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Alfalfa Growth under Changing Environments: An Overview

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CROP production is one of the most important agro-global issues, in particular questions of production under changing environmental conditions. This production needs to be increased to meet global needs for food, feed, fiber and fuel. Alfalfa is classified as "*the queen of the forage crops*" due to its high protein content and nutritional valueas well as its unique availability during the summer compared with other forage crops. The production of alfalfa under different stressful environments is a great challenge due toseveral problems with alfalfa crop production, which represent a serious threat to global food security.These stresses may cause a decline in the global feed production from alfalfa due to harmful effects resulting from stressesat the physiological, biochemical and histological levels. To improve the production of alfalfa under these stresses, there is a crucial need to understand the response of alfalfa plants to stresses, the mechanisms of tolerance and the management options. The bio-organic fertilizers derived from alfalfa plants are a crucial and sustainable solution in particular under stressful environments. This review represents an attempt to highlight the positive sides of alfalfa production, particularly the sustainable use of this crop in bio-organic fertilizer production. The chemical and anatomical properties of this plant will also bereviewed. The histology of alfalfa plants under changing environments still needsfurther investigation.

Keywords: Abiotic stress, Alfalfa Taxonomy and Anatomy,Climate change, Drought, Salinity.

Introduction

Global population has increased rapidly over the last few decades and may reach 9.8 billion by 2050 (Kopittke et al. 2019). The main challenge faced by agricultural scientists is producing enough healthy foods for all these people. The agricultural sector is the main source of food, feed, and fiber as well as a major source of fuel (Brevik et al. 2019). Global agriculture is facing many challenges including soil organic matter decline, low nutrient use efficiency, crop yield stagnation, multi-nutrient deficiencies and water scarcity (Raliya et al. 2018). Crop productivity is greatly influenced by manyabiotic stresses such as salinity, drought, waterlogging or flooding and heavy metals (Gopinath et al. 2018; Ullah et al. 2019). Furthermore, many environmental issuesrepresent serious threatsto crop production (e.g, climate change, pollution, water and energy scarcity).Therefore, there is an urgent need to address the problems caused by these environmental issues (Fahad et al. 2017;

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Lamaoui et al. 2018; Hussain et al. 2018; Sehgal et al. 2018; Dahal et al. 2019 and Rodrigues et al. 2019).

Alfalfa (*Medicago sativa* L.) could be considered the most important legume forage crop in the world and is primarily used as silage, hay and pastureto feed livestock (Hawkins and Yu, 2018 and Patra & Paul, 2019).This plant also called "lucerne" in Europe and other countries and its sprouts can be used as a staple crop for animals and humans due to its impressive nutritional content,including vitamins (*i.e.*, B, C, D and E),high protein content and other important minerals (Baker et al. 2019; Mattioli et al. 2019 and Michalczyk et al. 2019). Alfalfa was originally cultivated in south-central Asia (modern Iran)and it is well known as the "*Queen of Forage*" because of its high biomass yield, good quality of its forage and its palatability for ruminants (Lei et al. 2017). Alfalfa can grow in a wide range of soils and under several growing conditions,including nutrient-poor soils (Lei et al. 2017).More than 40 million hectares are cultivated worldwide (Luo et al. 2019). Alfalfa yields arehigher in light-textured soil conditions (e.g., sandy loam, silty loam and clay loam) than in heavy textured soils (Kavut and Avcioglu, 2015 and Mbarki et al. 2018). Alfalfa could be considered one of the most important cropsfor sustainable agriculture due to its promotion of soil fertility, abiltity to feed livestock in mixed production systems, N-fixation rate and ability to reduce greenhouse gas emissions (Annicchiarico et al. 2016; Luo et al. 2018 and Kulkarni et al. 2018). This plant can grow under arid and semi-arid conditions (200 mm annual precipitation) due to its relatively deep root system and enhancement of antioxidative protection and declining lipid peroxidation as a tolerant crop to water deficits (Lei et al. 2017 and Zhang et al. 2019). Based on its use in the production of organic acids and ethanol, alfalfa has great potential as a biofuel feedstock (Luo et al. 2019).

The cultivation of forage crops (in particular alfalfa) is an important issue for animal nutrition and the sustainability of thehuman food supply (Hedayetullah and Zaman, 2019). The production of alfalfa is totally controlled by environmental factors including biotic and abiotic stresses. These stresses can cause a significant reduction in the productivity of crops. Due to the importance of alfalfa, there has been increasing recent interest in cultivating alfalfa under stressful environments (Stritzler et al. 2018). In the past few decades, a considerable amount of literature has been published on the growth and production of alfalfa under different abiotic stresses. These studies focused on high-quality alfalfa production under stresses like salinity (Sandhu et al. 2017; Stritzler et al. 2018 and Luo et al. 2019), drought (Liu et al. 2018; Zhao et al. 2019 and Zhang et al. 2019) and heavy metals like cadmium (Kabir et al. 2016; Gu et al. 2018; Motaharpoor et al. 2019 and Yang et al. 2019) and copper (Samma et al. 2017; Chen et al. 2018; Duan et al. 2019 and Ju et al. 2019).

This study systematically reviews the data foralfalfa production intendingto provide a comprehensive overview on biochemical and anatomical aspects of the alfalafa plant. Drawing upon stressful environment research into alfalfa, this study attempts to present the several benefits of this crop under stress.

Alfalfa Taxonomy

Alfalfa (*Medicago sativa* L.) belongs to the genus *Medicago* within the Fabaceae family. Cultivated alfalfa plants are considered an outcrossing ploidy as tetraploid ($2n = 4\times = 32$) or diploid ($2n = 2x = 16$), where the pod shape may be coiled or falcate, the flower color is yellow (Greene et al. 2015), purple or variegated and glandular hairs may be absent or present (Monteros et al. 2014 and Hawkins & Yu, 2018). The botanical classification includes the following taxonomy (Bagavathiannan and Van Acker 2009):

Kingdom: Plantae (Plants)

Subkingdom: Tracheobionta (Vascular plants)

Superdivision: Spermatophyta (Seed plants)

Division: Magnoliophyta (Flowering plants)

Class: Magnoliopsida (Dicotyledons)

Subclass: Rosidae

Order: Fabales

Family: Fabaceae (pea family)

Genus: *Medicago*

Species: *Sativa*

Common name: alfalfa or lucerne

The genus *Medicago* contains at least 87 species, which are mainly distributedaround the Mediterranean basin. This genus is represented by flowering plants in the Fabaceae family and well known as medick or burclover. This name orginated from the Greek word *Median* or grass and is based onthe Latin name *medica*. The most important member in this genus is alfalfa. The general chromosome numbers in the *Medicago* genus may range from 2*n* = 14 to 48. Apart from *Medicago sativa*, there are many members in the*Medicago* genus such as*Medicago truncatula*. *M. truncatula* could be considered a model legume due to its small genome (50–550 Mbp; Gholami et al. 2014), short life cycle (about 3 months) and its ability to pollinate through both self-crossing and outcrossing (Burks et al. 2018; Roque et al. 2018). The members of the*Medicago* genus are characterized by their ability to produce many bioactive natural compounds, which can join in symbiotic interactions to preventattacks from herbivores and pathogens. These bioactive products have promising pharmaceutical properties for humans and are found in many secondary metabolite classes in *Medicago* species such as medicagenic acid (as a triterpenoid saponin) and medicarpin as a flavonoid (Gholami et al. 2014; Rafi□ska et al. 2017; Gill et al. 2018; Khalid et al. 2019). Many *Medicago* species have the potential to be used as green manure, sources of medicine, human food (e.g., sprouts) and in the biotechnology sector as a source of industrial enzymes (Gholami et al. 2014).

There is no possibility to hybridize the *Medicago* genus with any other genera.Nearly two-thirds of *Medicago* species are annual crops with the others being perennial crops, including cultivated alfalfa.Recently, some investigators have examined evidence of successful interspecific hybridization in *Medicago* species (e.g., Bagavathiannan and Van Acker 2009; Greene et al. 2015; Sousa et al. 2016, 2017; Eriksson et al. 2018). These efforts can be summarized for the most important species of the*Medicago* genus,aside from *Medicago sativa* and *Medicago truncatula*,as follows:

M. glomerata	(2n = 2x = 16)
M. rhodopea	(2n = 2x = 16)
M. rupestris	(2n = 2x = 16)
M. daghestanica	(2n = 2x = 16)
M. pironae	(2n = 2x = 16)
M. marina	(2n = 2x = 16)
M. hybrida	(2n = 2x = 16)
M. dzhawakhetica	(2n = 4x = 32)
M. saxatilis	(2n = 6x = 48)
M. cancellata	(2n = 6x = 68)
M. papillosa	(2n = 2x = 16; 2n = 4x = 32)
M. prostrata	(2n= 2x = 16; 2n = 4x = 32)

There is a growing body of literature that recognizes the importance of the plant genome and its database. The plant genome represents the genetic material of the plant or the collected genomic sequence of a plant species. The plant genome database is considered a storage platform system, in which more data could be included due to the rapid development of bioinformatics (Chen et al. 2018). The genome of *Medicago* speciesinvolving alfalfa has been investigated by many researchers recently,including exploring the structural variation in 15 *Medicago* genomes and their gene family (Zhou et al. 2017), genetic progress in alfalfa forage quality through mapping and genomic selection (Biazzi et al. 2017), the availability of genomic data (Burks et al. 2018), the sequenced angiosperm genomes and itsdatabase (Chen et al. 2018), alfalfa genomic prediction for 25 quality and agronomic traits (Jia et al. 2018), the discovery of theplastome traits within *Medicago* species (Choi et al. 2019), and improving the yield potential of alfalfa *via* quantitative trait loci mapping (Zhang et al. 2019).

Alfalfa Anatomy

Study of the internal structure of alfalfa and its parts (*i.e.,* the roots, stems and leaves) as well as its systems (e.g., the root, vegetative and reproductive system), could be called the plant's anatomy Crang et al. 2018). This science was established and developed many hundreds of years ago and still receives a large amount of effort, including original articles, reviews and books (examples of recent books: Beck 2010; Maiti et al. 2012; Steeves and Sawhney 2017; Crang et al. 2018). These efforts have real potential or importance in different fields, in particular the environmental and agricultural sciences (*e.g.,* Carriquí et al. 2019; Farooq et al. 2019; Lisztes-Szabó 2019; Wang et al. 2019; Zhong et al. 2019). Therefore, understanding of the plant's anatomy may guarantee sound knowledge of the plant's structural components and the function of each component. It is well documented that the plant's anatomy is a good guide toshow modifications in plant structure and further its development in response to environmental adaptations (Rosmala et al. 2016 and Steeves & Sawhney, 2017). Furthermore, the growing and developing of plant tissues and organs under undesired conditions could be monitored using the modern tools of plant anatomy. This modern anatomy also has been emphasized using the recent applications of molecular genetics (Wachsman et al. 2015; Chomickiet al. 2017 and Steeves & Sawhney, 2017). Therefore, understanding the anatomy of alfalfa is vital and required for several purposes such as understanding fiber content and its use in animal feed as well as the root structure and the resistance of alfalfa plants to stressful conditions.However, there is little work linking alfalfa anatomy to stressful conditions (e.g., Gronwald and Bucciarelli 2013; Printz et al. 2015; Moawed 2016; Mickky et al. 2018 and Nja et al. 2018).

Seeds of Alfalfa

Upon germination, the plant hasa slow emergence rate. The crown is formed first, followed by the establishment of a strong and deep root system. The crown of the alfalfa carries the shoot buds and has the ability to re-grow many times after cutting or grazing. The seeds of alfalfa are kidney shaped (**Fig. 1**). The alfalfa seeds are also an important source of sprouts,which areconsidered one of the most popular seed sprouts in recent decades (Kang et al. 2019).

Stem of Alfalfa

The stem supports the aerial portions of the alfalfa plant, in particular the leaves and flowers (Fig. 2). The main role of the stems is the proper distribution of leaves and flowers toguarantee the maximum absorption of light and successful pollination, respectively (Crang et al. 2018).The hemicellulose and cellulose contents in mature alfalfa stems average 120 and 310 g kg^{-1} dry basis, respectively, which might be hydrolyzed into sugars(Hojilla-Evangelista et al. 2017). The content of cellulose and hemicellulose increases with plant age, whereas the pectins decrease with increasing stem maturity (Printz et al. 2015).

Leaf of Alfalfa

Plant leaves are the greatest factories on earth, in which photosynthetic productsare produced (Fig. 3). This factory absorbs light through

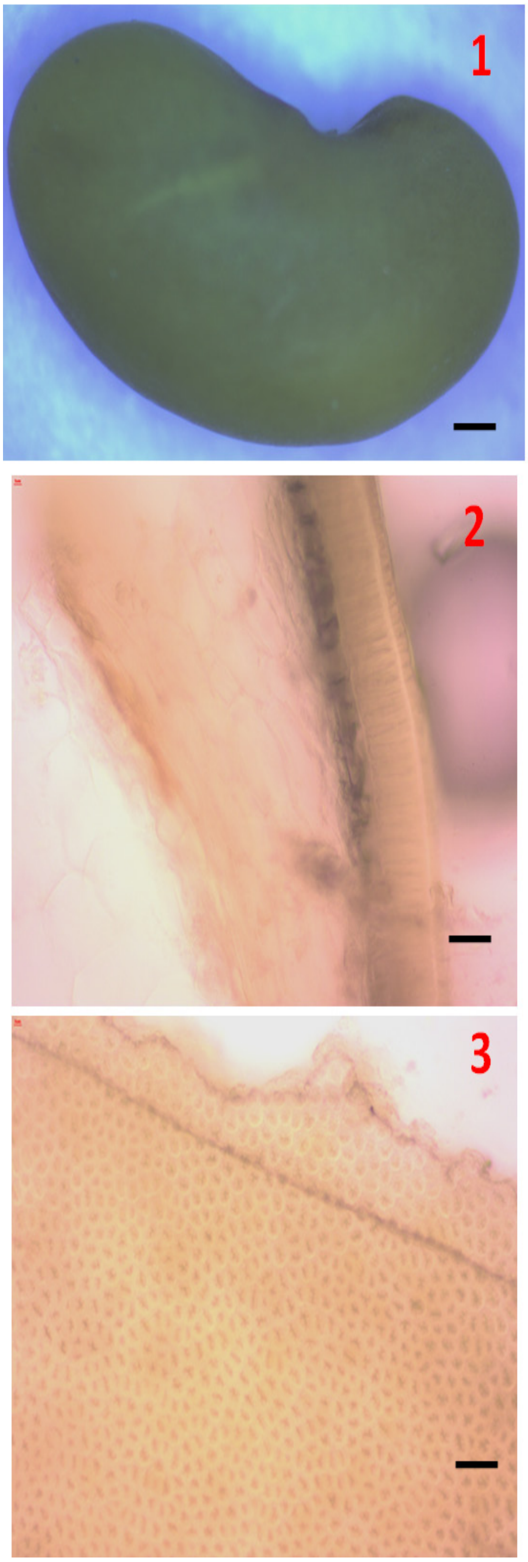

Fig. 1. The seeds of the Hungarian variety "Hunor" of alfalfa. Seed under stereo microscope (photo 1) and transection of seeds under a light microscope (photos 2 and 3). Scale bar for photos 1, 2 and 3 is 50, 5 and 5 µm, respectively.

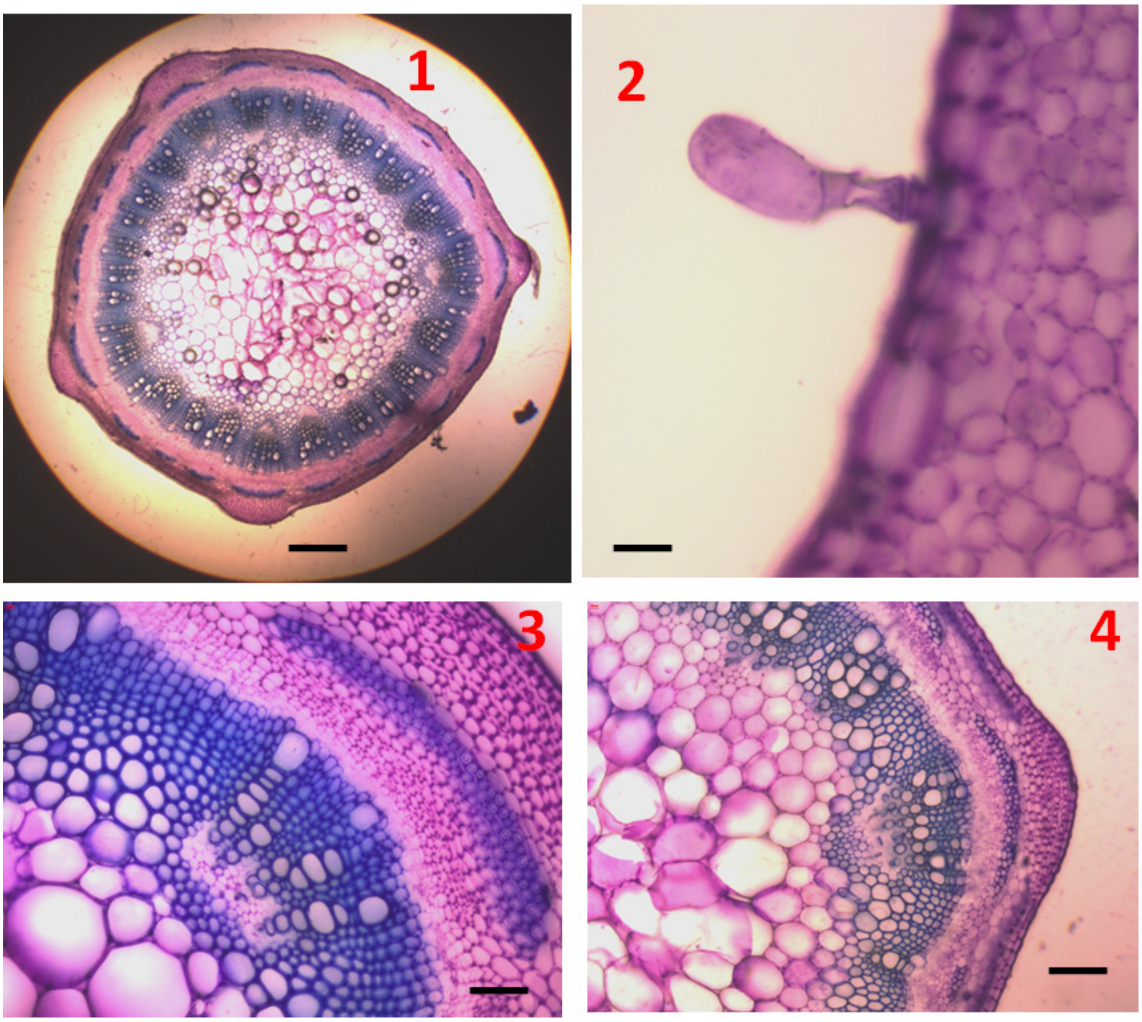

Fig. 2. The anatomical structure of the alfalfa stem in the Hungarian variety "Hunor". Whole cross- section of stem (photo 1), trichome type of stem (photo 2), and partial transection of stem (photos 3 and 4 in two different scales). Scale bar for photos 1, 2, 3 and 4 is 100, 5, 10 and 20 µm, respectively. All sections were stained by toluidine blue

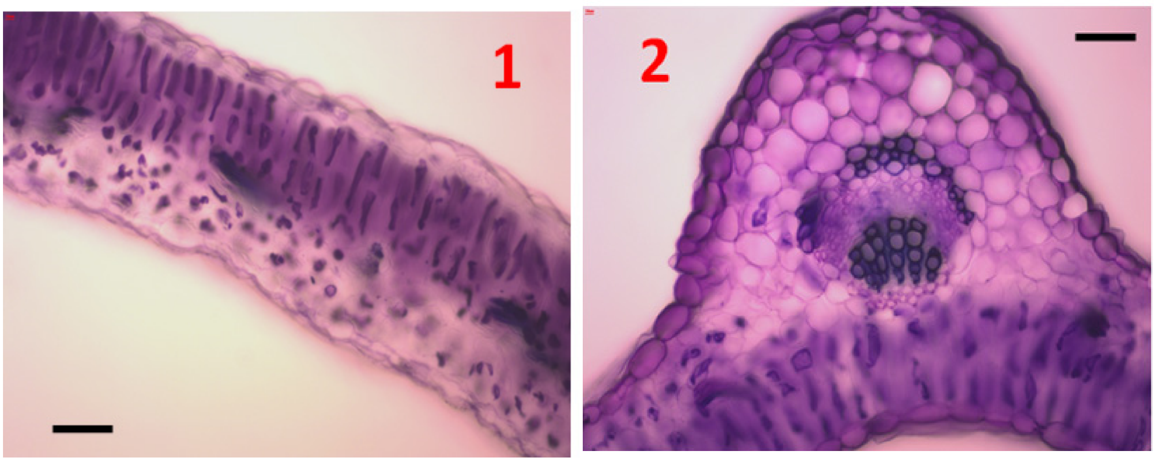

Fig. 3. The anatomical structure of the alfalfa leaf in the Hungarian variety "Olimpia". Cross- section of leaf arm (photo 1) and cross section of the main vein (photo 2). Scale bar for photos 1 and 2 is 10 and 20 µm, respectively. All sections were stained by toluidine blue

the thousands of chloroplasts and uptakescarbon dioxide to produce food (Crang et al. 2018).The most important component of alfalfa for animal feed is the leaves, which have the highest protein content (up to 300 g kg^{-1} DB)in comparing with protein content in the stems, which is up to 120 g kg^{-1} DB (Hojilla-Evangelista et al. 2017).

Alfalfa Productivity under Abiotic Stress

Production of alfalfa is mainly controlled by environmental and botanical factors as well as environmental stresses (Zahran, 2017). These adverse environmental conditions represent a serious threat to alfalfa growth, development, survival and productivity with marked impacts at biochemical, morphological, physiological and molecular levels (Zhang et al. 2018). The previouslymentioned plant levels of proteins and fiber are the responsible items for the quality and yield of alfalfa (Luo et al. 2019). Under abiotic stresses (*e.g.,* salinity, drought, heavy metals stress,and changes in climate) the production of alfalfa is projected to decrease,threatening global feed and food security (Reed et al. 2018). Due to the importance of environmental stresses, several studieshave addressed the impact of these stresses on alfalfa growth and productivity,including the affects of soil salinity (Sandhu et al. 2017; Wang et al. 2017; Lei et al. 2018; Noori et al. 2018; Stritzler et al. 2018; Gao et al. 2019b; Luo et al. 2019 and Yang et al. 2019), drought (Ma et al. 2016; Liu et al. 2018; Zhang et al. 2018; Zhao et al. 2019 and Zhang et al. 2019), irrigation water stress (Chen et al. 2018; Jia et al. 2018; Wang et al. 2018 and Baker et al. 2019),waste water stress (Rekik et al. 2017 and Elfanssi et al. 2018), water table stress (Berhongaray et al. 2019), fertilization stress (Gu et al. 2018) and general environmental issues (Bacenetti et al. 2018 and Ghaderpour et al. 2018).

Alfalfa Germination under Salinity Stress

Germination is considered the most important growth stage of cultivated crops and the most critical period in the plant's life, particularly under stressful environments. Alfalfa is moderately sensitive to soil salinity and relatively tolerant to drought (Ma et al. 2017). Alfalfa biomass yield can be reduced to50 % when soil salinity (EC) reaches 8.8 dS m^{-1}, whilstalfalfa seeds willgerminate well atsalinity levels ofabout 2.0 dS m^{-1} (FAO 2002). Salinity is considered one of the most serious stresses for alfalfa (Table 1). The main effects of salinity are ion toxicity (mainly sodium ions), hyper-osmotic pressure (which results in low water availability) (Luo et al. 2019), oxidative stress and nutrient deficiency (Boukari et al. 2019). Plants cultivated under salinity stress have certain defense mechanismstowards this stress in the form of a series of severe biochemical and physiological changes.Salinity stress may also disturb the regulation of plant hormones and photosynthesis processes causing an imbalance in plant nutritional status, reducing plant yield and quality (Farooq et al. 2017).The main effects of soil salinity on alfalfa reproduction include negative impacts on germination and growth, competition for the uptake of mineral nutrients, the photosynthetic process, the efficiency of biological nitrogen fixation and yield quality (Farooq et al. 2017). Alfalfa may adopt one or more of the following tolerance mechanisms towards salinity: ion homeostasis, osmotic and hormonal regulation, osmotic protection, the antioxidant defense system, and an increase in apoplastic acidification (Farooq et al. 2017).

The main management strategies for legume crops under salinity stress include the use of conventional breeding approaches, biotechnology and functional genomics, plant growth promoting rhizobacteria, application of exogenous hormones and osmoprotectants, inoculatingthe seeds with arbuscular mycorrhizal fungi, seed priming and nutrient management (Farooq et al. 2017).In general, changes in cultivated plants under salinity stress and the projected coping mechanisms of crops,including alfalfa,towards this stress include:

(1) Regulating water (Reef et al. 2015) and hormonal (Belmecheri-Cherifi et al. 2019) balances in the plant,

(2) Maintaining the integrity of plant cell membranes (Feng et al. 2018),

(3) Accumulating compatible solutes or osmolytes (*e.g.,* proline, glycine betaine and total soluble sugars) to adjust the cellular osmotic pressure (Kumar et al. 2017; Vyrides and Stuckey 2017 and León et al. 2018),

(4) Scavenging reactive oxygen species (ROS) and activating the antioxidant system,including non-enzymatic antioxidants (ascorbate, gluthatione, malondialdehyde, total phenolic compounds and total antioxidant flavonoids) and antioxidant enzymes (superoxide dismutase,

catalase, ascorbate peroxidase and glutathione reductase) (Kumar et al. 2017; Makavitskaya et al. 2018; Xiong et al. 2018 and Gao et al. 2019a),

(5) Balancing the uptake of nutrients like Na^+ and K^+ (Alsaeedi et al. 2019 and Jiang et al. 2019) and reinstating the cellular ionic equilibrium, then reducing the ionic or osmotic damage that was caused by salinity stress (Luo et al. 2019),

(6) Maintaining the performance of photosynthetic CO_2 assimilation and sinks (Penella et al. 2016). Preventing reduction in the photosynthetic attributes as well as alleviating stomatal parameters and chlorophyll fluorescence by adding H_2S under salinity stress (Jiang et al. 2019),

(7) Priming of alfala seeds in salicylic acid may improve the plant's tolerance to salinity stress and iron deficiency (Boukari et al. 2019),

(8) Due to water uptake inhibition and/or the specific toxic impact of ions in the embryo of legume seeds, seed germination may be reduced up to 50% or more under salt stress(Table 5) (Farooq et al. 2017; Gao et al. 2019b),

(9) Salinity stress may reduce the growth of legumes more than 70% and reduce the uptake of mineral nutrients as well as the yield from 12 to 100% because of the toxicity of specific ions and reduction in the photosynthesis process rate (Farooq et al. 2017),

(10) Microbes can play a large role in plants which influenced by salt stress (Salwan et al. 2019). The presence of certain bacteria or plant growth promoting rhizobacteria (Noori et al. 2018 and Ju et al. 2019) and the mycorrhizal association with arbuscular mycorrhizal fungi (Ben Laouane et al. 2019) can improve salinity tolerance because these microbes may help in thebioavailability of plant nutrients (Farooq et al. 2017).

During alfalfa plant growth and development, the germination and early seedling stagesare considered the most important and crucialfor plant establishment. Tolerance to salinity at the physiological and proteomic levels during the germination stage has been investigated by many researchers but still needs more studies (Gao et al. 2019b). Researchers have shown an increased interest in investigating the proteomic response of alfalfa to stressful environments (e.g., Gou et al. 2019; Li et al. 2018 and Singer et al. 2018), but little attention has been given to the behavior of alfalfa under salinity stress during the germination stage (*e.g.,* Amooaghaie and Tabatabaie 2017; Ma et al. 2017 and Gao et al. 2019b). This stress restricts water uptake, in which a hydrolysis of reserved food in the seed could occur due to enzyme activity(e.g., alcohol dehydrogenase and fructokinase activities). This step includes inhibiting the start of metabolism and interrupting the mobilization of starch in the germination stage (Gao et al. 2019b).

The response of alfalfa to salinity stress based on the genetic approach (Luo et al. 2019). That means breeding to emphasize the genes that are important candidates to improve

TABLE 1. Germination rate of some alfalfa cutivars at different levels of salt stress

Alfalfa variety or ecotype	Salt concentration and growth media	Germination rate (%)	The aim of the study	Reference
Gabes and Presmenti ecotypes	75 mM NaCl in Petri dishes for 3 days	------	Role of salicylic acid in improving seed tolerance to salinity	Boukari et al. (2019)
Zhongmu no. 1	200 mmol L^{-1} NaCl for 10 days in Petri dishes	60	The proteomic changes in two contrasting alfalfa cultivars	Gao et al. (2019b)
Zhongmu no. 3		80		
Zhongmu no.3 (salt-tolerant cultivar)	300 mM NaCl for 10 days in Petri dishes	30	Proteomic and physiological study at germination stage	Ma et al. (2017)
Hamedani (cv.)	150 mM NaCl for 5 days in Petri dishes	45	Low H_2O_2 alleviates salt stress during germination	Amooaghaie and Tabatabaie (2017)
Biaogan (cv.)	100 mM NaCl in Petri dishes for 3 days	50	Role of methane in alleviating NaCl toxicity during seed germination	Zhu et al. (2016)

the plant's resistance to salinity stress using genetic engineering (Luo et al. 2019). Therefore, the productivity of alfalfa could be improved through the use of molecular tools (Lei et al. 2017 and Singer et al. 2018) in general or under salinity stress (Ma et al. 2017; Gruber et al. 2017; Gao et al. 2019b and Luo et al. 2019). There has also been renewed interest in the role of mineral nutrients such as calcium, sulfur, potassium, silicon, selenium, etc. in supporting plants against salinity stress (Rizwan et al. 2015; Jabeen 2018; Xiong et al. 2018; Yang and Guo 2018a, b and Zhu et al. 2019). Further investigations are needed into the use of mineral nutrients and molecular geneticsto improve alfalfa production under salinity stress.

Alfalfa production under Drought Stress

Drought is a great threat that isconsidered one of the most important abiotic stresses and can limit the productivity of cultivated crops, causing enormous losses of yield (Zhang and Shi 2018; Laxa et al. 2019 and Zhang et al. 2019). Drought is common in arid and semi-arid regions, but is expected to increase in other areas due to global climate change, urbanization and deforestation (Zhang and Shi, 2018). Therefore, there is an urgent need for producing new varieties of major crops produced using plant breeding and molecular biology approaches thatare more tolerant to drought stress (Joshi et al. 2016 and Singh et al. 2019).

The investigation of alfalfa and its production under drought or water deficit stress is of interest to scientists all over the world due to the economic value of this crop. These studies include the physiological, biochemical and proteomic or molecualraspects of alfalfa varieties under drought stress (Fan et al. 2015; Quan et al. 2016; Ma et al. 2017; Singer et al. 2018; Zhang and Shi 2018 and Zhang et al. 2018)and its tolerance through enhancing the production of antioxidants and declining lipid peroxidation (Singer et al. 2018 and Zhang et al. 2019), pretreatment of alfalfa with priming agents such as phytohormones like jasmonateascorbic acid or polyethylene glycol (Salemi et al. 2019), reactive oxygen-nitrogen-sulfur species (Antoniou et al. 2016) and melatonin (Antoniou et al. 2017). These studies also reported on the crucial impact of drought stress on the nutritional composition of alfalfa and its yield (Liu et al. 2018). More studies on the metabolism of carbohydrates and photosynthesis efficiency of alfalfa seedlings in the presence of NO (Zhao et al. 2019), the phenotypic variations and genetic diversity related todrought tolerance inalfalfa accessions (Zhang et al. 2018) have been published.Themolecular or proteomic analysis of alfalfa under drought (Ma et al. 2016; Arshad et al. 2017; Li et al. 2017), during seed germination (Ma et al. 2017), also were involved. Some recent studies on the impact of drought on alfalfa production are summarized in Table 2.

In general, resistance of plants to drought stress can be classified into the following mechanisms (1) escaping from drought, (2) avoiding the drought, (3) tolerant to drought and (4) drought recovery (Fang and Xiong 2015 and Zhang et al. 2019). To be tolerant, alfalfa breeding should enhance the plant defence system, represented by high levels of enzymatic antioxidants (catalase, peroxidase, superoxide dismutase, glutathione reductase, ascorbate peroxidase) or non-enzymatic (ascorbate, proline, malondialdehyde, glutathione) (Zhang and Shi, 2018). It was concluded that further investigations are needed to understand the behavior of alfalfa under drought stress.In particular, the –omics approaches along with molecular biology and morpho-physiological analysis are needed for more elucidation of the alfalfa complex networks and identification ofproteins and theircore genes (Zhang and Shi, 2018).

The process of symbiotic N_2 fixation in alfalfa and other legumes is a distinguishing feature, which gives these legumes an economical benefit. This process is sensitive to many environmental factors particularly drought or water stress. The low availability of soil water content might decrease the N_2-fixation of field grown soybean and lentil (Parvin et al. 2018). Under semi-arid environments, the drying of soils is considered a significant constraint for N_2 fixation of legumes and the N uptake, where the N content in seed legumes will be controlled by the re-mobilization of the previous assimilated N-from vegetative tissues (Parvin et al. 2019b). Several studies have been handled the efficiency of N_2 fixation of legumes under drought stress (*e.g.,* Aung et al. 2017; Aldasoro et al. 2019 and Zhao et al. 2019). The improving efficiency of water use and N_2 fixation by enriching CO_2 under drought on of pea (*Pisum sativum* L.) also was investigated (Parvin et al. 2019a). The most common impact of drought on N_2 fixation of legumes may include accumulation of C and N compounds in leaves andnodule tissues, and decrease the activity of N_2-fixation of drought-stressed plants (Parvin et al. 2019b).

TABLE 2. Some studies on the production of alfalfa under drought stress

The study conditions	The aim of the study and response of plant	Reference
Seeds (cv. Ghomi) primed in AsA (1 mM) and PEG (7.8 Mm) for 6 h, water stress level -0.5 MPa	Priming seeds with AsA and PEG may help seedlings to ameliorate drought by increasing proline, soluble sugars, total phenolics, and antioxidant enzyme activities; by decreasing hydrogen peroxide and malondialdehyde in the seedlings	Salemi et al. (2019)
Three alfalfa varieties (Longzhong, Longdong, Gannong No. 3) under applied PEG-6000, osmotic potential of -1.2 MPa for 12 days	Drought decreased growth, photosynthetic capacity, increased malondialdehyde accumulation, ROS, osmolytes and antioxidant enzyme activities (e.g., APX)	Zhang et al. (2019)
Seeds (cv. Sanditi) immersed in dH_2O and PEG-6000 (5, 10, 15, 20, 25%), then 200 µM NO scavenger for 7 days	NO can regulate the response of alfalfa seedlings to drought by enhancing the metabolism of carbohydrate and photosynthesis efficiency	Zhao et al. (2019)
Two varieties (Longzhong and Gannong No. 3), seedlings were exposed to −1.2 MPa PEG-6000 for 15 days	Drought-tolerance may be attributed to higher osmotic adjustment capacity, enzymatic (GR, SOD, POD, CAT, APX) and non-enzymatic antioxidant (proline, MDA, AsA, GSH) to avoid oxidative damage	Zhang and Shi (2018)
Three alfalfa varieties (Longzhong, Longdong and Gannong No. 3); under osmotic potential: − 0.4, − 0.8, − 1.2, − 1.6 and − 2.0 MPa	Changes in biochemical and physiological characteristics under drought: increased lipid peroxidation, osmolytes contents, ROS production, levels of antioxidative enzymes and antioxidants with increasing drought stress	Zhang et al. (2018)
Field experiment, two cultivars (Gold Queen and Suntory) harvested early during a flowering stage or late at full bloom	Drought decreases alfalfa yield and its nutritional value; severe drought decreased the crude protein content and hay yield, increased the fiber, irrigation management and harvesting time can mitigate drought stress	Liu et al. (2018)
Field experiment for 6 years under drought, four P levels: 0, 9.73, 19.3, 28.9 kg P ha^{-1}	Forage yield and WUE increased under plastic film mulch, P fertilization at 16.1 or 17.5 kg P ha^{-1} improved soil water condition and forage yield under drought	Gu et al. (2018)
Pot experiment used under 10 µM melatonin, seedlings were imposed for drought stress by withholding watering for 7 days	Melatonin may ameliorate drought damage through regulation of reactive oxygen (CAT, APX, SOD, GR), modulates nitro-oxidative homeostasis and proline metabolism, regulates redox-related components and mRNA antioxidant levels	Antoniou et al. (2017)
Two varieties (Longdong and Algonquin), 6 days after germination the seedlings were transferred to plastic pots filled with vermiculite, drought-stress induced by withholding water for 18 days	Morphological, physiological, and transcriptional levels could be used in confirming the tolerant to drought stress, variety more tolerant to drought exhibits more proline and ascorbate content, more lateral roots, higher LWC, higher antioxidant enzyme activity, less cell membrane damage, less accumulation of H_2O_2, lower stomata density	Quan et al. (2016)

Abbreviations:PEG: Polyethylene glycol, AsA: ascorbic acid; MDA: malondialdehyde; SOD: superoxide dismutase; GR: glutathione reductase; POD: peroxidase; CAT: catalase; APX: ascorbate peroxidase; NO: Nitric oxide; AsA: ascorbate; GSH: glutathione; WUE: water use efficiency; LWC: leaf water content

Alfalfa and Its Potential in Phytoremediation

The modern world faces great challenges including non-enough clean water, natural resources depletion, the management of hazardous wastes and remediating polluted environments (Tilla and Blumberga 2018). Several studies have investigated environmental pollution and its remediation (*e.g.,* Wu et al. 2017; Tilla and Blumberga 2018 and Ye et al. 2019). Remediation is a process by which hazardous substances are removed from the environment to minimize threats to environmental and human health (Tilla and Blumberga, 2018). Remediation strategies should build on social, economic, environmental and sustainability issues. Sustainable remediation is important toprotect environmental and human health from a wide range of risks (*e.g.,* Emenike et al. 2017; Tilla and Blumberga 2018; Ashraf et al. 2019; Huysegoms et al. 2019 and Ye et al. 2019).

Environmental pollution has received considerable critical attention for many years and at all levels from people worldwide because it threatens human health. The remediation of this pollution includes bio- and phyto-remediation. Both types of remediation deal with organic or inorganic pollutants in co-contaminated sites in individualor collective forms. Phytoremediation is a bio-process by which several plants can uptake, destroy, remove, stabilize and/or trans fer pollutants from soils and groundwater. Phytoremediation has many processes or mechanisms including phytoextraction, phytostabilization, phytovolatilization, phytostimulation, rhizofiltration, phytodegradation, and phytodesalination (Rostami and Azhdarpoor, 2019). The management of environmental pollution through phytoremediation using invasive plants has received a large amount of attentionfrom researchers (Prabakaran et al. 2019) as has plant growth regulators (Rostami and Azhdarpoor 2019), bioenergy plants (Hunce et al. 2019),and sustainable and eco-environmental solutions (Ashraf et al. 2019 and Saxena et al. 2020).

Alfalfa hasconsiderable promisein the phytoremediation of contaminated sites due to its fast growth rate, high biomass production, deep and extensive root system, and potential to grow in many different soil types (Agnello et al. 2016). It hasalso potential in the phytoremediation ofa range ofcontaminantes, such as cadmium (Gu et al. 2018; Yang et al. 2019), copper (Chen et al. 2018a nd Ju et al. 2019), organic pollutants like polycyclic aromatic hydrocarbons (Alves et al. 2017 & 2018) and organochlorines (Teng et al. 2017 and Tu et al. 2017). The potential utilization of alfalfa in the phytoremediation of cadmium contaminated soils is presented in Table 3.

Alfalfa plants have a strong root system and have good potential for use in phytoremediation. Many investigations reported on this advantage of alfalfa, in particular using transgenic plants that are tolerant to different stressful environments.

Alfalfa Production under Climate Change

Climate change is a global problem that touches all human activities and impactsbiological systems (Hannah, 2020). Changing climate is increasingly recognized as a serious, worldwide public health concern (Tong et al. 2016). The agricultural field is considered one of the most important sectors, which may control by the climate and its factors (Upretyet al. 2019). Recently, a large body of literature has grown up around the impacts of climate change on agriculture. This literature touched on all agricultural issues including biodiversity (Filho et al. 2019), sustainability (Agovino et al. 2019), use efficiency of plant resources (Bhattacharya 2019), agricultural vulnerability (Neset et al. 2019), agricultural practices (Wagena and Easton, 2018), global food security (Doelman et al. 2019; Yadav et al. 2019), crop production (Chen et al. 2019), plant diseases and insects (Young et al. 2019) and agricultural ecosystems (Choudhary et al. 2019). Other studies have addressedclimate change and its impacts onagro-ecological issues including the impact of rising atmospheric carbon dioxide oncrop production (Lemonnier and Ainsworth 2019 and Dass et al. 2019), the variability in climate andenergy systems (Emodi et al. 2019), and climate variability in the agro-ecological zone (Aniah et al. 2019). The impacts of climate change, mitigation and its adaptations have also been reported in different countries and regions worldwide includingAustralia (Ireland and Clausen 2019), China (Wu et al. 2019), Europe (Hernández-Morcillo et al. 2018; Lungarska and Chakir2018), India (Sapkota et al. 2019), Italy (Pietrapertosa et al. 2019), Malaysia (Tang 2019), Nepal (Shrestha and Dhakal 2019), Pakistan (Hussain et al. 2018), Spain (Pasimeni et al. 2019), and for 192 countries (Sarkodie and Strezov. 2019). It is worth mentioning that there are also concerns about the impacts of climate change on livestock.

TABLE 3 . Survey about using alfalfa plants in cadmium (Cd) phytoremediation

The study conditions	Cd content in growth media	Remarkable results	Reference
Seeds (var. Baghdadi) in pots, soil pH (7.5), for 3 weeks	Added Cd (as $CdCl_2$): 100 mg kg^{-1}	Reduction of Cd in shoots inoculated with Rhizophagusirregularis (AMF)	Motaharpoor et al. (2019)
Plastic pots filled with soil contaminated with Cd, plants harvested after 60 days	Cd content: 9.01 mg kg^{-1}	Exogenous application of signaling molecules (H_2S and NO) enhanced plant growth by reducing phytotoxicity in Cd-contaminated soil	Fang et al. (2019).
Pot experiments used seeds of 20 alfalfa cultivars for 3.5 months growth period, soil pH: 6.83,	Cd content: 50 mg kg^{-1}	Under Cd stress, the content of free amino acid, proline and soluble protein were key to withstanding Cd toxicity in alfalfa.	Yang et al. (2019)
Pot experiment, soil pH: 8.07, seeds grew for 90 days	Cd content: 3 mg Cd kg^{-1} dry soil	Cd phytoextraction by alfalfa was 28 g ha^{-1}, adding 1.5 % (w/w) biochar enhanced plant growth	Zhang et al. (2019)
Seeds (cv. Biaogan) germinated, 4 day-old seedlings cultured in Hoagland added 1.30 mM CH_4	The added solution containing 100 µM $CdSO_4$	Methane (CH_4) may alleviate Cd accumulation, CH_4 promoted reduction lipid peroxidation, H_2O_2 accumulation under Cd stress	Gu et al. (2018)
Seeds (cv. Biaogan), seedlings pretreated with 10, 50, and 200 µM melatonin, exposed to Cd stress for 3 days	Seedlings were exposed to 0, 50, 100 and 200 µM $CdSO_4$	Pretreatment with exogenous melatonin may increase the content of melatonin in seedlings and alleviates Cd stress	Gu et al. (2017)

Alfalfa plants can grow as a perennial forage cropunder a range of climatic zones (Luo et al. 2019). It has high adaptability to different growing zone conditionsand high palatability for livestock with relatively high yields (Singer et al. 2018).Like all crops, the production of alfalfa is projected to change due to climate changes. These changes represent great constraints through typical abiotic and biotic sures on production. Common causes of climate change include rising sea level, changes in precipitation patterns,heat waves, and drought. These effects also are expected to escalate in their frequency and severity under the climate changes (Singer et al. 2018). Apart from changes in climate conditions, there are direct and indirect impacts of over-population on the global demand for alfalfa and other forage products for ruminants, whichare considered an important source offood (meat and dairy products)and animal products (i.e, leather) to meet human needs (Martin et al. 2017; Wilkinson and Lee 2018).To produce enough alfalfa to feed livestock populations, it is urgent that new novel approaches, which may guarantee higher yields under harsher conditions including marginal lands and extreme climates, be developed. The production of alfalfa was and still is a major area of interest under changing climateas follows:

(1) Alfalfa productivity is projected to decrease 15-35 % in sub-Saharan Africa due to climate change. Thisdecline will subsequently negatively impact the production of livestock. The impact of “forage-legume intercropping technologies” is one adaptation to climate change in Africa and its effects on the “mixed crop-livestock systems” (Hassen et al. 2017),

(2) Stresses due to climate change may decrease the fitness, fertility and longevity of grazing animalsunder drought and/or heat waves in arid and semi-arid rangelands. This impact mainly depends on the vulnerability of livestock to climate change, which varies based on many factors such asthe animal species, nutritional status, life stage and genetic potential (Hassen et al. 2017),

(3) Many impacts can be expected on alfalfa forage crops due to changing climate, such as changes in forage growth, quality, andthe content of carbohydrates and nitrogen (Hassen et al. 2017),

(4) The photosynthetic process and crop yield may increase with elevated CO_2 levels, whereas the response of alfalfa crop involves the decreaseof crop yield forthe long-term exposure to CO_2due to crop acclimation to elevated CO_2 concentrations or the down-regulation process (Kulkarni et al. 2018),

(5) There is a projected 25% increase in total non-structural carbohydrates or the primary photosynthates and an 8% N content decrease in alfalfa plant tissuesunder elevated CO_2 levels (Dumont et al. 2015),

(6) Alfalfa forage quality and yield under elevated CO_2 and temperatures were investigated by Baslam et al. (2012). They found that elevated carbon dioxide and temperature conditionsmay enhance fiber content, reducing crude protein and the digestibility of alfalfa forage. Due to the elevated CO_2, increased total mineralnutrient uptake alfalfa yield has been observed (Sanz-Saez et al. 2012),

(7) Total chlorophyll content in alfalfa leaves under elevated CO_2 conditions did not show a significant increase compared with ambientconditions (Al-Rawahy et al. 2013; Ksiksi et al. 2018),

(8) Ksiksi et al. (2018) studiedalfalfa production under water stress with CO_2 enrichment under greenhouse conditions. They reported that it is possible to reduce the amount of irrigation water used without a reduction in the forage yield of alfalfa if this production is coupled with CO_2 enrichmentunder greenhouse conditions,and

(9) Additional studies have investigated elevated CO_2 and its impact on alfalfa production under different conditions (e.g., Fischinger et al. 2010; Farfan-Vignolo and Asard 2012; Sanz-Sáez et al. 2013; Erice et al. 2014; Goicoechea et al. 2014; Irigoyen et al. 2014; Ariz et al. 2015).

Climate change is a major challenge facing our world and without an urgent global strategy, including measures to mitigate and adapt to this serious threat, more and more unexpected extreme events will sweep the world. The effects of climate change do not just include alfalfa yields and nutritional value but also the impact on livestock and its health. There are positive and negative sides to the changing climate. The elevated atmospheric CO_2 level enhances photosynthesis in some cultivated crops (C_3), but the adverse climatic circumstances can exertabiotic stresses (e.g., drought, heat, etc.) on alfalfa crops, which has negative impacts on the quality and quantity of the yield. Atmospheric CO_2 is projected to be 550 and 700 μmol mol^{-1} by 2050 and 2100, respectively with the current rate of increase at about 1.5 μmol mol^{-1} $year^{-1}$ (Ariz et al. 2015).

Conclusion

Alfalfa is a valuable food and feed crop for humans and animals. This crop can perform vital functions starting with green biomass and silage production for livestock and sprouts for human consumption. Alfalfa alsocan grow and produce desirable yields in stressful environments. Apart from the strong root system of alfalfa, the cultivated plants can grow well under abiotic stresses and in marginal lands.The histological approach of alfalfa is a good indicator for a sound understanding of the growth environments, in particular stressful conditions. Therefore, further anatomical investigations are needed concerning the production of alfalfa under changing environments.There are still some open questions concerning the productivity of alfalfa in particular under stressful conditions. Regarding different applications of alfalfa particularly for human nutrition, the current status of alfalfa research needs more investigations. What traits are important to improve the alfalfa resilience to climate-change or global warming. The derived materials, which produce from alfalfa are needed to investigate under cultivation of horticultural and agricultural crops.

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