1454 words

**Introduction**

Ethylene is a gaseous hormone with the molecular formula of C2H4. (Merchante et al, 2013). Ethylene is involved in many processes such as: seed germination; growth of seedlings; organ development/senescence; leaf/petal abscission; stress/pathogen responses and fruit ripening (Merchante et al, 2013). The main subject for this manuscript review is a paper focusing on ethylene’s effect on fruit ripening, specifically how degradation of ethylene receptors controls the ripening of tomato fruit (Kevany et al, 2007). An important model for the study of fruit development, specifically fleshy fruit, is tomato (Kevany et al, 2007). In the tomato genome, several genes that regulate ripening by ethylene signal transduction have been identified (Karlova et al, 2014). At the onset of ripening, tomato and other climacteric fruit like apples, bananas, pears, mangoes and papaya, will have a rapid rise in respiration and a burst of ethylene production (Karlova et al, 2014). The specific objectives of this paper are to show how a reduction of LeETR4 or LeETR6 levels, two of the six ethylene receptors, lead to an early-ripening phenotype (Kevany et al, 2007). Secondly, the paper proposes that receptors are degraded rapidly through the 26S proteasome-development pathway (Kevany et al, 2007). Thirdly, the paper proposes that by exposing ethylene to immature fruit and measuring the cumulative ethylene response, this proves receptor levels control the timing for the onset of fruit ripening (Kevany et al, 2007).

**Research Summary**

Figure 1 shows results of quantitative reverse transcription polymerase chain reaction (QRT-PCR) examining mRNA expression for all six ethylene receptors. There was low expression for all receptors in immature stages but a significant increase in NR, ETR4 and ETR6 transcripts when in mature stages. The increase in mRNA during an ethylene-dependent process suggests more receptors makes fruit less sensitive to ethylene, directly conflicting the receptor signalling model. Since it is known that ETR4 and NR are ethylene-inducible in fruit tissue (Ciardi et al, 2000), the rise in the mRNA transcripts may be due to increased ethylene production. They tested this by QRT-PCR of immature fruit tissue treated with ethylene, the results of which were shown in Figure 2. NR, ETR4 and ETR6 had high levels of mRNA transcripts while ETR1, ETR2 and ETR5 had a slight increase. Due to this, they decided that the earlier rise in expression for the NR, ETR4 and ETR6 receptors was due to ethylene produced as the fruit matured.

LeETR6 antisense lines were generated and compared to LeETR4 antisense lines. They grew plants with low levels of LeETR6 and observed epinastic leaf growth and premature flower senescence, similar phenotypes to LeETR4, as described in Figure 3 and Table 1. They concluded that either LeETR4 or LeETR6 triggers hypersensitivity to ethylene including premature fruit maturation and ripening, but no studies were done on other receptors.

They followed up with a comprehensive study of how mRNA and protein expression changes throughout fruit development as described in Figure 4. Receptor mRNA increased when ripening occurred and remained high till tomato turned fully red. Contrarily, receptor protein levels were high during immature fruit development but decline significantly when ripening starts. They claimed that RNA levels are not indicative of receptor protein content and ethylene is controlled from another level. They decided that ethylene binding induces receptor turnover since the decrease of receptor mirrors the onset of auto-catalytic ethylene synthesis.

With the objective of proving that ethylene binding causes receptor degradation, they treated immature leaf and fruit tissue with ethylene and observed an increase in NR, ETR4 and ETR6 mRNA, and a decrease in NR, ETR4 and ETR6 proteins as shown in Figure 5 and 6. When ethylene was removed, mRNA decreased to low levels similar to pre-treatment while protein levels remained low for twenty-four hours after treatment, suggesting mRNA expression was reversible while protein levels were irreversible. For seedlings, a similar pattern was observed; increase in mRNA for NR, ETR4 and ETR6 and respective receptor protein level decreased. Therefore, in both vegetative and reproductive tissues, ethylene exposure causes the sudden decline in receptor protein level independent of transcript levels.

To determine if 26S proteasome-dependent pathway causes the turnover of ethylene receptors, proteasome inhibitor MG132 was treated to seedlings. After ethylene treatment, the protein levels increased as shown in Figure 6. They assumed ubiquinated protein are extracted from the membrane, then degraded by cytoplasmic 26S proteasome complex. MG132 results are consistent with ubiquitin mediated receptor degradation, however, no receptors could be detected in the soluble fraction and no larger, ubiquitinated forms of receptors in microsomal membrane fractions were found.

Instead of just causing degradation, to prove that the process is a necessity, 1-methylcyclopropane (1-MCP), the competitive inhibitor to ethylene, was treated onto seedlings. The attachment of 1-MCP to the receptor is irreversible, so 1-MCP stabilises the receptor protein by preventing ethylene-induced receptor protein degradation. As shown in Figure 6, tomato seedlings with 1-MCP did not undergo receptor degradation and there was no increase in mRNA. To act as further proof, never ripe (Nr) mutant seedlings was treated to ethylene and since the mutant Nr protein cannot bind to ethylene, while a significant decrease occurred in ETR4 and ETR6, a less significant change was observed in NR protein level. So, the research provided proof that ethylene binding is required for receptor degradation, but they did note a possibility for ethylene-induced receptor degradation machinery

To see whether ethylene-induced receptor degradation causes early-ripening phenotype, immature fruits attached to the plant were treated with ethylene. on average, the treated fruits ripened three days earlier, shown in Table 2. To test for a connection between lower protein levels and reduced time for ripening, protein and mRNA samples were collected and with the results provided in Figure 7, proved that there is a correlation.

**Discussion**

This scientific paper has been cited two hundred and nine times; it is credible and has made some valid conclusions. The ethylene-signalling cascade does start with ethylene binding to receptors and they are negative regulators that actively repress ethylene response in the absence of ethylene (Merchante et al, 2013). Proteasome-mediated protein degradation is important to the ethylene-signalling cascade (Merchante et al, 2013). At the receptor level, they found homology in Arabidopsis since ethylene induces ETR2 degradation through the 26S proteasome (Merchante et al, 2013). The paper being referenced, directly cites the paper under this review and then claims that transcriptional regulation and proteasome-mediated degradation of receptors could be a piece of the desensitising mechanism of ethylene in response to stress (Merchante et al, 2013).

The paper under review has directly influenced other research in this area outside of conclusions being cited, as three of the original authors of the paper followed up with a paper on fruit specific-suppression of LeETR4 (Kevany et al, 2008). They validated the model that if receptors are not replaced after ethylene-mediated degradation like in immature fruit, the fruit is more sensitive to subsequent ethylene exposure and ripen earlier using results provided from both papers (Kevany et al, 2008). Following on from both papers, it has been proven that LeETR4 is multiply phosphorylated in vivo and the phosphorylation level depends on ripening stage and ethylene action (Kamiyoshihara et al, 2012; Gapper et al, 2013). The phosphorylation state of receptors like LeETR4 may mark receptors for ubiquitination (Gapper et al, 2013). Furthermore, this led into identifying a tomato TPR protein SITPR1 which interacts with NR and LeETR1 in yeast and in vitro which may act as an adaptor for receptor degradation, causing enhanced ethylene sensitivity (Lin et al, 2009; Gapper et al 2013). SITPR1 is a type of tetratricopeptide repeat proteins (TPRs) and TPRs label proteins for degradation via 26S proteasome by ubiquitination (Gapper et al, 2013). However, overexpression of SITPR1 did not affect fruit ripening in terms of colour or textural changes, so there may be additional regulators in receptor turnover (Gapper et al, 2013).

In terms of methods used in this paper, comparing mRNA and protein levels are a central aspect of this paper so it is good that QRT-PCR and protein blot was used since they are two industry standard methods to handle transcriptomic and proteomic research, respectively. ORT-PCR is quicker and cheaper than RNA-Sequencing and while RNA-Sequencing may provide more detailed information, there does not appear to be a strong reason to use it, as QRT-PCR provides enough data. However, mass spectrometry would have been more useful in identifying any ubiquitinated forms of receptors from MG132 treated seedlings that were not detected in the soluble fraction. Also, quantitative data could have been obtained if different amounts of ethylene was used to create a scale of how plants will ripen early, providing information on upper and lower limits of ethylene required for early-ripening phenotype, instead of just using 50ppm of ethylene as described in Figure 7.

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