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Molecular characterization of autophagy in transgenic *Solanum lycopersicum* during fruit ripening

By

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٤. Summary and conclusion

Autophagy is an essential part of the development of most organisms. It not only recycles nutrients, but also plays a significant role in maintaining homeostasis during various phases of developmental processes in most organism. However, its role during development and ripening of the fleshy fruit has not been characterizedyet. The role of autophagy process in tomato fruit identified tomato homologs for several autophagy genes, including genes involved in autophagy regulation (TOR, PI٣K (VPS٣٤)), induction (ATG١, ATG١٣, ATG١٧, ATG١٠١), vesicle nucleation (ATG٢, ATG٦, ATG٩, ATG٢٧) expansion and completion (ATG٣, ATG٥, ATG٧, ATG٨, ATG١٠, ATG١٢). These genes exhibited a high degree of homology with other plant autophagy genes, indicating they are conserved during the plant evolution. RNA-Seqtranscriptome analyses of tomato fruits at the breaker (onset of ripening) and fully ripe (٨ days after breaker) stages of fruit ripening have been used to analyze the role and regulation of autophagy duringfruit ripening process. Several autophagy genes including ATG١٣b, APG٣, ATG٥a, ATG٧, ATG٨a, ATG٨b, ATG٨d, ATG٨f and ATG١٢ were upregulated during ripening, suggesting a role of autophagy in tomato fruit ripening.

Wild-type long shelf-life processing tomato fruit cv. Ohio٨٢٤٥ as the genetic background for jasmonate impaired (*SlLoxB)* and polyamine rich tomato genotypes (٥٥٦HO) and a genetic cross between them (SAMLOX) have been utilized to evaluate the roles of jasmonate or polyamines in the fruit ripening associated autophagy process. Jasmonate reduction enhanced steady state levels several ATGs, includingATG٦, ATG٧, ATG٨b, ATG٨c, ATG٨g, ATG٨f, PI٣K but also reduced transcript levels of ATG٥a, ATG٨e, and ATG١٣b. Enhanced spermidine/spermine had a limited effect on

transcript levels of ATG and showed increase in ATG٨b but decrease in ATG١٣b. Fruits have a simultaneous increase in spermidine/ spermine and reduction in jasmonate in the double transgenic mutant (LOXSAM) exhibited patterns similar to SAM٥٥٦HO suggesting a dominant role of polyamines in determining the expression pattern of the autophagy genes.

The changes in the expression patterns of the autophagy geneshave been characterized in isogenic lines having ripening mutations *rin*, *nor*, and *Nr* and compared them to their parental wild-type genotypeto understand if the autophagy has a role in fruit ripening.These investigations were carried out in a short shelf- life salad tomato fruit from cv. Ailsa Craig and its isogenic ripening impaired tomato genotypes for *rin*, *nor*, and *Nr* mutants. The patterns of autophagy gene expression in Ailsa Craig were similar to that Ohio ٨٢٤٥ confirming a role for autophagy in tomato fruit ripening. The three ripening mutation affected patterns of ATGs expression. All ripening impairing mutations, *Nr, nor* and *rin* significantly upregulated transcript levels of the autophagy genes in B٨ stage compared to WT fruits likely to maintain homeostasis during extended shelf life period.

This thesis is divided into three chapters:

Chapter one: autophagy as a recycling process in the living cell.

Chapter two: Effect of transgenically enhanced impaired lipoxygenase and polyamines and on the autophagy process during fruit ripening.

This chapter includes:

١- Identification of autophagy homologues in *Solanumlycopersicum.*

٢- Jasmonate reduction enhances transcript levels of autophagy genes.

٣- Effect of high polyamines on the transcript levels of autophagy genes during fruit ripening.

٤- High polyamines overcome some of the induction caused by LOXB silencing.

Chapter three: Autophagy is, an integral part of tomato (*Solanumlycopersicum* L) fruitduring ripening process.

This chapter includes:

١- Changes in autophagy gene expression during fruit ripening.

٢- Effect of impaired ripening on autophagy gene expression:

a) Effect of *Nr* mutations on ATG gene expression during fruit ripening process.

b) Effect of delayed-ripening mutants, *nor*, on the transcript levels of autophagy genes during fruit ripening.

c) Effect of RIPENING INHIBITOR (*rin*) mutation on the transcript levels of autophagy genes during fruit ripening.

Conclusion:

Chapter ٢

١. Identification of ٢٢ putative autophagy genes in *Solanumlycopersicum*. However, transcripts of ١٧ putative autophagy genes were present in ripening fruit RNAseqtranscriptome. These autophagy genes included two ATG١٣ and ATG١٠١ from the induction, ATG٦ from PI٣K core complex, APG٩, ATG٢ and ATG٢٧ from the membrane delivery and seven ATG٨ two ATG٥, and ATG١١from the phagophore assembly. Expression of tomato homologues for ATG٣, ATG٧, ATG١٠ and ATG١٢ were also detectable in ripening fruits based on the RNAseq analyses. The homologues for ATG١, ATG٤, ATG١٤, ATG١٥, ATG١٦, ATG١٧, ATG١٨, ATG٢٠, ATG ٢٣ and VSP١٥ were absent in tomato genome.

٢- Autophagy complex in tomato fruit has not yet been characterized.The absence of these genes transcription can be interpreted in several ways, including the proteins encoded by the absent genes are not required/ essential for autophagy in the tomato fruit. Other possibility is that other proteins, not homologues to yeast autophagy genes, are involvedin this process.

٣- Jasmonic acid production impairment has an effect on the expression of autophagy genes during fruit ripening. During fruit ripening, transcripts levels of many autophagy genes in the *SlLOXB* silenced fruits were significantly up regulated compared to wild-type. Jasmonate influences expression of autophagy genes and in the absence of this phytohormones they are expressed at higher levels. Jasmonate also negatively affects expression of many autophagy genes. However, more experiments are needed to show that the increased levels of transcript also enhance the formation of more autophagosome leading to higher autophagy.

٤- Increased spermidine/spermine levels during the ripening of ٥٥٦HO fruits exhibited significant upregulated transcript of several ATGs patterns similar to that observed in ripening *SlLOXB*-silenced fruits. However unlike *SlLOXB* fruit, ٥٥٦HO fruits exhibited a significant decline in other genes, suggesting that LOXB-silenced fruits and high polyamine fruits differentially regulate expression of autophagy genes.

٥- Using crosses among high polyamine and impaired jasmonate plants elucidated the respective role of high polyamine phenotype as stimulators of the autophagy process.

Chapter three:

١- All autophagy proteins implicated in the autophagy process are not present in ripening fruit from tomato cv. Ailsa Craig. The results indicate a limited role of autophagy process during fruit ripening. However, the autophagy process is more active at the onset of ripening in wild type fruit than the latter stages of fruit ripening.

٢- The ethylene-impaired *(Nr*) fruits continued to maintain active autophagy gene expression which likely allowed *Nr* fruit to maintain homeostasis.

٣- *nor* gene has limited effect on autophagy process during the early ripening stages but modulates it during later stages of ripening.

٤- Regulation of the autophagy process during aging of *rin* fruits was similar to that obtained in *nor* fruits.

٥- Based on the steady state levels of various ATG gene transcripts,the results indicate that autophagy is active during fruit ripening and more research is needed to draw specific conclusions on the role of autophagy in tomato fruit ripening.