CHAPTER 1

INTRODUCTION

1. Introduction

Polyamines are ubiquitous nitrogenous compounds necessary for growth and development of most of the organisms including plants (Sobieszczuk-Nowicka et al., 2016). They are present in both free as well as in conjugated forms with phenolic compounds, proteins and nucleic acids (Yatin 2002). Common polyamines exist amongst all organisms are diamine putrescine (Put), triamine spermidine (Spd) and tetraamine spermine (Spm) (Kumar et al., 1997). Some uncommon polyamines such as thermospermine, homospermidine, homospermine, norspermine have also been reported in some microorganisms and plants (Sagor et al., 2013).

In plants, polyamines are involved in a range of plant growth and developmental processes including cell division, embryogenesis, reproductive organ development, seed germination, root growth, tuberization, floral initiation, fruit development and ripening as well as leaf senescence (Bais and Ravishankar 2002, Evans and Malmberg 1989, Galston et al., 1997, Guo et al., 2018, Mellidou et al., 2016). Also they participate in regulating abiotic and biotic stress (Bais and Ravishankar 2002, Galston et al., 1997, Liu et al., 2015, Sagor et al., 2012, Tiburcio et al., 1993, Walters 2003a). Polyamines can act by stabilizing membranes, by scavenging free radicals, affecting nucleic acid and protein synthesis, by interacting with hormones and ethylene biosynthesis (Slocum et al., 1984). Because of these numerous interactions of polyamines in plant system, their precise role in growth and development is very difficult to determine. Due to uniform distribution of positive charge under physiological pH, polyamines can bind to almost all negatively charged macromolecules such as nucleic acids, phospholipids and proteins and alter their structure and functions, moreover protect them from degradation and modifications (Jiménez Bremont et al., 2014). The simplicity in their structure, consistent distribution in all the cellular compartments and involvement in various developmental and physiological processes make polyamines an interesting group of compounds for investigation.

Precursor of all the polyamines is putrescine, which is synthesized from ornithine by the enzyme ornithine decarboxylase (ODC) (EC 4.1.1.17) in all the organisms, except in plants, where putrescine may be alternatively formed via the activity of arginine decarboxylase (ADC) (EC 4.1.1.19) through arginine. Further putrescine is converted to triamine spermidine through spermidine synthase (SPDS) (EC 2.5.1.16) and subsequently to tetraamines spermine or thermospermine via spermine synthase (SPMS) (EC 2.5.1.22) and thermospermine synthase (ACL5) (EC 2.5.1.79), respectively. Enzyme *S*-adenosylmethionine decarboxylase (SAMDC) (EC 4.1.1.50) transfers an aminopropyl group from s-adenosyl methionine (SAM) to spermidine, spermine and thermospermine synthases sequentially to produce spermidine, spermine and thermospermine, respectively (Kusano et al 2008).

Catabolism of polyamines is typically carried out by two enzymes; diamine oxidases (DAO) (EC 1.4.3.22) and polyamine oxidases (PAO) (EC 1.5.3.14) (Edreva 1996). DAO breaks down putrescine, while PAO degrades spermidine and spermine to release ammonia and H_2O_2 (Bagni & Tassoni 2001, Smith & Best 1978). Degradation of polyamines gives rise to different compounds such as 4-aminobutanal and 1,3-diaminopropane which are biochemically active during different sets of reactions (Bouchereau et al., 1999, Federico and Angelini 1991, Šebela et al., 2001, Walters 2003b) and can be further metabolized to β-alanine and GABA, respectively, which are associated with stress tolerance in plants (Bouche & Fromm 2004, Koc et al 1998). Thus these enzymes are important in preventing polyamine levels from getting very high in cells (Cervelli et al., 2003, Wang et al., 2004).

Due to the importance of polyamines in regulating plant development and abiotic and biotic stress responses, genes involved in their biosynthesis has been identified and characterized in several plants (Hu et al., 2016, Sagor et al., 2016, Sekula et al., 2016, Sequera-Mutiozabal et al., 2016). One of the *Arabidopsis* polyamine biosynthetic gene SPDS was shown to be essential for embryo development (Imai et al., 2004). Also, ADC gene is required for seed development in *Arabidopsis* (Urano et al., 2005). In Poplar and *Arabidopsis* it is observed that thermospermine synthase is essential for vascular tissues development (Milhinhos et al., 2011, Muñiz et al., 2008). In addition to the developmental roles, polyamines are also involved in regulating abiotic and biotic stresses in plants (Liu et al., 2015, Pál et al., 2015, Sagor et al., 2012, Saha et al., 2015, Sequera-Mutiozabal et al., 2017, Walters 2003a). Among the polyamines, spermine is reported to have an assured role against salt and drought stress in *Arabidopsis*. *Arabidopsis* spm mutant (Atspm) plants lacking spermine were observed to hypersensitive to those stresses (Kusano et al., 2007). In rice, *Datura* ADC overexpression lead to drought tolerance (Capell et al., 2004). *Capsicum annum* SAMDC overexpression in *Arabidopsis* also showed enhanced drought tolerance (Wi et al., 2014). Similarly, tomato overexpressing SAMDC were found to be more heat tolerant than control plants (Cheng et al., 2009). Also, it was observed that during chilling stress, expression of ADC was mainly induced in *Arabidopsis* (Cuevas et al., 2008).

Roles of polyamines during plant pathogen interactions have also been investigated (Jiménez Bremont et al., 2014, Marini et al., 2001). *Arabidopsis* overexpressing SAMDC reported to have increased tolerance against *Pseudomonas syringae* (Marco et al., 2014). Also several reports demonstrated the participation of spermine in plant defense against pathogens (Mitsuya et al., 2009, Urano et al., 2003, Yamakawa et al., 1998). However the role of its isomer thermospermine in plant defense has not been investigated. Though a recent report suggested that exogenous application of thermospermine decreases *Cauliflower mosaic* virus (CaMV) infection and increases the expression of defense related genes in *Arabidopsis* (Sagor et al., 2012). However, the basic mechanism how thermospermine is protective against CaMV has yet to be elucidated.

Thermospermine which is a structural isomer of spermine was first reported from extreme thermophile *Thermus thermophilus,* where it is shown to protect thermophiles under extreme growth conditions by stabilizing their nucleic acids (Oshima 2007). In *Arabidopsis* thermospermine is synthesized by thermospermine synthase which is encoded by a single gene ACAULIS5 (ACL5) (Kakehi et al., 2008). Recently, existence of thermospermine has also been reported from wide range of plants including tomato (Marina et al., 2013). In *Arabidopsis,* ACL5 has been shown to be involved in prevention of premature cell death of vascular tissues (Hanzawa et al., 2000, Muñiz et al., 2008). However, how ACL5 and its regulatory pathway regulating the development and stress responses has yet to be elucidated in detail.

Inspite of extensive work done on polyamines, our understanding about the underlying molecular mechanism of role of polyamines is still very unclear in plants. Further investigations are needed to understand the role of polyamines in epigenetic control of gene expression, how polyamines activate signaling cascade during stress conditions, how they cross talk with PCD related genes, how polyamines acts during autophagy. Although, the various mechanisms of regulation of intracellular polyamines are being elucidated in some detail, however an overall understanding of the roles of polyamines and including their regulatory network during development is still lacking in plants. Considering these facts, in present study we proposed to identify and characterize the role of some of the polyamine biosynthetic genes in tomato (*Solanum lycopersicum* L.). Tomato is a model plant and a major crop and its genome is also sequenced, therefore we selected this plant to carry out our investigation. In present study, we planned to carry out a genome-wide molecular analysis of tomato to identify candidate genes involved in polyamine biosynthetic pathway followed by their detail expression profiling both during development and stress responses. Some of the differentially expressed polyamine genes were also selected to further analyze their roles in plants using functional genomics based approaches.

Therefore, to identify and characterize the roles of polyamine biosynthetic genes in tomato following objectives have been undertaken for present work:

1. Identification of candidate polyamine biosynthetic genes in tomato.
2. Expression analysis of candidate polyamine biosynthetic genes during plant development and stresses.
3. Characterization of tomato ACL5 gene function by gene silencing and/or overexpression in plants.