CHAPTER 5

DISCUSSION

5. Discussion

5.1 Genome-wide identification, molecular characterization and phylogenetic analysis of polyamine biosynthetic genes in tomato

Polyamines are essential for all the living organisms (Yatin 2002). In plants, they are important modulators of cellular and developmental processes. They are known to stabilize DNA, RNA, membranes and regulate cell division, root development, tuberization, embryogenesis, fruit development, and senescence (Martin-Tanguy 2001, Kusano et al,. 2007). Polyamines are also involved in regulating programmed cell death and autophagy (Seiler and Raul 2005, Thomas and Thomas 2001). In addition to these, polyamines are also known to protect plants under various stresses (Ahmad et al., 2012, Groppa and Benavides 2008, Walters 2003).

In the present study, we performed the genome-wide analysis to identify genes involved in polyamine biosynthesis in tomato. As the polyamines are prevalent in plant kingdom, but the number of genes involved in their biosynthesis varying in different plant species. Our molecular analysis revealed that there are eighteen candidate polyamine biosynthetic genes in tomato genome, whereas in *Arabidopsis thaliana* there were reported only ten polyamine biosynthetic encoding genes (Figure 5.1). Contrary to our results, previous report suggested that only eight genes were involved in the polyamine biosynthesis in tomato (Tsaniklidis et al., 2016). Furthermore, in a recent genome-wide identification in apple, it is reported that there are eighteen genes participating in polyamine biosynthesis (Gong et al., 2018). Multiple sequence alignment and phylogenetic analysis between polyamine biosynthetic genes of *Arabidopsis* and tomato showed that all the identified candidate tomato genes from gene families, viz., arginine/ ornithine decarboxylase family, S-adenosyl methionine decarboxylase family and spermidine/ spermine synthases family were closely related with homologous genes and corresponding gene families of *Arabidopsis* and their protein sequences and domain structure were highly conserved. For instance, ADC genes of tomato and *Arabidopsis* shared 70% sequence similarity at the protein level. Likewise, SAMDC genes have around 60%, SPDS genes have 60-80%, SPMS has 80% and ACL5 members shared about 60-70% sequence similarity with *Arabidopsis* homologs.

Our phylogenetic analysis revealed that tomato genome contain two genes encoding ADC, similar to *Arabidopsis*. We also identified two genes encoding ODC in tomato

genome, however contrastingly, *Arabidopsis* genome does not encode any gene for ODC (Hanfrey et al., 2001). Similar to tomato, apple genome also contains two genes each for ADC and ODC (Gong et al., 2018). Similar duplicated ADC and ODC genes have also been reported in *Datura* and *Hyoscyamus* (Hashimoto et al., 1998). However, *Citrus clementina* reported to have one ADC gene and two ODC genes (Trénor et al., 2010). Our results showed that tomato has five SAMDC paralogs, whereas *Arabidopsis* genome encoded by four SAMDC. Similar to *Arabidopsis*, *Citrus clementina* genome also reported to have four SAMDC encoding genes (Trénor et al., 2010). However, surprisingly apple has eight paralogs of SAMDC in its genome (Gong et al., 2018). Unlike tomato, *Arabidopsis* genome contains two SPDS encoding genes, whereas tomato has five genes for SPDS. Moreover, SPMS in both plants encoded by only a single gene (Hanzawa et al., 2002, Imai et al., 2004, Panicot et al., 2002). On the other hand, *Citrus clementina* genome is encoded by single gene each for SPDS and SPMS, while *Malus hupehensis* genome has six genes for SPDS (Gong et al., 2018).

Previously, Solyc08g061970.2 was designated as spermine synthase (Tsaniklidis et al., 2016) however our analysis revealed that it is the closest homolog of *Arabidopsis* ACL5 than SPMS gene, therefore we renamed it as SolycACL5. In our study, Solyc09g075900.2 and Solyc07g041300.1 were also found phylogenetically more closer to SolycACL5 than the SolycSPMS, hence named as SolycACL5-Like1 and SolycACL5-Like2. In contrast to tomato where we have identified three ACL5 like genes, *A. thaliana* and *C. clementina* are encoded by only a single ACL5 gene (Hanzawa et al., 2000, Knott et al., 2007). Unlike tomato, *Malus* is represented by two ACL5 like genes (Kitashiba et al., 2005). Presence of three ACL5 like genes in tomato genome suggests that it may be a consequence of species-specific gene duplication events during evolution of tomato. Furthermore, it seems that ACL5 homologs are widespread in the plant kingdom but are not yet reported from fungi and animals. Molecular evidence suggested that ACL5 gene is of bacterial origin acquired by the plants through horizontal gene transfer (Minguet et al., 2008).

Our analysis revealed that the identified candidate genes belonged to three different families namely, arginine/ ornithine decarboxylase, S-adenosyl methionine decarboxylase and spermidine/ spermine synthases. Out of eighteen genes, ADC and ODC belongs to arginine/ ornithine decarboxylase gene family; whereas SAMDC belongs to S-adenosyl methionine decarboxylase gene family and rest SPDS, SPMS

and ACL5 are members of spermidine/ spermine synthases family. Besides these, we also reported that putative genes of specific families also have specific domains which are conserved between tomato and *Arabidopsis*. ADC and ODC members have Orn/DAP/Arg decarboxylase2, N-terminal and Orn/DAP/Arg decarboxylase2, C-terminal domains. Whereas, SPDS, SPMS and ACL5 members contains polyamine biosynthesis domain and spermidine synthase, tetramerisation domains.

Gene structure analysis revealed that polyamine biosynthetic genes involved in the synthesis of diamines consist of only one exon, whereas, the genes involved in triamine and tetraamine synthesis consist of nine and ten exons, respectively. This suggests that number and length of exons and introns are specific for each type of candidate genes and conserved during evolution.

Cis-regulatory elements play important roles in regulation of gene expression. Information gained from *in silico* analysis of cis-elements can be useful for further functional characterization of polyamine biosynthetic genes in tomato. In the present study, we have identified that there were a total of eighty two (82) cis-regulatory elements in the 1kb of genomic sequences upstream of 5' UTR of polyamine biosynthetic genes. These cis regulatory elements might play essential roles in regulating dvelopmental and stress specific roles of polyamines. Some cis elements like HD-Zip1, HD-Zip2 and CCGTCC-box were involved in regulating leaf morphology and meristem development. A series of well-known stress responsive elements such as HSE, LTR, TC-rich repeats, Box-W1, MBS, WUN-motif, and TCA-element were detected in the promoters of several candidate genes. These elements might play important roles in regulating the expression of these genes in a range of stress conditions. Indeed we have found nearly all polyamine biosynthetic genes showed change in expression during stress treatments. In addition to these, other reported cis-elements, like, TGA-element, ABRE, GARE-motif, P box, TATC-box, ERE etc. were hormone responsive, thus our results suggested that various stress and hormones responsive cis-elements may regulate the transcription of polyamine biosynthetic genes during development and various stress responses in tomato.

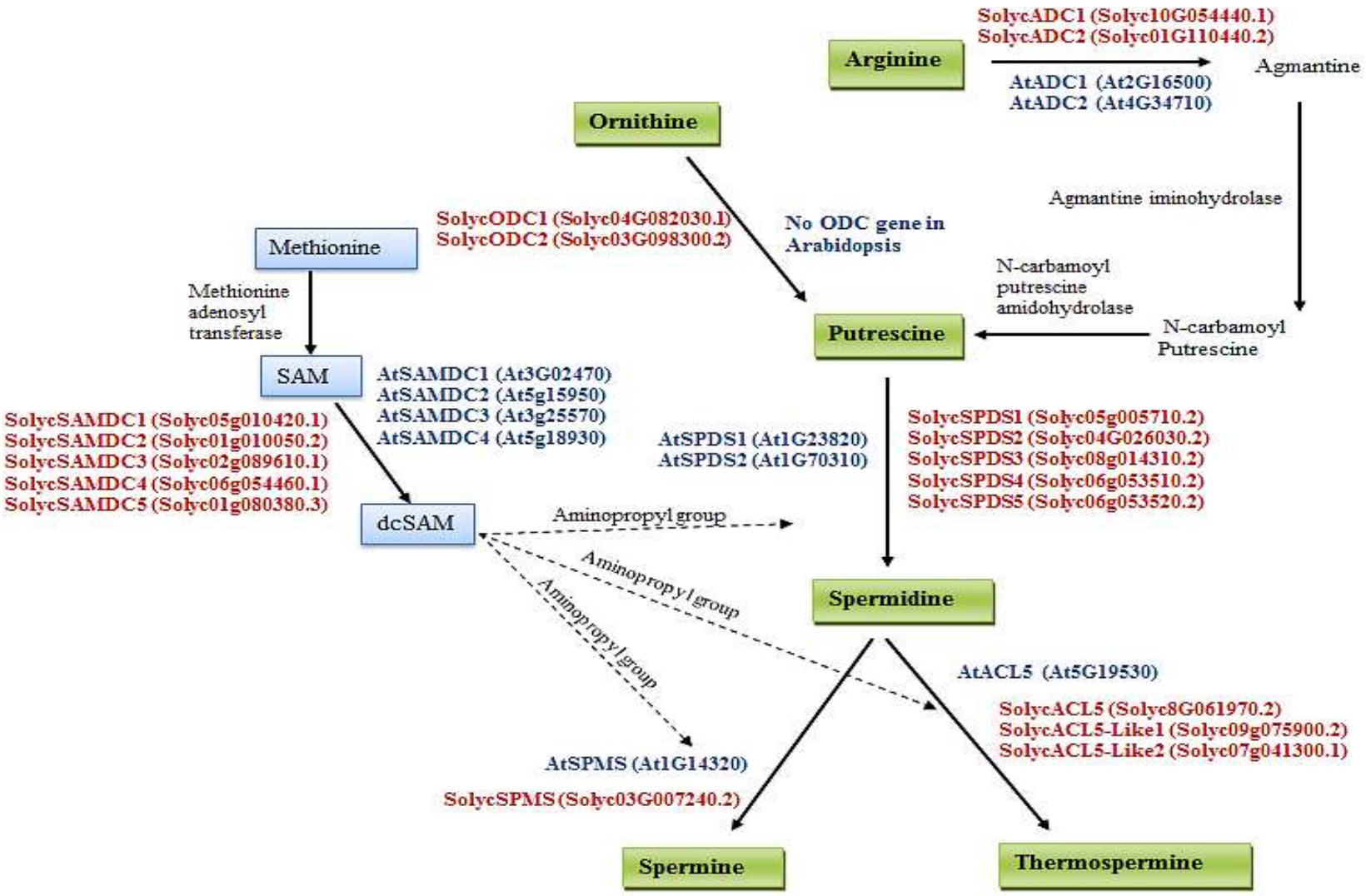

Figure 5.1:. **Comparative polyamine biosynthetic pathway in *Arabidopsis* (At) and tomato (Solyc).** ADC; Arginine decarboxylase, ODC; Ornithine decarboxylase, SAMDC; S-adenosyl methionine decarboxylase, SPDS; Spermidine synthase, SPMS; Spermine synthase, ACL5; Thermospermine synthase, SAM; S-adenosyl methionine, dcSAM; Decarboxylated S-adenosyl methionine. Blue representing *Arabidopsis* and red representing tomato enzymes.

5.2 Expression pattern of polyamine biosynthetic genes in different tissues of tomato

The importance of polyamines during various plant developmental processes have been extensively reviewed (Alabadí and Carbonell 1999, Liu et al., 2006, Shi and Chan 2014, Urano et al., 2005). Although polyamines are cosidered as growth promoting compounds but the underlying mechanisms regulating the stimulation of growth by polyamines during plant development is still not very clear. Therefore, expression profiles of all identified polyamine biosynthetic genes during tomato plant development was analysed. We observed that SolycADC1 and SolycADC2 were highly expressed during early stages of fruit development and afterwards its expression declined during later stages of fruit maturation. These results are in line with the previous report that activity of ADC in most cases gradually decreased during tomato fruit maturation (Rastogi and Davies 1990). Our results showed that both the ADC1 and ADC2 of tomato have higher expression in the outer layer of stem indicating their roles in stem tissues development. In addition, both the genes also have higher expression during early flower developmental stages.

SolycODC1 and SolycODC2 also showed differential expression in different tissues of tomato. A recent study also shown that ODC has important role in facilitating growth and development in tobacco (Dalton et al., 2016). Downregulation of ODC in tobacco lead to have significant morphological defects including reduced size of leaf, delayed flowering, delayed seed germination, early senescence (Choubey et al., 2017), signifying ODC roles in plant development.

We observed that SolycSAMDC1 and SolycSAMDC2 has significant higher expression in different vegetative and reproductive tissues of tomato, whereas SolycSAMDC1 expression was higher during initial stages of flower development and in fruits undergoing ripening, thus it is speculated that SAMDC might be involved in maturation and ripening of fruits. Similarly, it has been reported that SAMDC is differentially expressed during fruit development (Hao et al., 2005). Furthermore, SAMDC is shown to increase shelf life of fruits in litchi (Das et al., 2016). Other SolycSAMDC3, SolycSAMDC4 and SolycSAMDC5 genes were found to be expressed at low level in some tissues of tomato.

Our expression analysis showed that both SolycSPDS1 and SolycSPDS2 were differentially expressed during fruit development. Our result are in line with earlier

report where two of the spermidine synthase genes in pea plants were also differentially expressed during early stages of fruit development (Alabadí and Carbonell 1999). Exogenous appliaction of spermidine also reported to improve fruit set, yield and quality in sweet orange (Saleem et al., 2006). Furthermore, our results showed that SolycSPDS1 and SolycSPDS2 also expressed highly during the early staegs of flower development suggesting their participation at the time of flower development. This result is in agreement with a previous report where GtSPDS genes were involved in induction of flowering in *Gentiana triflora* (Imamura et al., 2015). We found that SolycSPDS3, SolycSPDS4 and SolycSPDS5 did not show any significant expression in the vegetative and reproductive tissues of tomato.

qRT-PCR analysis showed that SolycSPMS was considerably expressed in the vegetative tissues of tomato, whereas in the reproductive tissues it had highest expression during the maturation stages of fruits. Recently, it is reported that exogenously applied spermine increased the shelf life of fruit (Sharma et al., 2017). Thus, we speculate that SolycSPMS might also involved in development of fruits in tomato.

Arabidopsis ACL5 is reported to be highly expressed in root cells and involved in xylem cells specification and in prevention of premature cell death of xylem (Muñiz et al., 2008). Our analysis also showed similar expression of SolycACL5 in roots. This indicates that SolycACL5 might also be playing similar role in development of root in tomato. SolycACL5 and its another paralog SolycACL5-Like1 showed more expression in the stem, confirming its role during the stem development which is in support of earlier report in *Arabidopsis* where ACL5 is required for stem elongation (Kakehi et al., 2008). Furthermore, it is also reported that mutation in ACL5 caused defect in the development of both inflorescences and leaves (Tsukaya et al., 1993). We also found that all ACL5 like genes in tomato expressed differentially in developmental stages of leaves and early flowering stage indicating their possible role in the development of leaf tissues and flower. Thus, expression analysis of polyamine biosynthetic genes revealed that most of these genes are differentially expressed during development of tomato plants (Table 5.2).

5.3 Expression pattern of tomato polyamine biosynthetic genes in response to stress and hormone treatments

Polyamines are known to play direct or indirect role in protecting the plants from unfavorable stress conditions (Alcázar and Tiburcio 2014, Khare et al., 2018, Liu et al., 2015). We also analyzed the expression of identified biosynthetic genes in responses to various stresses and hormone treatments in tomato. We observed that most of the polyamine biosynthetic genes were upregulated during heat, cold, UV-C and drought treatments, whereas expression of some of the genes were either downregulated or remain unchanged in response to hormones JA, SA and ABA treatments. Chemical treatments showed differential expression of most of the genes. Expression of SolycADC1 and SolycADC2 genes was upregulated during drought stress in tomato. A similar upregulation of ADC was also observed in *Datura* which resulted in protection of plants against drought stress (Capell et al., 2004). In another report, ADC2 overexpression in *Arabidopsis* resulted in drought tolerance of plants (Alcázar et al., 2010). Thus, it can be said that ADC might have some role in providing drought tolerance to plants. We observed that SolycADC1 expression was also upregulated during flood. A protective role of ADC under food have also been reported in plants (Yiu et al., 2009). ADC also found to be the main enzyme induced during cold stress to the plants (Hummel et al., 2004). In *Arabidopsis*, a mutation in ADC increased the plants sensitivity to low temperature with significant decreased putrescine content (Cuevas et al., 2008). Polyamine content was reported to increase during UV-C stress and protect the plants by stabilizing the membrane structure under stress conditions (Kramer et al., 1991). This study is also in agreement with our results as SolycADC1 and SolycADC2 expression was upregulated by UV-C stress. Our analysis also showed that SolycADC2 expression was highly downregulated by the defense hormone JA and SA treatments. Additionally, SolycADC2 was downregulated by salt treatment which is consistent with the earlier observation that putrescine which is synthesized by ADC was decreased in response to salinity stress in plants (Zapata et al., 2004). In another report, overexpression of ADC improved salt tolerance of lotus plants (Espasandin et al., 2018). Results also showed that JA treatment caused extremely high downregulated expression of SolycODC2 in tomato. Similarly, MeJA treatment also reported to decline the level of ODC in tobacco (Imanishi et al., 1998).

SolycSAMDC1, SolycSAMDC3 and SolycSAMDC4 were significant upregulated during heat stress, which is in agreement with previous report, where overexpression of *S. cerevisiae* SAMDC gene in tomato lead to higher degree of heat tolerance (Cheng et al., 2009). SolycSAMDC1 also upregulated during cold stress which is consistent with prior work where overexpression of *Dianthus* SAMDC enhanced the plants tolerance to low temperature in tobacco (Wi et al., 2006). Another SolycSAMDC3 gene was found to express significantly high during drought stress in tomato. An earlier report also showed that overexpression of *Capsicum annum* SAMDC in *Arabidopsis* resulted in increased drought tolerance (Wi et al., 2014). Similarly, overexpression of *Salvia* SAMDC gene in tobacco lead to enhanced drought tolerance (Liu et al., 2017). These results indicate that SAMDC1 and some other SAMDC genes in tomato and other plants are involved in tolerance against heat, drought and cold stresses.

Overexpression of SPDS enhanced multiple environmental stresses in *Arabidopsis* (Kasukabe et al., 2004). Overexpression of SPDS1 in tomato also enhanced their salt tolerance significantly (Neily et al., 2011). Similarly, SPDS overexpressing sweet potato plants were found to be salt and drought tolerant (Kasukabe et al., 2006). We observed that several members of SPDS gene family were also showing differential expression patterns in response to various stresses. SolycSPDS1 was highly downregulated by mannitol which is a contrary result to the earlier report, where a higher accumulation of spermidine was reported in response to mannitol in *Pyrus communis* (He et al., 2008).

It has been reported that spermidine positively regulate SA biosynthesis and signaling (Anwar et al., 2015). Our results also showed that one of the spermidine biosynthetic genes SolycSPDS2 expression was increased during SA treatment.

Spermine is known to protect the *Arabidopsis* from heat induced damage (Sagor et al., 2013). Our expression analysis is also in agreement with previous report as expression of SolycSPMS was significantly upregulated during the heat stress. However, contrary to the earlier report, where spms mutant in *Arabidopsis* was shown to be hypersensitive to salt and drought stress (Kusano et al., 2007, Yamaguchi et al., 2006), our results revealed that SolycSPMS expression was highly downregulated by the salt and drought. Furthermore, spermine enhances the genes for biosynthesis of ethylene and JA but downregulated the genes for GA and ABA

biosynthesis (Anwar et al., 2015). However, in our case expression of SolycSPMS was not affected by ABA and JA, while it was highly upregulated by SA treatment.

We observed that expression of SolycACL5 and SolycACL5-Like1 was highly upregulated by heat stress, this is consistent with the previous report where thermospermine increases in plant at the time of heat stress (Roy and Ghosh 1996). Moreover, ACL5 is also reported to have protective role during drought stress to plants (Yamaguchi et al., 2007). We also confirmed this observation as SolycACL5-Like1 was significantly upregulated during drought stress. Although the correlation between ACL5 and defense related hormones is not yet explored, however our analysis showing that the expression of both SolycACL5 and SolycACL5-Like1 was highly upregulated after the treatment with JA and SA, indicating their possible cross-talk with defense hormones. Thus, our study showed that expression of most of the candidate polyamine biosynthetic genes was modulated during stress responses in tomato (Table 5.2).

5.4 Silencing of SolycACL5 in tomato

On the basis of expression analysis, we have selected SolycACL5 for further characterization in plants. In the present study, gene silencing of SolycACL5 in the tomato was achieved by using amiRNA based approach (Schwab et al., 2006). The amiRNA mediated gene silencing is a powerful approach to manipulate genes in plants (Li et al., 2013). MicroRNAs (miRNAs) are small, non coding RNAs, regulating gene expression during plant growth and development and also in response to various stresses (Baldrich et al., 2015). Similar to miRNA, amiRNA are single stranded, about 21nt long, designed by replacing mature miRNA sequences within pre-miRNAs (Tiwari et al., 2014). Like natural miRNAs, amiRNA targets a particular gene or group of endogenous genes (Schwab et al., 2006).

In present study, we showed that silencing of SolycACL5 by amiRNA resulted in abnormal growth of tomato shoots. This gene silencing caused severely dwarf shoots which failed to grow beyond 3-5 mm in length and finally after few weeks of growth resulted in death of shoots. A similar phenotype was also reported in *Arabidopsis,* where, knockout of ACL5 gene was responsible for severe defect in stem elongation and casued dwarf phenotype (Imai et al., 2006). Similarly, silencing of ACL5 in cotton was shown to be defective in stem elongation (Mo et al., 2015). Due to the

severe phentype, no further characterization of SolycACL5 gene silencing in tomato was performed.

5.5 Overexpression of SolycACL5 in tobacco

To gain further insight into the developmental role of SolycACL5, we ectopically overexpressed the tomato SolycACL5 in tobacco and analyzed altered phenotypes in 35S::SolycACL5 overexpressing lines (OE). We generated several independent tranformed OE lines in tobacco. Our phenotypic analysis showed that OE were taller plants with larger leaves and more chlorophyll pigmentation relative to control plants. Also, the OE plants have a more number of green leaves and lesser number of senescencing leaves comparatively to control plants indicating that there was delay in senescence of their leaves. Overall, OE lines showed superior phenotypes and more sturdier growth than the control. Our results are in line with the previous report where overexpression of Cotton ACL5 resulted in an increased plant length (Mo et al., 2015). However, its knockout in both *Arabidopsis* and cotton plants showed dwarf phenotype (Kakehi et al., 2010, Kakehi et al., 2008). Moreover, knockout of ACL5 in *Arabidopsis* resulted in premature cell death of xylem cells (Muniz et al., 2008). In contrast to our observation, overexpression of ACL5 in *Arabidopsis* exhibited the fairly normal phenotype of plants (Marina et al., 2013).

Exogenous application of polyamines shown to reduce generation and accumulation of H_2O_2 in plants (Mandal et al., 2013). We also found that overexpression of SolycACL5 resulted in the lesser accumulation of H_2O_2 in OE plants. DAB staining also supported this result, suggesting that there was less H_2O_2 accumulation in OE plants. Previously, it is already reported that polyamines are powerful hydroxyl radical scavengers (Das et al., 2004). Thus, it might be possible that overexpression of SolycACL5 caused reduction in H_2O_2 accumulation in OE lines. Generally, ROS generation is compensated by the activity of antioxidant enzymes and polyamines are known to moderate the activities of scavenging enzymes (Kubiś 2008). We have also determined the activity of antioxidant enzymes such as CAT, SOD, GP, and APX in the OE plants and showed that there is a modulation in the activity of some of these enzymes. Specifically, we observed that there was reduction in the enzymatic activity of APX, and to some extent, increase in the CAT and GP activity in the OE lines. Moreover, there was no significant change in the activity of SOD. Recently, it was reported that tomato fruits having higher polyamine content

exhibited lower ROS generation and higher CAT and SOD activities, signifying that higher polyamine level positively regulated antioxidant enzyme system (Sánchez-Rodríguez et al., 2016). Thus, there was less ROS particularly H_2O_2 generated in OE lines and the enzymatic activity of some of the enzymes was also modulated in plants.

In addition, MDA content was also analysed to measure lipid peroxidation which measures the level of oxidative degradation of lipids in cells. Recently, it was seen that oxidative activity of MDA was decreased in *Calendula officinalis* after the exogenous application of spermine (Baniasadi et al., 2018). We also observed that lipid peroxidation was less in the OE plants. It is suggested that delayed leaf senescence in plants is associated with higher polyamines accumulation and reduced ROS generation (Sequera-Mutiozabal et al., 2016). Therefore, a lower activity of the antioxidant enzyme and lower lipid peroxidation could be due to less generation of ROS in SolycACL5 OE plants. Moreover, polyamines are reported to inhibit lipid peroxidation in dark incubated oat leaves, thus preventing the leaves to undergo senescence (Borrell et al., 1997). Our results also suggested that inhibition of lipid peroxidation in OE plants may be one of the mechanisms responsible for their antisenescence effect in leaves.

Exogenous application of polyamines is known to prevent loss of chlorophyll during leaf senescence in barley (Cohen et al., 1979). Earlier, exogenous application of spermidine and spermine prevented the loss of chlorophyll in oat leaves (Besford et al., 1993). In a recent report, it was observed that increased polyamine level in tobacco plants lead to increased chlorophyll content (Nölke et al., 2018). Similarly, we also observed that OE plants were more pigmented and having more chlorophyll. In a earlier report, putrescine treatment of the callus culture of *Daccus carrota*, enhanced the anthocyanin production (Sudha and Ravishankar 2003). Recently, it was reported that strawberry fruits overexpressing SAMDC had increased anthocyanin content (Guo et al., 2018). We also showed that overexpression of SolyACL5 resulted in higher accumulation of anthocyanin in plants, thus it can be speculated that overexpression of SolycACL5 leads to more anthocyanin accumulation in OE plants. Hence, it could be the probability that overexpression of polyamines in plant led to enhanced pigmentation.

Leaf senescence is a degradative process leading to the breakdown of proteins and nucleic acids, the disintegration of chloroplast along with the loss of chlorophyll and photochemical activities and increase in the number of hydrolytic enzymes (Thomas and Stoddart 1980). Various reports have shown that polyamines, besides having a role in growth and development, are also involved in regulating plant senescence (Altman 1982, Altman et al., 1977, Popovic et al., 1979). They stabilize thylakoid membrane (Popovic et al., 1979) and inhibits RNases and proteases (Kaur-Sawhney and Galston 1979). All of these protective effects of polyamines may be due to their cationic nature and because of their binding ability; they stabilize the membrane structure and delay senescence.

We observed that SolycACL5 OE plants showed delayed senescence of leaves. In order to further verify whether developmental senescence is delayed, we analyzed the expression of senescence markers, SGR1 (Stay Green 1) and SAG12 (Senescence-Associated gene12) in OE plants (Breeze et al., 2011). Results showed significantly lower expression of SGR1 and SAG12 in OE plants, suggesting suppression of leaf senescence program. Thus, it can be suggested that delayed senescence of leaves of SolyACL5 OE plants might be due to late initiation of senescence program.

Chloroplasts are known to play a central role in the evolution of plant senescence (Thomas et al., 2009). It is also known that disintegration of chloroplast and slowing down of chloroplast activity is an important sign of the leaf senescence process (van Doorn and Yoshimoto 2010), therefore we have analyzed the expression pattern of various chloroplast associated marker genes such as rpoA, rbcL, and petB in OE plants. Increase in rpoA might be the result of the increased molecular activity of chloroplast (Krause et al., 2003). Moreover, high expression of rbcL gene suggests a high accumulation of RUBISCO enzyme which is required for the photosynthesis (Patel and Berry 2008), also high petB expression may lead to more electron transfer for photosynthesis (Song et al., 2014). The higher transcript level of these chloroplast encoding genes in OE plants showed that plants have a higher rate of chloroplast activity that leading to delayed senescence of leaves. As plastid RNA polymerase activity is essential for chloroplast biogenesis and function (Kremnev and Strand 2014), increased expression of rpoA suggests an important role of SolycACL5 gene in chloroplast function. Increased expression of rbcL and petB also indicates high functioning of photosynthesis and electron transport chain in SolycACL5 OE lines.

These results emphasize that overexpression of SolycACL5 delays leaf senescence process by postponing chloroplast degradation process and in increasing its activity which resulted in better and healthy growth of the plants.

In conclusion, a total of eighteen polyamine biosynthetic genes were identified and systematically analyzed in tomato using various bioinformatics tools. Most of the identified tomato polyamine biosynthetic genes showed differential expression pattern during vegetative and reproductive development of tomato plants. Expression analysis revealed that most of candidate genes were highly regulated by stresses and defense hormones. Promoter sequence analysis showed presence of a relatively large number of hormone and stress responsive cis-regulatory elements in promoters of candidate genes. Three close homologs of *Arabidopsis* ACL5 genes in tomato were identified which showed higher expression in roots, stem, and early developmental stages of leaves. Silencing of selected SolycACL5 gene could not be functionally characterized further because of severely dwarf phenotype. Overexpression of SolycACL5 in tobacco resulted in vigorus plant growth with higher accumulation of chlorophyll and anthocynin pigments and delayed leaf senescence. The phenotype exhibited by OE plants may also be due to the lower accumlation of H_2O_2. Expression of genes responsible for initiating senescence namely, SGR1 and SAG12 was also downregulated in the OE plants. The higher transcript level of the chloroplast encoding genes rpoA, rbcL, and petB in OE plants also indicated that plants have a higher rate of chloroplast activity that leading to delayed leaf senescence. Thus, it could be anticipated that overexpression of SolycACL5 in plants leads to higher pigmentation and delayed senescence of leaves. Thus the study provide a comprehensive analysis of polyamine biosynthetic genes in tomato and that laid a foundation for further functional characterization of these genes in plants.

Table 5.1Expression domains of polyamine biosynthetic genes during development and stress responses in tomato

Polyamine biosynthetic gene	Expression during development	Expression during stress
SolycADC1	Mature leaf, outer layer of stem, abscision zone, developing flower bud, mature flower	Upregulated under heat, cold, UV-C, flood, drought, wounding and FB; downregulated by mannitol.
SolycADC2	Immature leaf, abscision zone, developing flower bud, mature flower	Upregulated under heat, cold, UV-C, flood, drought, wounding; downregulated by JA, SA, ABA and mannitol.
SolycODC1	Immature leaf, outer layer of stem	Upregulated under heat, cold, UV-C, flood, drought, wounding, mannitol, ABA and SA; downregulated by RB, FB1 and JA.
SolycODC2	Outer layer of stem, mature flower, immature fruit	Upregulated under FB1; Downregulated under heat, cold, UV-C, flood, drought and wounding;
SolycSAMDC1	Leaf, stem, developing flower bud, mature fruit	Upregulated under heat; downregulated under drought, ABA, NaCl and mannitol.
SolycSAMDC2	Mature leaf, inner layer of stem, developing flower bud, mature flower	Upregulated under JA, SA; downregulated under NaCl, mannitol, MV, RB, FB1
SolycSAMDC3	No significant expression	Upregulated under heat, drought and SA; downregulated under cold, UV-C, NaCl, mannitol, MV, RB, FB1 treatments
SolycSAMDC4	No significant expression	Upregulated by heat, drought, wounding, SA and MV
SolycSAMDC5	No significant expression	Upregulated by heat, wounding and SA
SolycSPDS1	Immature leaf, outer and inner layer of stem, developing flower bud, mature red fruit	Upregulated under FB1; downregulated under wounding, ABA, JA, NaCl, mannitol, RB
SolycSPDS2	Immature leaf, outer and inner layer of stem, developing flower bud, immature fruit	Upregulated under JA, SA and FB1; downregulated under flood and mannitol
SolycSPDS3	Cotyledon, mature flower	No significant expression
SolycSPDS4	Hypocotyl, immature fruit	Upregulated under flood
SolycSPDS5	No significant expression	No significant expression
SolycSPMS	Immature leaf, inner layer of stem, mature fruit	Upregulated under heat, cold, UV-C, SA, RB and FB1; downregulated under drought, flood, NaCl and mannitol
SolycACL5	Root, leaf, inner layer of stem, abscision zone, developing flower bud	Upregulated under heat, wounding, JA, SA and MV, downregulated under cold, UV-C, flood and mannitol
SolycACL5-Like1	Immature leaf, inner layer of stem, developing flower	Upregulated under heat, drought, wounding, JA; downregulated ABA and mannitol
SolycACL5-Like2	Mature leaf, immature fruit	Upregulated under flood, ABA; downregulated under heat, cold, drought, wounding