggcaccgacaccaaagctataggagagcttggagg
cgtagctgtggggtcagctgtatgtatctcgtccatctttctgggccaatatcgggtggatcgatgaaccagcaagatc
aataggaccagccattgcaagttcacggttaccgaaggaatgtgggtgtacatgatcgggtccagttacaggaaccttgcctt
catcattttcatataatctcatagcagcactgaaaaacacaccattcactctcgttacattga
```

Supplementary Table 1. Lipoxygenase genes

№	Primer	Sequence	Tm	Length	bp	Source
1	CsLOX1B F	AGGACTTCCTGTTCCATTGGA	55.66	21	227	<a href="http://cucurbitgenomics.org/feature/mRNA/Cucsa.091300.2">http://cucurbitgenomics.org/feature/mRNA/Cucsa.091300.2</a> Cucsa.091300.2 <i>Cucumis sativus</i> (Cucumber (Gy14) v1)
	CsLOX1B R	TGGAGGAAATTCCTTAAGACGA	52.76	22		
2	CsLOX2B F	TCCAATTCATAAGCTACTTGTTCT	53.21	25	200	Cucsa.091340.1 <i>Cucumis sativus</i> (Cucumber (Gy14) v1) <a href="http://cucurbitgenomics.org/feature/mRNA/Cucsa.091340.1">http://cucurbitgenomics.org/feature/mRNA/Cucsa.091340.1</a>
	CsLOX2B R	ACCTTTCTCTCTCTTGATGAGATCA	54.49	25		
3	CsLOX4 F	GCAACTCATGGGTTTACCC	53.81	19	156	Yang et al., 2012 (LOX4) (LOC101207163), XM_011650527.1 <a href="http://cucurbitgenomics.org/feature/mRNA/Cucsa.091290.1">http://cucurbitgenomics.org/feature/mRNA/Cucsa.091290.1</a>
	CsLOX4 R	CTTGACGCTCTCCTTTTCC	53.40	19		
4	CsLOX8 F	TCCTGCTGACAAATACAAAACC	52.85	22	297	<a href="http://cucurbitgenomics.org/feature/mRNA/Cucsa.091380.1">http://cucurbitgenomics.org/feature/mRNA/Cucsa.091380.1</a> <i>Cucumis sativus</i> (Cucumber (Gy14) v1) Cucsa.091380.1
	CsLOX8 R	CTTTTGGATCTTTTTGGAAGG	49.77	23		
5	CsLOX9 F	TCGTCTCCTAGAGTTCCTCC	57.84	21	395	<a href="http://cucurbitgenomics.org/feature/mRNA/Cucsa.185530.1">http://cucurbitgenomics.org/feature/mRNA/Cucsa.185530.1</a> <a href="http://cucurbitgenomics.org/feature/mRNA/Cucsa.185530.2">http://cucurbitgenomics.org/feature/mRNA/Cucsa.185530.2</a> <i>Cucumis sativus</i> (Cucumber (Gy14) v1)
	CsLOX9 R	AATCATTACGGCGACATAA	51.36	20		
6	CsLOX10 F	TGGGAGAAGAAGCATACTTAGAGG	55.59	24	286	<a href="http://cucurbitgenomics.org/feature/mRNA/Cucsa.091390.2">http://cucurbitgenomics.org/feature/mRNA/Cucsa.091390.2</a> <a href="http://cucurbitgenomics.org/feature/mRNA/Cucsa.091390.1">http://cucurbitgenomics.org/feature/mRNA/Cucsa.091390.1</a> <i>Cucumis sativus</i> (Cucumber (Gy14) v1)
	CsLOX10 R	GGAAGATAGCTCTGGTTGAGAAAG	54.94	24		
7	CsLOX16 F	TATAGCGCAAAGAAATTGTTCG	51.05	23	255	<a href="http://cucurbitgenomics.org/feature/mRNA/Cucsa.153610.1">http://cucurbitgenomics.org/feature/mRNA/Cucsa.153610.1</a> Cucumis sativus (Cucumber (Gy14) v1); Cucsa.153620.1 <a href="http://cucurbitgenomics.org/feature/mRNA/Csa4G286960.1">http://cucurbitgenomics.org/feature/mRNA/Csa4G286960.1</a>
	CsLOX16 R	TGGGCTTAGCAAGCCTC	55.48	18		



						<a href="#">Cucumis sativus (Cucumber (Chinese Long) v2)</a>
8	CsLOX17 F	CCTCGTCGAATAAGA AACTCTTC	52.48	22	188	CsaV3_7G027160 <a href="http://cucurbitgenomics.org/feature/gene/CsaV3_7G027160">http://cucurbitgenomics.org/feature/gene/CsaV3_7G027160</a>
	CsLOX17 R	GTGGAAGGTTGATAGTTTAGGG	52.59	22		
9	CsLOX19 F	CTCATTGGCTGAGAACTCATTG	53.55	22	271	<a href="http://cucurbitgenomics.org/feature/mRNA/Cucsa.065250.1">http://cucurbitgenomics.org/feature/mRNA/Cucsa.065250.1</a> <i>Cucumis sativus</i> (Cucumber (Gy14) v1)
	CsLOX19 R	TGAATTAAGTCAGCAGGTAAAGCT	53.55	24		
10	CsLOX20 F	TTGCTTCCAATAAGATGGGAC	52.81	21	264	<a href="http://cucurbitgenomics.org/feature/mRNA/Csa4G288610.1">http://cucurbitgenomics.org/feature/mRNA/Csa4G288610.1</a> <i>Cucumis sativus</i> (Cucumber (Chinese Long) v2)
	CsLOX20 R	TCCCGTTACAGGATCAACCT	55.43	20		
11	CsLOX22 F	TCTCTAATAAGCCTTATCTTCCAGGT	54.19	26	260	<a href="http://cucurbitgenomics.org/feature/mRNA/Csa7G449420.1">http://cucurbitgenomics.org/feature/mRNA/Csa7G449420.1</a> <i>Cucumis sativus</i> (Cucumber (Chinese Long) v2) <a href="http://cucurbitgenomics.org/feature/mRNA/Cucsa.360190.1">http://cucurbitgenomics.org/feature/mRNA/Cucsa.360190.1</a> <i>Cucumis sativus</i> (Cucumber (Gy14) v1)
	CsLOX22 R	TGCAGTCATATCAGTCTCGGA	55.07	21		
12	CsLOX23 F	TAAATCATTGGTTAAGGACTCAG	52.07	24	285	<a href="http://cucurbitgenomics.org/feature/mRNA/Cucsa.075520.1">http://cucurbitgenomics.org/feature/mRNA/Cucsa.075520.1</a> <i>Cucumis sativus</i> (Cucumber (Gy14) v1)
	CsLOX23 R	CCATACCCCTTCGAATAAGATC	52.26	22		

Lox1

Prediction: Cytoplasm, Soluble

# DeepLoc-1.0

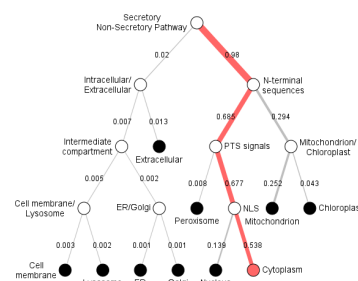
Predicted proteins



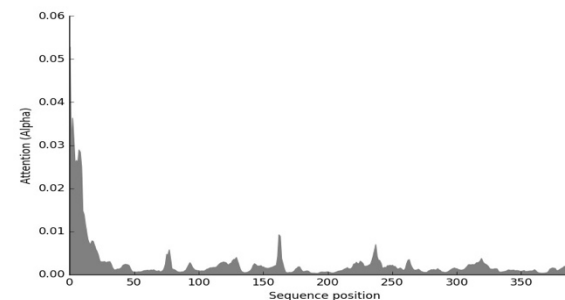
Localization	Cytoplasm	Mitochondrion	Plastid	Peroxisome	Nucleus	Extracellular	Lysosome/Vacuole	Endoplasmic reticulum	Golgi apparatus	Cell membrane
Likelihood	0.4539	0.2496	0.0956	0.0857	0.0517	0.0248	0.014	0.0089	0.0085	0.0073

Type	Soluble	Membrane
Likelihood	0.8832	0.1168

Hierarchical Tree.



Position Importance



CELLULOSE-BINDING DOMAIN OF LYSION CBS (PDB: 1LX1)

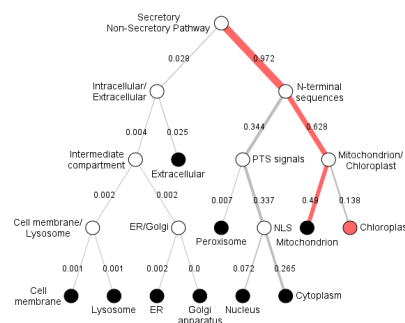
Lox2

Prediction: Mitochondrion, Soluble

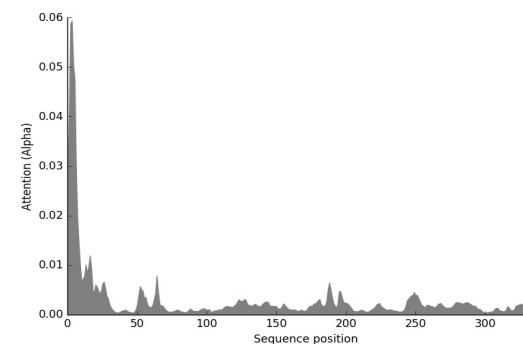
Localization	Mitochondrion	Plastid	Cytoplasm	Nucleus	Extracellular	Peroxisome	Endoplasmic reticulum	Cell membrane	Golgi apparatus	Lysosome/Vacuole
Likelihood	0.5322	0.2437	0.1268	0.0242	0.0239	0.0168	0.0147	0.0085	0.0055	0.0038

Type	Soluble	Membrane
Likelihood	0.7473	0.2527

Hierarchical Tree.



Position Importance



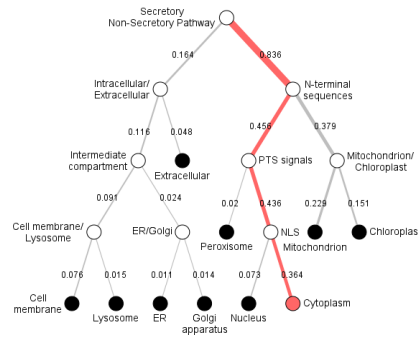
Lox4

Prediction: Mitochondrion, Solubl

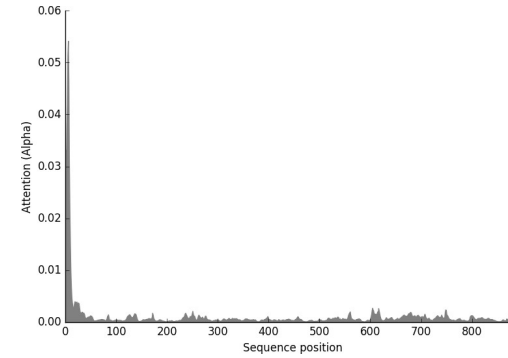
Localization	Mitochondrion	Cytoplasm	Plastid	Nucleus	Cell membrane	Peroxisome	Extracellular	Lysosome/Vacuole	Golgi apparatus	Endoplasmic reticulum
Likelihood	0.3669	0.228	0.2049	0.0556	0.0455	0.0286	0.0277	0.0173	0.0163	0.0092

Type	Soluble	Membrane
Likelihood	0.7797	0.2203

Hierarchical Tree.



Position Importance



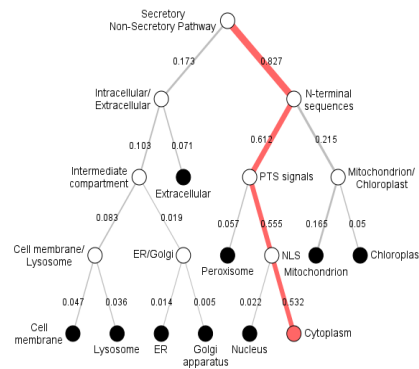
Lox8

Prediction: Cytoplasm, Solubl

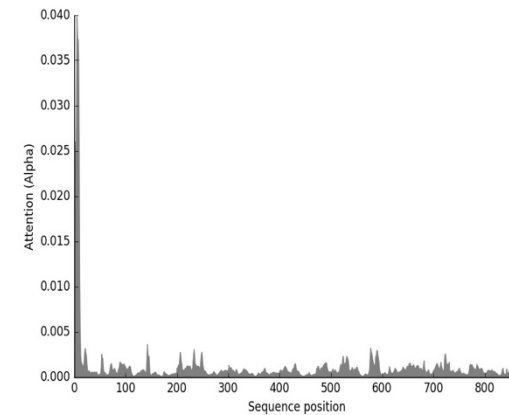
Localization	Cytoplasm	Mitochondrion	Plastid	Peroxisome	Cell membrane	Extracellular	Lysosome/Vacuole	Endoplasmic reticulum	Nucleus	Golgi apparatus
Likelihood	0.4093	0.213	0.1032	0.0893	0.0571	0.0488	0.038	0.0185	0.0135	0.0091

Type	Soluble	Membrane
Likelihood	0.8041	0.1959

Hierarchical Tree.



Position Importance

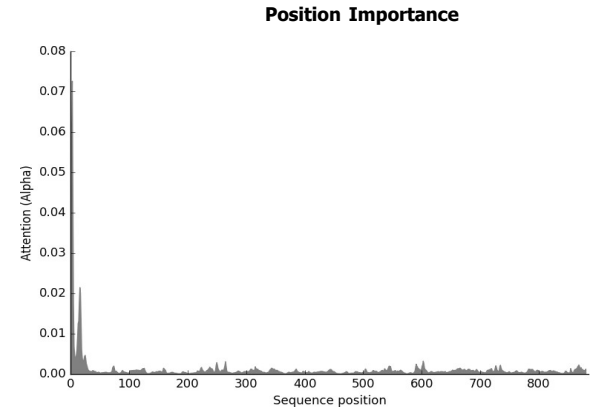
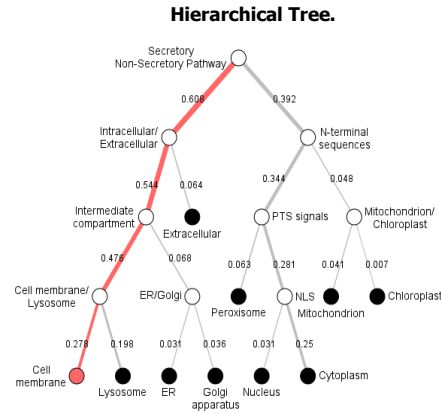


**Lox9**

**Prediction: Cell membrane, Membrane**

Localization	Cell membrane	Lysosome/Vacuole	Peroxisome	Cytoplasm	Mitochondrion	Golgi apparatus	Endoplasmic reticulum	Extracellular	Nucleus	Plastid
Likelihood	0.3129	0.1806	0.174	0.1194	0.0657	0.0506	0.0375	0.0347	0.0127	0.0119

Type	Soluble	Membrane
Likelihood	0.3924	0.6076

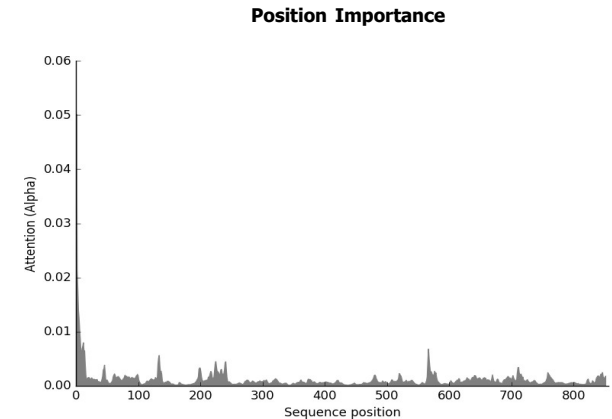
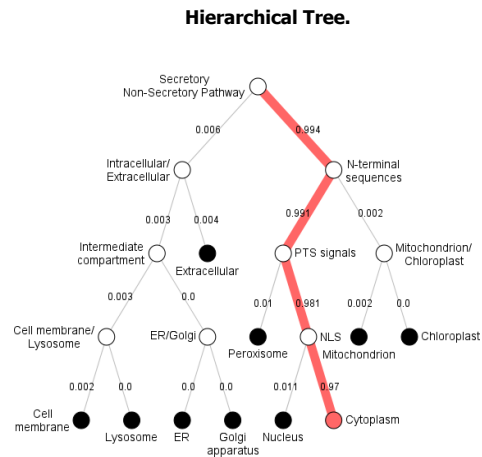


**Lox10**

**Prediction: Cytoplasm, Soluble**

Localization	Cytoplasm	Peroxisome	Cell membrane	Mitochondrion	Extracellular	Plastid	Endoplasmic reticulum	Nucleus	Lysosome/Vacuole	Golgi apparatus
Likelihood	0.8461	0.0589	0.0222	0.021	0.0161	0.0141	0.0072	0.0066	0.0063	0.0015

Type	Soluble	Membrane
Likelihood	0.9563	0.0437



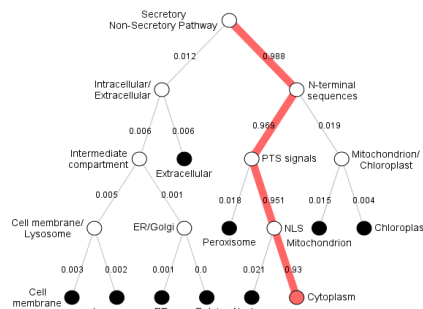
Lox16

Prediction: Cytoplasm, Soluble

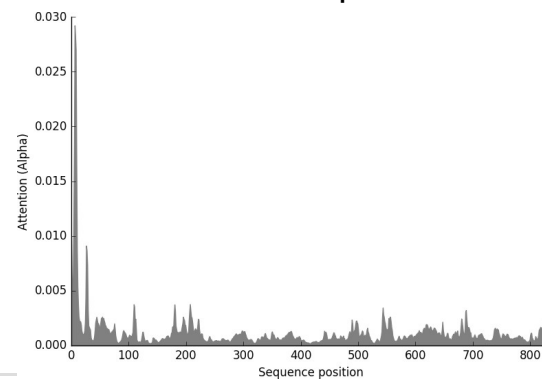
Localization	Cytoplasm	Peroxisome	Plastid	Mitochondrion	Nucleus	Extracellular	Cell membrane	Lysosome/Vacuole	Endoplasmic reticulum	Golgi apparatus
Likelihood	0.8444	0.0814	0.0247	0.0167	0.0113	0.0072	0.0056	0.0043	0.0029	0.0015

Type	Soluble	Membrane
Likelihood	0.9833	0.0167

Hierarchical Tree.



Position Importance



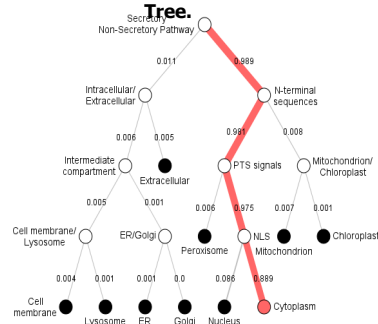
Lox17

Prediction: Cytoplasm, Soluble

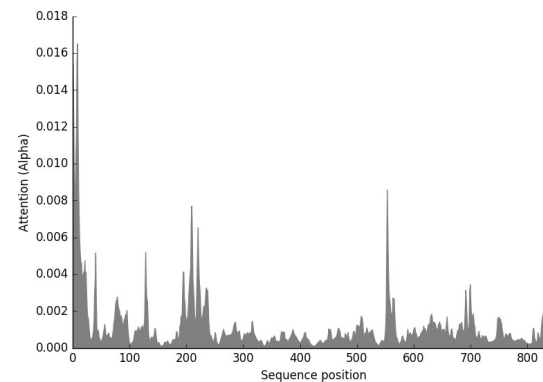
Localization	Cytoplasm	Nucleus	Peroxisome	Cell membrane	Endoplasmic reticulum	Lysosome/Vacuole	Mitochondrion	Extracellular	Golgi apparatus	Plastid
Likelihood	0.8411	0.075	0.0414	0.0087	0.0082	0.0077	0.0076	0.0049	0.0028	0.0027

Type	Soluble	Membrane
Likelihood	0.9828	0.0172

Hierarchical Tree.



Position Importance

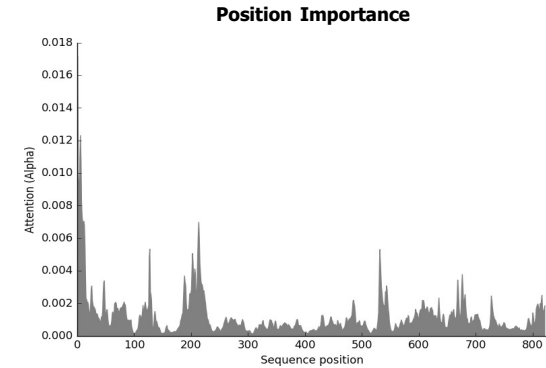
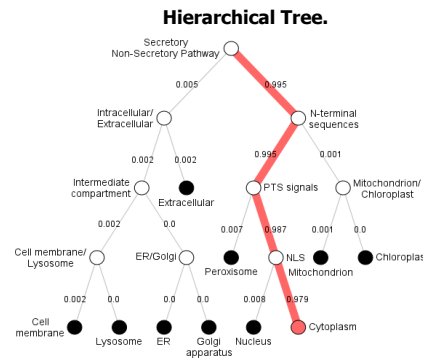


**Lox19**

**Prediction: Cytoplasm, Soluble**

Localization	Cytoplasm	Peroxisome	Lysosome/Vacuole	Nucleus	Endoplasmic reticulum	Extracellular	Cell membrane	Mitochondrion	Plastid	Golgi apparatus
Likelihood	0.9289	0.0404	0.0086	0.0063	0.0045	0.0036	0.0033	0.0021	0.0016	0.0007

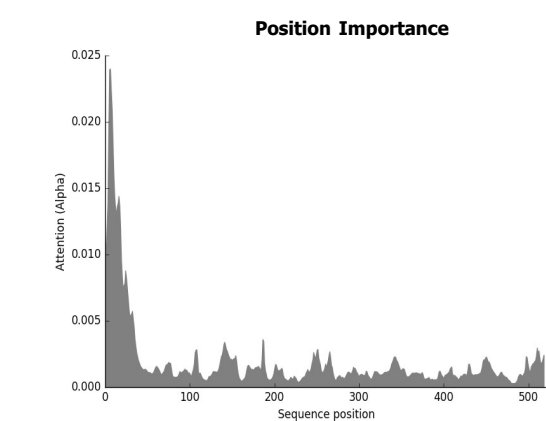
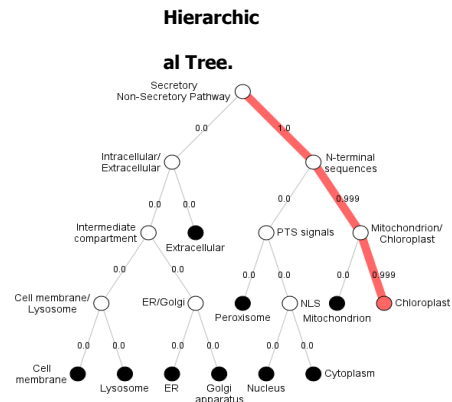
Type	Soluble	Membrane
Likelihood	0.9945	0.0055



**Lox20**

**Prediction: Plastid, Soluble**

Localization Type	Plastid Soluble	Nucleus Membrane	Mitochondrion	Cytoplasm	Endoplasmic reticulum	Lysosome/Vacuole	Extracellular	Peroxisome	Golgi apparatus	Cell membrane
Likelihood	0.9995 0.9046	0.0002 0.0954	0.0002	0.0001	0	0	0	0	0	0

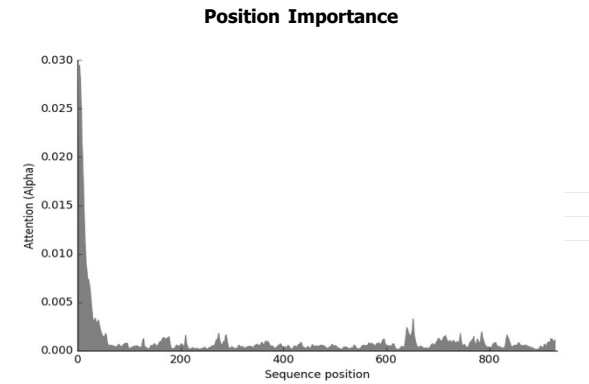
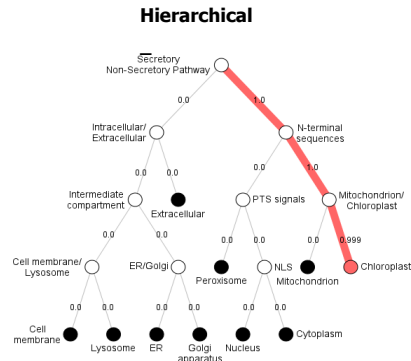


Lox22

Prediction: Plastid, Soluble

Localization	Plastid	Mitochondrion	Cytoplasm	Nucleus	Peroxisome	Endoplasmic reticulum	Extracellular	Lysosome/Vacuole	Golgi apparatus
Likelihood	0.9972	0.0026	0.0001	0	0	0	0	0	0

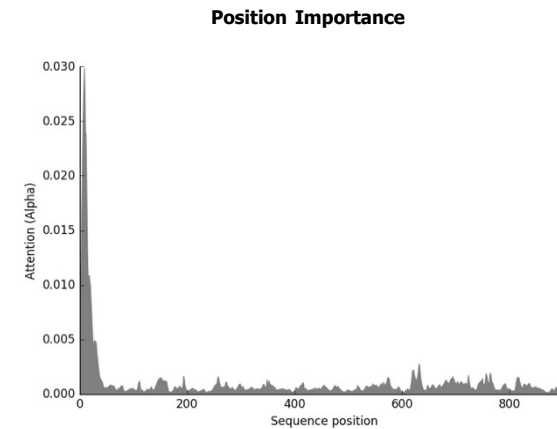
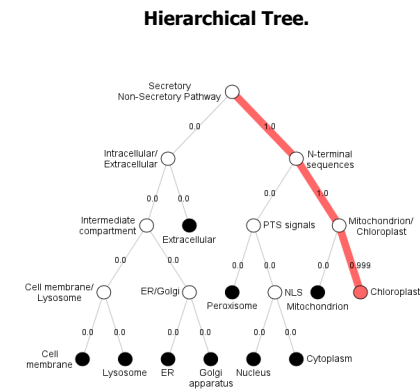
Type	Soluble	Membrane
Likelihood	0.8806	0.1194



Lox23

Prediction: Plastid, Soluble

Localization	Plastid	Mitochondrion	Cytoplasm	Nucleus	Endoplasmic reticulum	Extracellular	Lysosome/Vacuole	Golgi apparatus	Peroxisome	Cell membrane
Type	Soluble	Membrane								
Likelihood	0.9997	0.0001	0.0001	0	0	0	0	0	0	0
Likelihood	0.8733	0.1267								



## 11. ACKNOWLEDGEMENTS

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