CHAPTER - 1

GENERAL INTRODUCTION

Plants are considered to be an excellent source of bioactive components which have potential nutritional and therapeutic applications (Shetty and Labbe, 1997; Gupta *et al*., 2014). Understanding the nutritional and therapeutic role of dietary intake of these plants in any form is an important scientific agenda which is essential for developing conventional foods with enhanced health benefits (Shetty, 2001).

Free radicals or reactive oxygen species are predominantly reactive and are known to reduce the concentration of molecular oxygen in the cell and create a physiological state called oxidative stress (Williams and Jeffrey, 2000). They damage the major macromolecules such as nucleic acids, proteins and membrane lipid and consequently trigger a series of aging-related problems (Halliwell *et al*., 1992). Free radical-mediated oxidative stress is believed to be the primary cause of many disorders, such as cardiovascular diseases, brain dysfunction, cataract, diabetes mellitus, arthritis, cancer and ageing (Enayde *et al*., 2006). In the treatment of these diseases, antioxidant therapy has gained utmost importance in the recent years.

Antioxidant compounds are capable of mitigating the negative effects of oxidative stress as they are efficient scavengers of the free radicals. The trend of using natural products has increased and the active plant extracts are frequently screened for new drug discoveries for the presence of potential antioxidant components (Ramma *et al*., 2002). The food resources which are considered to be a good source of natural antioxidants has been found to be beneficial for protection against the diseases related to oxidative stress (Chinnici *et al*., 2004).

Seeds have been the vital component of the human diet since early age (Ranal and de Santana, 2006). Cereal grains alone, which comprise 90% of all cultivated seeds, contribute up to half of the global per capita energy intake (Nonogaki *et al*., 2010). Not surprisingly then, seed biology is one of the most extensively analyzed research areas in plant physiology (Chen *et al*., 2002). With the seed, the independence of the next generation plant begins. The seed, containing the embryo as the new plant in miniature, is structurally and physiologically equipped for its role as a dispersal unit and is well provided with food reserves to sustain the growing seedling until it establishes itself as a self-sufficient, autotrophic organism, because the function of a seed is to establish a new plant (Bewley, 1997). Similarly, seed sprouts have also been included in the diet as health food and it has been scientifically proved that along with being a good source of basic nutrients, sprouts also are found to possess various bioactive components with disease preventive and health promoting properties (Kurtzweil, 1999).

Trigonella foenum-graecum commonly known as fenugreek has been reported to possess several pharmacological and folkloric applications. Its leaves have been reported to show potential antioxidant property, antimicrobial as well as antidiabetic activity. The *in vivo* hypoglycemic activity of fenugreek seeds has been established in various animal model systems. In addition, fenugreek seeds possess potential hypocholesterolemic effect, antioxidant property and are also very effective in the treatment of diabetic disorders (Meghwal and Goswami, 2012).

The consumption of nutritional food forms is not sufficient enough for the long term survival at present era due to increasing population explosion which is creating several new forms of disease with time and the limited resources are not to be ignored. Therefore, it has become very important to develop strategies for the enhancement in the nutritional qualities of the healthy foods including sprouts to serve the rapidly increasing population with limited resources and combat wide spectrum of diseases and disorders. Additionally, the prevention and management of diseases and ailments through dietary intake becomes the most effective strategy to lead healthy life and minimum health-care costs.

The environmental factors are known to have significant impact on the morphological, biochemical attributes along with the growth and development of plants. When any of these factors exceeds the tolerance level, a stress is imposed on the plant which influences its development along with structural, physiological and biochemical processes (Jaleel *et al*., 2007). The increase in the salt content above optimum level, which creates salinity stress is considered one among these environmental factors which are responsible for threatening the crop productivity worldwide (Manivannan *et al*., 2007).

The deleterious effect of salinity which affect the normal growth and development of the plant is attributed to a reduced osmotic potential, specific ion toxicity and nutrient deficiency of the substratum (Luo *et al*., 2005; Bhattacharjee, 2008). The reduced osmotic potential due to salinity affects water availability due to the prevention of water uptake by the plants, leading to a condition known as physiological drought (Kim *et al*., 2009). In addition, salinity is reported to induce the generation of reactive oxygen species which further leads to membrane disruption and metabolic toxicity in plant system (Mittler, 2002). To protect themselves from the oxidative stress mediated damages, plants are found to develop scavenging mechanisms from these destructive free radicals. This involves detoxification processes regulated an integrated system of non-enzymatic antioxidants such as ascorbic acid and glutathione (Sharma, 2004), and the enzymatic system which comprises of efficient antioxidants such as catalase ascorbate peroxidase, superoxide dismutase and glutathione reductase (Reshmi and Rajalakshmi, 2012).

So, not only humans, plants are also equally facing several environmental stresses which includes both natural and anthropogenic. Plants are also affected by the free radicals, which damages the physiological and biological systems. They also come across the oxidative stress mediated adverse conditions, during which their growth and development are hugely affected (Ozdener and Kuttbay, 2008). Consequently, the retardation in the growth and development might also lead to significant reduction in the nutritional quality of the plant. These free radicals by virtue of their highly unstable state, cause serious damages to the membranes and tissues which adversely affect the functioning of the plant system, thus affecting the plant productivity (Maevskaya and Nikolaeva, 2013). Therefore, there is also a need to adopt effective strategies and techniques to protect the plant resources from such hazardous environmental stresses, in order to maintain an ecological balance in nature. Seed priming is considered as one of the pre-sowing techniques in which seeds are subjected to the low external water potential that limits hydration which does not allow the protrusion of radicle through the seed coat. This technique is known to enhance the primary development of seeds under unfavourable environmental conditions (Rozbeh *et al*., 2011; Nasri *et al*., 2011). The priming of seeds with various substances *viz*. water, inorganic salts, osmolytes, and hormones has been successful and reported as a cost-effective strategy to enhance tolerance under saline conditions (Joshi *et al*., 2013).

Nitric oxide is a bioactive molecule, which functions both as a pro-oxidant as well as antioxidants in plant system (Kopyra and Gowzdz, 2003). The chemical properties of nitric oxide make it a versatile signalling molecule that functions via interactions with several cellular components (Lamattina *et al*., 2003). It is also considered as an RNS and its concentration-dependent impacts on different systems were reported to be either protective or toxic (Beligni and Lamattina, 1999). Calcium (Ca^{2+}) is another important secondary messenger and signalling molecule which is actively involved in various physiological and developmental processes. In previous studies, Ca^{2+} has also shown a protective effect against stress by mitigation of oxidative damages and membrane stabilization (Larkindale and Knight, 2002).

However, literature suggests that reports on the role of calcium ion and nitric oxide in enhancement in the nutritional quality of fenugreek sprouts and also their effect on growth and metabolism of fenugreek during the developmental phases under salinity stress are not studied till date. Considering this fact, the present study was undertaken to investigate the role of nitric oxide and calcium ion in improving the free radical scavenging activity and antidiabetic activity along with related phenolics and also developing stress tolerance during early developmental phases through priming techniques. Also, the cross talk among these signalling molecules, polyamines and other growth regulators were also studied in connection with the tolerance mechanism.

The results obtained will lead to a better understanding of fenugreek's responses towards salinity and the role of the two important signalling molecules, nitric oxide and calcium in the enhancement of the therapeutic potential and stress tolerance during early germination stages and also such priming techniques will be of good aid in enhancement of the nutraceutical quality of other plant species which are usually consumed in sprout form.

Objectives of Research

The objectives of research work are:

1. Evaluation of antioxidant activities of the sprouts of fenugreek.
2. Phytochemical screening as well as their quantitative changes at different stages of seed germination.
3. *In-vitro* anti-diabetic activity of the sprouts of fenugreek.
4. Investigation of the effect of seed priming with different elicitors of nitric oxide and calcium signaling on morphology and various germination parameters.
5. Alteration of free-radical scavenging activity during seed germination under the influence of elicitors of nitric oxide and calcium signaling.
6. Biochemical changes during seed germination under the influence of exogenous nitric oxide and scavenger under saline condition.
7. Biochemical changes during seed germination under the influence of exogenous calcium, its chelator and channel-blocker under saline condition.
8. Interaction of calcium and nitric oxide with other growth regulators during germination under saline condition.
9. Determining the correlation between elicitor mediated alteration of biochemical metabolites and their association with activities of antioxidant enzymes.