



Abiotic Stress and Red Clover: A Less Explored Area of Research

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Authors' contributions

This work was carried out in collaboration among all authors. Author AP designed the study and wrote the first draft of the manuscript. Author VS managed the literature searches. Author MB managed the data presentation and final editing of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Red clover (*Trifolium pratense* L.) is one of the main forage species from temperate regions and its centre of origin is located in southern Europe and southern Eurasia. Although red clover is Mediterranean in origin, it is a widely adapted species grown in many climatic conditions around the world. It is a perennial, medicinal herb from legume family and it grows best in calcium, phosphorus and potassium rich soils. This medicinal plant is in symbiotic association with bacteria present in its root nodules, thus the plant is capable of fixing the atmospheric nitrogen into the soil thereby increasing the quality of the soil. Red clover is typically used to treat a number of respiratory ailments such as asthma, bronchitis, and bronchitis, skin disorders such as eczema and psoriasis, inflammatory conditions like arthritis, and to treat women's health problems especially in giving relief from menopausal symptoms. However, the response of Red Clover under abiotic stress conditions is a less explored area of research. The present review highlights the existing potential of Red clover in fighting abiotic stress conditions and also explains the need of developing resistant varieties of this plant to meet the future challenges.

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1. INTRODUCTION

Red clover (*Trifolium pratense*) is a perennial short duration herb from legume family and it grows best in calcium, phosphorus and potassium rich soils. This medicinal plant is in symbiotic association with bacteria present in the root nodules, thus the plant is capable of fixing the atmospheric nitrogen into the soil thereby increasing not only the fertility but also the quality of the soil [1]. For this reason, this pharmaceutically important medicinal plant is also extensively used in crop rotation. Red clover (*Trifolium pratense* L.) is one of the main forage species from temperate regions and its centre of origin is located in southern Europe and southern Eurasia [2,3,4].

In herbal medicine, red clover is typically used to treat a number of respiratory ailments such as asthma, bronchitis, and bronchitis, skin disorders such as eczema and psoriasis, inflammatory conditions like arthritis, and to treat women's health problems especially in giving relief from menopausal symptoms [5].

Red clover's brightly colored flowers contain many nutrients including calcium, chromium, magnesium, niacin, phosphorus, potassium, thiamine, and vitamin C [6].

2. ABIOTIC STRESS IN PLANTS

Abiotic stresses such as salt, flood, drought, cold, heat, frost and mineral toxicity reduce the growth, development, seed quality and yield at all stages. In future it is predicted that fresh water scarcity will increase and ultimately intensity of abiotic stresses will increase [7]. Development of crop varieties that are resistant to abiotic stresses is the need of the hour to ensure food safety and security. Roots are a plant's first line of defense against abiotic stress. As the plants are sessile they have naturally developed highly organized molecular mechanisms to sense and respond to various environmental changes occurring in the environment [8,9,10]. The responses generated by the plants under abiotic stress conditions are both reversible and irreversible. Plants under abiotic stress trigger a number of plant responses ranging from altered cellular metabolism, gene expression, growth rate and finally the crop yield. The plant's response to the stress depends upon a number of factors viz., severity of the stress, duration of the exposure and rate of the stress. Under the

physiological and metabolic response, plants produce osmoprotectants ex., proline, sugars, sugar alcohols and glycine betaine [11] that leads to cellular osmotic homeostasis, changes in the lipid composition [12] regulation of ions by transport proteins across the membranes [13] and regulation of stomatal aperture [14].

Plants experience a number of abiotic stresses which negatively impact the crop productivity worldwide. These abiotic stresses are interrelated with each other and may result in osmotic stress, malfunction of ion distribution and plant cell homeostasis. The growth rate and productivity is affected by a complex responses generated by a group of genes by changing their expression patterns. So, the identification of responsive genes against abiotic stresses is necessary in order to understand the abiotic stress response mechanisms in crop plants [15].

3. SALT STRESS IN PLANTS

Stress in plants refers to external conditions that adversely affect growth, development or productivity of plants. Stresses trigger a wide range of plant responses like altered gene expression, cellular metabolism, changes in growth rates, crop yields, etc. A plant stress usually reflects some sudden changes in environmental condition. However in stress tolerant plant species, exposure to a particular stress leads to acclimation to that specific stress in a time time-dependent manner [16].

As per the survey conducted by FAO Land and Plant Nutrition Management Service in 2010, over 6% of the world's land is under salt stress [17]. Approximately 20% of cultivated land worldwide is affected by salinity. It is one of the major stress limiting the crop productivity and yield [18,19]. As per Central Soil Salinity Research Institute of India total 6744968 ha land in India is under salt stress [20]. Soil salinity is not only restricted to arid and semi arid regions but also occurs extensively in the coastal regions . In arid and semi arid regions it is mainly due to mal irrigation practices and in coastal regions, salinity is attributed to sea water [21].

Salinity is a condition which is characterized by high salt concentration. It is one of the most significant environmental factors limiting plant growth. A paste of saturated soil having electrical conductivity 4 dsm^{-1} is said to be saline soil. It signifies the accumulation of salts mainly sodium chloride and sodium sulphate in the soil or water

above a threshold level. The other salts responsible for increasing the salinity of the soil include chlorides and sulphates of calcium and magnesium.

Salinity is caused by a number of factors including decomposition of rocks, sedimentation in marine waters, mal irrigation practices, ground water in water logged soils, flowing of flood water over saline areas. Salinity causes effect on all the developmental stages of plant including germination, photosynthesis, ionic balance, oxidative stress, respiration and finally yield. Excess salinity changes the rate of imbibitions of water by seeds at the time of germination due to lower osmotic potential of surroundings [22]. It alters metabolism of proteins [23], changes the activities of enzymes of nucleic acid metabolism [24], causes hormonal imbalance and reduces the utilization of seed reserves [25] Salinity causes reduction in the growth rate [26] as well.

One of the primary responses to high salinity is the imbalance of the Na^+/K^+ ratio in the cytoplasm of the plant cell. The salt enters the roots of the plant and triggers many cascade of reactions thereby causing water stress due to intake of excessive sodium (Na^+) and chloride (Cl^-) leading to development of cytotoxic conditions. Plant responses to salinity have been divided into two phases. First, an ion-independent growth reduction, which takes place within minutes to days, causing inhibition of cell expansion in shoots and stomatal closure in leaves [27]. A second phase takes place over days or even weeks and pertains to the build-up of cytotoxic ion levels, which slows down metabolic processes thereby causing premature senescence, and ultimately cell death [28].

Plants adopt a number of strategies to combat the salt stress. Predominant ones include, activation of several signal cascades that generate ionic tolerance by restricting (net) Na^+ influx into the root and reduce (net) Na^+ translocation and tissue tolerance is enhanced by compartmentalization of toxic ions into vacuoles to avoid detrimental effects on cytoplasmic processes. The phytohormone abscisic acid (ABA) also plays an important role during plant adaptation to environmental stress such as high salinity [29]. The above strategies has been clearly observed in halophytes making them tolerant to high salt concentrations [30,31].

4. RED CLOVER AND SALT STRESS

The behavior of red clover plant under salt stress is a less explored area. The effect of salt stress

was studied on germination of 28 red clover populations taken from Turkey and it was found that population 17 gave the highest promptness index while the population 19 produced the longest roots at 180mM NaCl concentration [32]. The researches have proved that Red clover is a highly salt sensitive plant. Several reports concluded that salt concentrations when applied to Red clover seeds had a critical influence not only on the germination of seeds but also on plant establishment [33]. Salinity reduced water potentials in the leaves of clovers (*Trifolium sp.*), reduced length and dry mass of the stem and affects the length and conductivity of the root [34]. Phenolic compounds have received great attention in recent times for their ability to prevent plant from oxidative stress induced harmful effects. The phenolic compounds in red clover roots were characterised by high performance liquid chromatography and mass spectrometry and the response of root phenolics was studied under various stress factors. It was found that root phenolics in Red clover were not only sensitive to biotic but also abiotic stress conditions. Red Clover is found to be salt sensitive especially during seed germination and early seedling growth stages. It has been observed that the germination energy, percentage of dead or infected seeds, normal seedlings, root length, shoot length, fresh weight, dry weight and seedling vigor index were decreased considerably with increasing dosage of NaCl [35].

5. RED CLOVER AND COLD STRESS

Studies were conducted to improve the freezing tolerance of Red Clover under cold climate conditions [36]. The freezing tolerance was assessed by comparing the proteome analysis of non-acclimated and cold-acclimated plants of two initial cultivars of red clover: Endure (E-TF0) and Christie (C-TF0) and of populations issued from these cultivars after three (TF3) and four (TF4) cycles of phenotypic recurrent selection for superior freezing tolerance. The freezing tolerance was significantly improved by recurrent selection and was closely associated with the accumulation of cold regulated proteins. These cold regulated proteins play a key role in increasing the level of freezing tolerance.

6. RED CLOVER AND HEAVY METAL STRESS

The effect of different concentrations of arsenate was studied on red clover plants and it was observed that with increasing dosage of

arsenate, the superoxide dismutase activity, peroxidase activity, carotenoid and chlorophyll content increases. This clearly indicates that higher concentrations of arsenate causes oxidative damage in red clover shoot [37].

7. CONCLUSION

The researches stated above clearly indicate that Red Clover and abiotic stress is less explored area of research till now. The future research should be carried out to not only study the effect of various abiotic stress conditions on this important medicinal plant but also to develop resistant varieties of this plant. Red clover is a pharmaceutically important plant and has many therapeutic uses.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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