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Role of ethylene receptors during senescence and ripening in horticultural crops

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The past two decades have been rewarding in terms of deciphering the ethylene signal transduction and functional validation of the ethylene receptor and downstream genes involved in the cascade. Our knowledge of ethylene receptors and its signal transduction pathway provides us a robust platform where we can think of manipulating and regulating ethylene sensitivity by the use of genetic engineering and making transgenic. This review focuses on ethylene perception, receptor mediated regulation of ethylene biosynthesis, role of ethylene receptors in flower senescence, fruit ripening and other effects induced by ethylene. The expression behavior of the receptor and downstream molecules in climacteric and non climacteric crops is also elaborated upon. Possible strategies and recent advances in altering the ethylene sensitivity of plants using ethylene receptor genes in an attempt to modulate the regulation and sensitivity to ethylene have also been discussed. Not only will these transgenic plants be a boon to post-harvest physiology and crop improvement but, it will also help us in discovering the mechanism of regulation of ethylene sensitivity.

Introduction

Sessile organisms like plants cannot move and are dependent on themselves for their requirements and to combat environmental assaults. Where after they are equipped by nature with the simplest known yet powerful phytohormone "ethylene", a gaseous plant hormone, which is known to produce myriad effects on plant physiology and its growth and development.[1] Ethylene is known to produce marked phenotypic changes in etiolated seedlings. It is globally characterized by the so called "triple" response reported for the first time in pea demonstrating the inhibition of epicotyl and root elongation, radial swelling of epicotyls and root cells, diagravitropical (horizontal) growth pattern and apical hook formation.[2] Ethylene is perceived by a family of ethylene receptors in the presence of a copper cofactor.[3] Ethylene receptors and the immediate follower of receptor elements, Constitutive Triple Response (CTR1) element are the negative regulators of the cascade. The ethylene receptors are classified into 2 subfamilies. Subfamily I (ETR1, ethylene receptor1/ethylene resistant1 and ERS1, ethylene response sensor1) receptors have three membrane spanning domains at the N-terminus of the hydrophobic sensor domain where as subfamily II (ETR2, ERS2, EIN4, ethylene insensitive 4) have four domains, 3 of which are the membrane spanning domain and one is a putative signal peptide. Subfamily I receptors show histidine kinase (HK) activity[4,5] while, subfamily II receptors possess serine/threonine kinase activity.[5] ERS1 is known to possess both the activities. Reversion to ethylene sensitivity (RTE1), co localized on endoplasmic reticulum (ER) with ETR1 acts as a positive regulator of ETR1 and is specific to ETR1, barring other receptors. It regulates ethylene signaling by physically interacting with ETR1.[6] ETR1 is found to be both essential and sufficient for nutations among the ethylene receptors. However, it was concluded that ETR1 HK activity and phosphotransfer through the receiver domain are not needed to rescue nutations.[7] Of the five Arabidopsis ethylene receptor, ETR1 is the best and most exhaustively studied. Almost every possible information about it has been deciphered Whether, it is ethylene binding activity,[8] need of a copper co factor for better affinity,[3] HK activity,[4] localization in ER,[9] interaction with CTR1[10] or its disulfide linked dimer formation.[8] The other companion of this receptor in subfamily I i.e.ERS1 has also been shown to depict ethylene binding activity.[11] Binder (2008)[12] has elaborated on the models for receptor function and output. Epistatic studies have revealed that ethylene receptors namely ETR1, ETR2, ERS2, ERS1and EIN4 act upstream of the CTR1, whereas the EIN2, EIN3, EIN5, EIN6 and EIN7 act downstream of CTR1.[13,14] EIN3 and related EIN3-LIKE (EIL) are known to function as transcription regulators of ethylene primary response genes.[15] CTR1 activates Ethylene Insensitive 2 (EIN2), cytoplasmic protein which is related to Nramp family of metal transporters.[16] Ethylene Insensitive 3 (EIN3) has a DNA binding ability and is located in nucleus. It acts upon Ethylene response Factor1 (ERF1) gene which encodes for an ethylene response element binding protein (EREBP) now known as ERF (ethylene-responsive element binding factor) that binds a GCC-box, a *cis* element of many ethylene response genes.[15]

A short post-harvest longevity has been a detrimental factor for many crops. Separation from plant leads quickly to ripening of fruits and senescence of flowers. In many species ripening and senescence are ethylene regulated. Thus, attempts have been made to retard the post-harvest processes by applying chemicals

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that inhibit ethylene synthesis like 1-MCP (1-Methylcyclopropene) marketed as antisenescence agent EthylBloc, silver thiosulfate, AVG (amino ethoxyvinylglycine hydrochloride) marketed as Retain. 1-alkyl-cyclopropenes and $AgNO_3$ are the most prominently used ethylene antagonists.[17-22] Another class of strained alkenes, cyclobutenes and *trans* cyclooctene have recently been brought into picture because of their greater convenience in handling and administration.[23] Kazemi et al. (2011)[24] reviewed the effect of various chemicals at different concentrations on Gebrera cut flowers. However, the long-term solution will probably be based on genetically modified plants with genes that either suppress synthesis or reduce sensitivity to ethylene. A few mutants that impart ethylene insensitivity are known e.g., tomato *Nr* and dominant mutants of Arabidopsis ethylene receptor sensor genes *ETR* and *ERS.* Genetically modified crops with *etr1–1* allele have shown delayed fruit ripening, flower senescence and flower abscission of tomato and petunia. Mutants of ETR1 and ERS1 receptor from *Zea mays* has shown to confer ethylene insensitivity in Arabidopsis but in the presence of subfamily I Arabidopsis ethylene receptors.[25] This is an example of functional conservation of ethylene receptors across the genus. ERS1 not only represses ethylene responses but also promotes ethylene responses in ETR1 dependent manner implying collaboration among ethylene receptors and regulation of receptor mediated signaling.[26] Recent finding by Liu and Wen (2012)[26] showed a difference in ethylene responses when dominant ethylene-insensitive receptor was used in the background of wild type ethylene receptor. It was opined from this observation that different combination of ethylene receptors can facilitate differential receptor signal output thus regulating the ethylene activity. Collaboration among the receptors was further substantiated by the finding that they form protein complexes to relay signal as per the specific cellular environment and responses.[25] Characterization of ethylene receptor genes provide clues to understand how plants regulate their ethylene sensitivity. Therefore, an alteration of ethylene action is a valuable target for the genetic engineering of crops. Manipulation of ethylene biosynthesis or perception allows us to modulate these processes and thereby create plants with more robust and desirable traits, giving us a glimpse into the role of ethylene in the plant. Having known about the ethylene biosynthetic pathway and signaling molecules, the approach has been to manipulate the perception of hormone not directly involving the biosynthesis.

Modification in the levels of plant hormones and their signaling have augmented the life and yield of many agriculturally important crops as reviewed by Csukasi et al. (2009).[27] Ethylene is not just a ripening hormone in addition, the phytohormone plays role in physiological processes throughout the life cycle of the plant.[1,28] Its impact on nodulation in legumes mediated by manipulated ethylene biosynthesis is reviewed in detail by Shaharoona et al. (2011).[29] Its involvement in such agronomically important processes such as senescence, abscission and fruit ripening has made ethylene a target for manipulation by genetic engineering methodologies.[16] The role of ethylene in controlling abscission has been reviewed by Binder and Patterson (2009).[30] Key aspects of research on regulatory networks controlling ethylene synthesis, its role in flower development and fruit ripening integrating knowledge from the model plant Arabidopsis and other plant species has been focused on by Lin et al. (2009).[31] This review aims at findings involving regulation of ethylene perception and its hormonal levels mediated mainly by the receptors and the downstream molecules involved in the signaling cascade. Expression studies have also been covered to get a better insight into the role of these molecules in regulating senescence and ripening in a crop specific manner.

Targeting Ethylene Perception, and Not Ethylene Biosynthesis

There have been many prolific attempts to procrastinate senescence by exploiting ethylene biosynthetic pathway. Little et al. (2009)[32] have elaborated on how an altered ethylene manufacturing modified reception by its receptors or an intervened signaling keeps a check on senescence, stress and disease, at the same time regulating ripening and flowering. Stearns and Glick (2003);[33] Czarny et al. (2006)[34] have documented how the biosynthetic pathway rate limiting enzyme ACC synthase, ACC oxidase along with other enzymes on being suppressed using antisense approach or overexpressed have fetched with the desired outcome. The approach has been put to use in different crops like tomato, tobacco, potato, carnation, begonia, torenia, cantaloupe, broccoli, canola, melon, etc. Reverse genetics approach using TILLING platform was exploited in melon to identify novel alleles involved in ripening by Mardas et al.,[35] Blocking ethylene production by targeting enzymes has been an approach to combat senescence to good extent but with contraindicated repercussions. Another approach that has gathered attention in recent years is reducing the sensitivity of plant for ethylene without altering the biosynthetic pathway.

A few points are enumerated that could have led to the paradigm shift to modulate the plant's sensitivity to ethylene instead of manipulating the biosynthetic pathway.

(1) ACC synthase, the rate limiting enzyme and ACC oxidase enzyme of the pathway are coded by a multigene family and undergo posttranslational modifications to give rise to different isoforms which are active under different physiological conditions.[1,36,37-39] Different isoforms of these enzymes limits the success of antisense suppression of the genes coding for these enzymes in other crops, e.g., Oeller et al. (1991)[40] observed limited success while using antisense ACC synthase to lower plant ethylene levels in tomato. In addition, ACC oxidase is present in meagre amounts in plant tissues.[41]

(2) Antisense suppression of carnation ACC oxidase resulted in noticeable decline in ethylene production during flower senescence and enhanced longevity of flowers. This could only check the endogenous production of ethylene[42] and application of exogenous ethylene which would re-establish the impact of ethylene. However, the same idea did not work in case of begonia.[43]

(3) S-adenosylmethionine (SAM), initiator of ethylene biosynthetic pathway acts a universal methylating agent for proteins, nucleic acids, lipids, carbohydrates and other biomolecules. In an

attempt to reduce the availability of SAM for ethylene production by overexpression of SAM hydrolase[44] or sense and antisense expression of SAM decarboxylase (this enzyme produces decarboxylated SAM, used for polyamine biosynthesis) in potato leads to unusual phenotype.[45]

(4) There are certain events in flower development like flower opening, ovary development or pedicel elongation which are elicited by ethylene even in the flowers where petal senescence is virtually irresponsive to ethylene. So, it would not be a good idea to disturb the biosynthetic pathway thus hampering the normal development of the flower.[21]

(5) Pollination induces increased ethylene production leading to enhanced senescence, which is described as a mechanism to terminate the life of flower after successful pollination to benefit the survival of species.[46] Decreased ethylene sensitivity by using mutated ethylene receptors would retard senescence.[47]

(6) Czarny et al. (2006)[34] and Hoffman et al. (1999)[48] have reviewed that blocking the ethylene biosynthetic pathway can put a check on scattering effect of damage caused due to an infection or stress in plant. But it seems unreckonable to check the upsurge of second ethylene peak which leads to more profound and collateral damage. The second peak of ethylene is originated due to faster transcription of ACC synthase genes and antisense suppression of such a large pool of ACC synthase genes does not seem feasible.

(7) Numerous studies have also revealed that antisense expression of ethylene synthesis genes varied according to organ,[49] developmental stage, age of organ[50] and enzyme induction factors.[49] Transgenic expression will further vary according to the promoter employed, transformation method, species and other variable factors e.g., transgenic petunia were developed using antisense broccoli ACC synthase (*BoACS1*) and ACC oxidase (*BoACO1*) genes. *BoACO1* was able to reduce ethylene biosynthesis and delay flower senescence of *Petunia hybrida* more efficiently than the other gene.[51]

(8) Dandekar et al. (2004)[52] used antisense versions of apple ACC synthase and ACC oxidase to transform the apple with the aim of studying the role of ethylene in apple ripening. Ethylene production was drastically hampered and fruits ripened very slowly but with a side effect of decreased concentration of flavor ester forming enzymes.

(9) Transgene flow through pollen dispersal is one of the major concerns because it could lead to introduction of undesirable traits in other plants and weeds. Transgenics raised by targeting ethylene biosynthetic pathway did not offer any solution to the problem. However, using mutated ethylene receptor, the issue was sorted out.

Role of Ethylene in Climacteric and Non-Climacteric Plants

Flowers can be categorized as being ethylene sensitive or ethylene insensitive. In climacteric or ethylene sensitive flowers such as carnations and orchids, senescence is accompanied by a sudden transient increase in ethylene production and respiration, while treatment of non-senescent flowers with ethylene rapidly induces petal senescence. In non climacteric flowers such as Gladiolus, tulip, and iris, generally, no increases in ethylene production and respiration are apparent during flower senescence, and exogenous ethylene application has little or no effect on petal senescence.[21,53] Several studies to date suggest that abscission and senescence of flowers may be triggered by the perception of endogenous ethylene by ethylene receptors. Abscission is a typical ethylene response induced through ethylene receptors and is influenced by mutations in ethylene receptors.[54] Therefore, investigations of ethylene receptors and associated signal transduction pathways are essential for understanding ethylene perception in flowers. However, these changes are not understood in best capacity when it comes to non climacteric fruits. The molecular mechanism of fruit ripening is a complex phenomenon, as the dynanism and degradation of ethylene receptor has a significant part to play. The major difference between climacteric and non-climacteric plants is marked by the presence or absence of autocatalytic ethylene production. There are several aspects which are focused on to modulate ripening and senescence in plants and authors have reviewed these different perspectives. For example, Palma et al. (2011)[55] has focused on the total proteome involvement in the molecular physiology of fruit ripening in important climacteric and non climacteric crops. Bapat et al. (2010)[56] has enlisted the cases where ripening of fleshy fruits is arrested by targeting the ripening related genes. Here, we have listed all the horticultural crops which are either genetically modified using ethylene receptor gene for altered perception and senescence (**Table 1**) or in which the expression of these receptors and transcription factors involved in signaling cascade has been studied.

Genetically Modified Ornamental Horticultural Crops and Role of Ethylene Receptors, Downstream Molecules in Regulating their Senescence and Ripening

Campanula. *Campanula carpatica* is an ethylene-sensitive ornamental horticultural crop, which wilts in three days on ethylene exposure. The plant was transformed exploiting *etr1-1* gene from Arabidopsis under the influence of a flower specific fbp1 promoter from Petunia. Enhanced tolerance to exogenous ethylene was observed in transgenic lines and this reduced sensitivity to ethylene was stably passed on to the next generation. The better part of the result was that there was no compromised disease resistance, no interference with root forming ability and fertility of the plant. On crossing, a two T-DNA containing transgenic plant with an ethylene sensitive cultivar, the T1 progeny obtained plants were with high tolerance to ethylene. The degree of ethylene tolerance in plants with a single or double copy number did not vary at all showing that the sensitivity is independent of the copy number of the transgene.[57]

Carnation. During natural and pollination-induced senescence of the flowers, ethylene is first produced in the gynoecium (pistil) and the evolved ethylene acts on petals and induces the expression of genes for ethylene biosynthetic enzymes and cysteine proteinase resulting in the autocatalytic ethylene production from the petals and wilting of the petals.[58-60] Also, exogenous ethylene applied to carnation flowers, which have not yet started

Table 1. Transgenic crops with altered phenotype, senescence and ripening due to overexpression and suppression of ethylene receptor and downstream signaling molecules

Crop	Gene	Function of the Gene	Expression	Effect/Phenotype	Conclusion	References
Tomato	NR (Receptor gene) Semi Dominant	Functionally redundant ethylene insensitive in all tissues.	Antisense suppression	Normal ripening as in wild type fruit. No effect on ethylene sensitivity, increased levels of LeETR4 mRNA. No effect on ethylene sensitivity of seedlings	Not essential for ripening. Ethylene perception and signaling via the NR receptor is consistent with the receptor inhibition model. Functional compensation of LeETR4 for reduced NR expression	Hackett et al., 2000[172] Lanahan et al., 1994[163] Tieman et al., 2000[167]
	NR (Never Ripe) Semi Dominant	Functionally redundant ethylene insensitive in all tissues.	Overexpression in LeETR4 suppressed lines	Eliminates ethylene sensitivity, lack of epinasty, flower senescence and normal fruit set, rescued the ethylene response phenotype.	Proves the functional redundancy of the receptors. Negative Regulators of ethylene response. Only associated with ripening.	Tieman et al., 2000[167]
	Nr (Mutant NR gene)	Unable to bind ethylene, delay senescence, imparts ethylene insensitivity.	Antisense suppression	Activated ethylene responses and onset of normal ripening, plant with two homozygous copies of transgene produced wild type fruit. Single gene in a homozygous state gave intermediate phenotype.	Threshold level of mutant receptor is needed to suppress the ethylene response pathway. Support receptor inhibition model.	Hackett et al., 2000[172] Lanahan et al., 1994[163]
	LeEIL(1-3) Transcription Factor	Functionally redundant, positive regulator of ethylene signaling.	Antisense suppression	Single LeEIL suppression results no change. Multiple LeEIL gene suppression delays leaf epinasty, flower abscission, flower senescence.	Normal levels of expression of the other two LeEILs can compensate for the loss of single gene.	Tieman et al., 200[123]
	LeEIL1 in tomato Nr mutant	Functionally redundant, positive regulators of multiple ethylene receptors.	Overexpresession in ethylene insensitive non ripening Nr mutant of tomato	Partial restoration of ripening. Seedling triple response, upregulation of all ripening related genes were not restored.	LeEIL1 may not have expressed at sufficiently high levels to restore these functions,LeEIL2, LeEIL3 are also required.	Chen et al., 2004[175]
	LeEIN2	Positive mediator of ethylene signaling and fruit development	Suppression (VGIS)	Delayed fruit development and ripening with fewer seeds and locules, suppressed ethylene inducible and ripening related genes, partially blocked ripening with large green parthenocarpic sector on fruit	Probable involvement of LeEIN2 in a crosstalk between ethylene and auxin during fruit development	Zhu et al., 2006[174]
	LeETR4	Imparts ethylene in sensitivity, delay the onset of ripening until the seeds have matured.	Suppression	Increased ethylene sensitivity, fruits ripen prematurely flower senesce early without an increase in ethylene level. Severe epinastic curvature of petioles, no change in the levels of NR.	Negative regulator of the ethylene signaling pathway, lowering the receptor levels increases sensitivity to ethylene. An important regulator of ripening	Tieman et al., 2000[167]
	LeETR4 using immature fruit specific *Tfm7* promoter	Delays onset of ripening in immature fruits	RNAi mediated suppression	Early ripening, total yield, fruit size and flavour related chemical composition had no negative effect. Leaves and other tissues were unaffected.	Tissue specific approach to hasten fruit development without unwanted effects and enhanced ethylene response by ethylene receptor depletion.	Kevany et al., 2008[171]
	LeETR1	Functionally redundant	Antisense expression of receiver domain and the 3′ UTR of LeETR1	Delayed abscission and shorter internodes with no effect on ethylene sensitivity, normal triple response, and reduced auxin movement. No effect on NR transcripts.	Receiver domain of LeETR1 and LeETR2 has a role to play in signaling and can not be compensated by NR receptor	Whitelaw et al., 2002[176]
	etr1-1 (mutated ethylene receptors from Arabidopsis)	Imparts dominant ethylene insensitivity	Overexpression	Long thin hypocotyls, normal root growth, more open and expended apical regions. Fruits with delayed ripening and senescence similar to Nr mutants	Confers ethylene insensitivity in heterologous plants	Wilkinson et al., 1997[112]

Table 1. Transgenic crops with altered phenotype, senescence and ripening due to overexpression and suppression of ethylene receptor and downstream signaling molecules (continued)

Crop	Gene	Function of the Gene	Expression	Effect/Phenotype	Conclusion	References
	LeETR1 and LeETR2	Non redundant, plays role in ethylene perception	Antisense suppression	Shortened, thickened hypocotyls, enhanced abscission of petiole explants, faster senescence of flowers both in presence and absence of flowers.	LeETR1 might have a more specific role to play in signaling, each member has a specific role to play and are not totaly redundant.	Wang et al., 2003[170]
Carnation	*etr1-1* using CaMV 35S promoter	Imparts global ethylene insensitivity	overexpression	Diminished ethylene insensitivity and diseased resistance with delayed senescence. No petal inrolling, firm petals.	Heterologous expression imparts insensitivity in carnation	Bovy et al., 1999[79]
	etr1-1 using flower specific *fbp1* promoter	Imparts global ethylene insensitivity	overexpression	Delayed flower senescence with increased disease resistance. Ethylene sensitivity increased in other parts of flower apart from petals, better seed germination and reduced leaf abscission.	Flower specific promoter fixes the compromised disease resistance status	Bovy et al., 1999[79]
Petunia	*etr1-1/Nr*	Imparts global ethylene insensitivity	overexpression	Ethylene insensitivity with delayed flower senescence. Turgid corollas of flower, delayed abscission.	Cross species transfer	Wilkinson et al., 1997[112]
	etr1-1 using *flower binding protein* from Petunia (*fbp1*) or *apetala 3* promoter from *Arabidopsis*	Imparts global ethylene insensitivity	overexpression	Diminished disease resistance and reduced adventitious root hair formation, increased ethylene insensitivity with delayed senescence, seedling root hair formation observed. 73% of fbp1 and 32% of ap3 plants showed more than 100% increase in flower life.	Flower specific promoter enhances disease resistance in cross species transfer	Cobb et al., 2002[114]
	boers (mutated form of BOERS from *Brassica oleracea*)	Imparts ethylene insensitivity	overexpression	Plants retained turgidity and pigmentation longer, larger flowers, extended flower longevity, diminished disease resistance with higher mortality, increased ethylene evolution and reduced ethylene sensitivity, self incompatible.	boers is a dominant allele imparting insensitivity in the heterozygous transformed plants	Shaw et al., 2002[177]
	PhEIN2 (downstream signaling protein)	Involved in regulation of accumulation of EIN3 and EIL1 proteins. Positive regulators of ethylene cascade	Suppression by expressing sense RNA and RNAi mediated silencing	Transgenic lines using sense RNA expression showed moderate delay in flower senescence and fruit ripening with no premature death reported. RNAi construct suppressed lines showed great delay in flower senescence and fruit ripening with great deal of premature death. Corollas were turgid even after the ovaries were turgid.	Reduced or inhibited adventitious root formation (sense RNA expression) Reduced ethylene sensitivity (RNAi suppression)	Shibuya et al. 2004[119]
Coriander	*AtERS1* (mutated dominant receptor)	Confers ethylene insensitivity	overexpression	Delayed leaf senescence with prolonged total chlorophyll content in transgenic leaves, delayed flower senescence.	Delayed senescence is not due to drastic reduction in ethylene biosynthesis rather it is due to the effect of dominant negative ERS1 transgene. Distantly related crop species can have extended shelf life exploiting heterologus gene transfer.	Wang and Kumar 2004[91]

Table 1. Transgenic crops with altered phenotype, senescence and ripening due to overexpression and suppression of ethylene receptor and downstream signaling molecules (continued)

Crop	Gene	Function of the Gene	Expression	Effect/Phenotype	Conclusion	References
Campanula	*etr1-1* with fbp1	Imparts global ethylene insensitivity	Overexpressed	Enhanced tolerance to exogenous ethylene and reduced ethylene sensitivity, stably passed to next generation of plants, weak expression of etr1-1 in leaves, intact fertility and no compromise on disease resistance.	Sensitivity to ethylene in plants is independent of the copy number of the T-DNA.	Srikandarajah et al., 2007[57]
Nemesia	*CmETR1/H69A*, missensed mutated ethylene receptor from *Cucumis melo*	Imparts reduced ethylene sensitivity	Overexpression	Delayed flower senescence by 1–3 days and lesser root hair formation compared to non transgenic wild type.	Artificially mutated ethylene receptor gene conferred reduced ethylene sensitivity in heterologus plants	Cui et al., 2004[109]
Lettuce	*CmERS1/H70A*	Imparts stable sterlity, reduced fertility along with ethylene insensitivity	Overexpressed	Apart from reduced ethylene sensitivity, induced sterility in vegetatively propagated plants and pollen propagated plants	This mutated receptor gene checks the pollen dispersal from transgenic plants and prohibit unwanted development of traits in other plants and weeds, can induce sterility in heterologus transgenic plants.	Takada et al., 2007[140]
Chrysanthemum	*mDG-ERS1 (etr1-4)*, mutated nucleotide substitution corresponding to etr1-1.	Imparts ethylene insensitivity	Overexpressed	Delayed leaf yellowing on exposure to exogenous ethylene and leaves on lower part of stem remain attached to it without yellowing, reduced ethylene sensitivity, detached shoots in darkness without ethylene treatment showed reduced senescence.	Heterologus gene transformation remained unsuccessful; the mutated receptor could work for compositae family members.	Narumi et al., 2005b[85] Satoh et al., 2006, 2007, 2008[87,88,89]
	Cm-ETR1/H69A (missensed mutated ethylene receptor from *Cucumis melo*)	Imparts ethylene insensitivity, male sterility, prevents transgene flow via pollen	Overexpression	Drastic reduction in number of pollen grains at temp 20–35 °C but observed at 10-15 C. Due to suppression of *Cm-ETR1/H69A* expression at low temperature and optimal growth temp for plant at 15–20 °C. Female fertility was also reduced.	Optimal growth temperature for Chrysanthemum collides with its vegetative, reproductive growth resulting in mature pollens to flourish	Shinoyama et al., 2012[90]
Lotus	*Atetr1-1*	Mutated dominant ethylene insensitive ethylene receptor	Overexpression	Delayed abscission and senescence of petals, ethylene insensitive, twisted coiled hypocotyls, no triple response, fewer lateral roots, 7-fold increased nodulation in highly ethylene insensitive transformants, 1.7-fold increases in bacteroid infection.	Multiple roles of ethylene in nodule initiation by influencing root cell interactions and radial positioning, independent of autoregulation and nitrate inhibition of nodulation	Lohar et al., 2009[105]
	Cm-ERS1/H70A	Inhibits ethylene sensitivity, promotes root nodulation.	Overexpression	Confers ethylene insensitivity and fixes the transgene in T3 generation, reduced ethylene sensitivity due to 1-ACC resistance, increased flowering	Transgenic alteration of ethylene perception alters the rhizobial infection and nodulation phenotype.	Nukui et al., 2004[104]
Cucumber	*At-etr1-1* with CaMV 35S, AP3, CRC promoter	Imparts dominant ethylene insensitivity	Overexpression	Prevents carpel development resulting in male flowers with CaMV35S promoter. Increased femaleness and conversion of bisexual to female buds with CRC promoter. Exclusive male flower production with AP3 promoter.	Heterologus ethylene insensitivity. Ethylene perception is required to promote femaleness in melon. Dual role of ethylene in sex expression and carpel maturation. Site for ethylene perception lies in stamen primordia and not in carpel primordial.	Little et al., 2007[145]

ethylene production, induces autocatalytic ethylene production in petals, resulting in wilting of the petals.[61,62] Thus, carnation (*Dianthus caryophyllus* L.) flowers are considered to be an excellent model system for the study of ethylene perception and signaling. Three ethylene receptor genes have been isolated in carnation namely *DC-ERS1*,[63] *DC-ERS2*[64] and partial sequence of *DC-ETR1*.[65] The expression analysis revealed that only *DC-ERS2* and *DC-ETR1* are involved in flower opening but in a tissue specific manner as the mRNAs of both the genes are present in abundance in petals at the fully opened stage and level of both the mRNAs, *DC-ERS2* in particular declined as the senescence progresses. However, the level of *DC-ERS2* increased slightly in ovaries and that of *DC-ETR1* remained constant whereas, expression was observed to be constant during opening and senescence in the style.[66] Flowers treated with 1, 1-dimetly-4-(phenylsulfonyl)-semicarbazide (DPSS) are known to be blocked in ethylene production[67] and result in decreased expression of the genes in petals independent of ethylene production. In presence of exogenous ethylene, the expression stayed the same as it was under the influence of increase in endogenous ethylene production. The decrease in *DC-ERS2* mRNA in petals with the progress of senescence goes in accordance with the negative regulation model of ethylene signaling but further work needs to be done at the protein level to confirm this.[66] An EIN3-like protein (*DC-EIL1*), downstream to receptors was identified in carnation by Waki et al. (2001).[68] *DC-EIL1* transcript levels decreased in petals during natural senescence, marginal increase in *DC-EIL1* expression has been reported in ovaries as the senescence approaches, which otherwise was almost constant in styles and ovaries even during senescence. Exogenous ethylene and ABA treatment showed increased expression initially followed by a decline as the senescence progresses. However, small amount of RNA accumulated on ABA and ethylene treatment. Since the exogenous application of ethylene or ABA leads to declined *DC-EIL1* mRNA level and increased ethylene production during natural senescence demonstrated the same results. Therefore, it can be interpreted that *EIN3* level decreased with the increase in ethylene production, contradicting the findings of Chao et al. (1997)[69] and Kosugi and Ohashi (2000)[70] which states that *EIN3* and *TEIL* mRNA levels in Arabidopsis and tobacco respectively are not affected by ethylene. This indicates that there could be other senescence factors apart from ethylene resulting in decline of *DC-EIL1* expression. It is known that EIN3 and its homologs act as positive regulator of ethylene response, the *DC-EIL1* transcripts were therefore expected to increase or remain unchanged in the presence of ethylene. However, the declined *DC-EIL1* mRNA levels indicate the decrease in ethylene response of petal tissues, thus mitigating the ethylene response in senescing carnation petals in response to exogenous and natural ethylene produced. This contradicts the predicted enhanced ethylene response in the tissues due to decreased *DC-ERS2* and *DC-ETR1* expression in carnation.[66]

Overexpression of several EIN3/EIL members confers constitutive ethylene responses without significant changes in transcript levels suggesting that EIN3 may be regulated at the protein level[71] as shown by Guo and Ecker (2003);[72] Potuschak et al. (2003)[73] and Yanagisawa et al. (2003).[74] Apart from *DC-EIL1* three more EIN3-like (EIL) genes *DC-EIL1/2*, *DC-EIL3*, *DC-EIL4* were isolated, cloned and analyzed for their expression in vegetative and flower tissues (petals, ovaries and styles) during growth and development under natural and ethylene induced senescence in carnation (*Dianthus caryophyllus*) by Iordachescu and Verlinden (2005)[75] to get a better insight into ethylene responsiveness during flower development and senescence leading to ethylene climacteric. *DC-EIL3* mRNA levels in flower petals and style increased both under normal flower development and exogenous ethylene application. The EIN3 like proteins are also known to interact with ethylene responsive elements (EREs).[15] These observations suggest that DC-EIL3 may be playing a role in regulating gene expression, therefore regulating senescence. However, dip in *DC-EIL3* expression in wounded plant suggests the suppression of onset of senescence in leaves either by decreasing the tissue sensitivity to ethylene or activation of some defense mechanisms as observed in ovaries during senescence with a temporary decline in *DC-EIL3* mRNA status when higher ethylene production is reported in the flower (negative correlation of ethylene production and *DC-EIL3* mRNA accumulation). Thus, showing differential regulation of *DC-EIL3* in different parts of the flower. Another evidence of dynamism of *DC-EIL3* was supported by the unexpected maintained increase in its mRNA level in sucrose treated ovaries of the flower. Sucrose and other sugars are known to extend the vase life of flowers,[76] however sucrose induced the decline of ethylene responsiveness in carnation petals,[77] raising the expected possibility of delayed increase in *DC-EIL3* mRNA. Also the elevated levels of *DC-EIL1/2* and *DC-EIL4* mRNA in flowers treated with sucrose were in contradiction with depleted ethylene responsiveness, prompting tissue dependent and complex ethylene signaling.

Exploiting the fact that ethylene production shoots up post pollination in carnation flowers at different intervals of time,[78] an attempt was made to interpretate the regulation of *DC-EINs* with the ethylene production in flower. NBD (an ethylene inhibitor) treated pollinated flowers showed higher accumulation of *DC-EIL1/2* and *DC-EIL4* transcripts (at 12 and 48 h after pollination) which was expected to show lower *DC-EILs* mRNA levels. *DC-EIL3* transcript levels showed subtle differences between NBD non treated flowers and only pollinated flowers. It was therefore concluded that *EIN3* like genes and *DC-EIL3* gene in particular is involved in significant regulation by ethylene in initiating and sustaining of the senescence process in Carnation petals.[75]

Transgenic carnation plants were obtained by using etr1-1 allele driven by constitutive CaMV 35S or flower specific petunia fbp1 promoter,[79] CMB2 (Carnation MADS box) containing promoter.[80] Lower transformation efficiency along with diminished disease resistance and lower ethylene sensitivity[48,81]was observed using constitutive promoter compared with flower specific promoters, which took care of both the above stated drawbacks.[79,82] Symptoms like petal in rolling phenotype, hallmark of ethylene-dependent carnation flower senescence was not observed in transgenic carnation flowers. Instead, the petals remained firm throughout the senescence process and gradually became brown

on the edges (loosing color) and died from rotting.[79] Transgenic carnation cut flowers had three times the vase life of non transformed flowers and lasted longer than flowers treated with inhibitors of ethylene biosynthesis or ethylene antagonists.[79,80]

Catharanthus. The *ETR1* gene deciphered in *Catharanthus roseus* showed highest transcript levels in petals and ovaries. The expression level was almost unperturbed by ethefon treatment, under any abiotic stress or by other hormonal actions. However, the amount of monoterpene indole alkaloid Ajmalicine in the cells subjected to exogenous ethylene was reduced on being treated with inhibitors of HK. This prompted the involvement of this receptor protein in ethylene related enhancement of alkaloid biosynthesis in Catharanthus.[83]

Chrysanthemum. Chrysanthemum is an ornamental plant known to be insensitive to ethylene. However, some cultivars of Chrysanthemum are ethylene sensitive. Narumi et al. (2005a)[84] observed that in an ethylene insensitive cultivar of Chrysanthemum the *DG-ERS1* transcript level was highest and declined consistently thereafter. The same was not observed in an ethylene-insensitive cultivar, though. The observed difference between the two cultivars is probably related to the variation in sensitivity to ethylene between them and this suggests that the *DG-ERS1* gene and its resultant DG-ERS1 protein may be involved in the perception of ethylene signal in flower and leaf tissues of the cut Chrysanthemum.

DG-ERS1 gene was subjected to one nucleotide substitutions corresponding to *Arabidopsis etr1-1, etr1-2, etr1-3, etr1-4* and tomato *Nr*. These mutated *DG-ERS1* receptors were transformed into Chrysanthemum (ethylene-sensitive cultivar) to attain reduced ethylene sensitivity. Failure with heterologous transgene expression in Chrysanthemum, led to this idea of mutating the Chrysanthemum ethylene receptor for imparting reduced ethylene sensitivity. Out of the five *DG-ERS1* transgenes tested, *DG-ERS1* (*etr1-4*) emerged the best resulting in 37% reduced ethylene sensitive transgenic Chrysanthemum leaves.[85] Sumitomo et al. (2008)[86] used the transgenic Chrysanthemum expressing a mutated ethylene receptor genes, *DG-ERS1* (*etr1-4*) and *DG-ERS1(Nr)* with reduced ethylene sensitivity to assess the involvement of ethylene response pathway in temperature induced dormancy of Chrysanthemum. Satoh et al. (2008)[87] evaluated ethylene sensitivity of the transgenic grown in soil. They observed delayed leaf yellowing in shoots after being detached from soil grown plants on ethylene treatment under continuous light. Also, these shoots when kept in darkness without ethylene treatment resulted in reduced senescence. Natural senescence in shoots was also analyzed from soil grown plants by Satoh et al. (2006, 2007).[88,89] All the observations and failure of heterologus transformation in Chrysanthemum suggests that the mutated ethylene receptor *DG-ERS1 (etr1-4)* could be used to combat senescence in composite leafy vegetables plants, which cannot be stored for a long time at cool temperatures because of cold injury to plants.

Transgene flow from transgenic plants to wild types (horizontal gene transfer) has always been a concern. To tackle the problem male sterility needs to be introduced to prevent transgene flow via pollen. Shinoyama et al. (2012)[90] overexpressed the mutated ethylene receptor gene from melon (CmETR1/H69A) into Chrysanthemum which resulted in delayed tapetum degradation of anther sac and reduced pollen grain formation. However, the concurrence of healthy growth pattern and flourished pollen formation at a temperature of 15–20°C was a hindrance to put a check on pollens from genetically modified chrysanthemum to mingle with its wild type counterparts.

Coriander. Delaying senescence in coriander leaf and flower using a dominant negative mutant *ERS1* of Arabidopsis is yet another attempt of heterologous expression. Expression analysis of ACO and *ERS1* homologs in both transgenic and wild-type plants revealed subtle downregulation of ACO in transgenic plants compared with wild type. The marginal downregulation of ACO was attributed to the failure of feedback control mechanism of ethylene synthesis by heterologous dominant negative receptor. Thus, the delay in senescence in transgenic plant resulted due to the effect of dominant negative *ERS1* and not due to reduction of ethylene biosynthesis. Also *ERS1* homolog in coriander was not altered significantly indicating no co-suppression by the transgene to confer its function.[91]

Delphinium. Delphinium flowers show a climacteric-like rise in ethylene production before abscission of sepals. These are abscised by exposure to exogenous ethylene.[92] Two ethylene receptor genes in Delphinium have been isolated namely *Dl-ERS1* type1 and *Dl-ERS1* type 2 differing by three amino acids.[93] Two more cDNA encoding ethylene receptors *Dl-ERS1–3* and *Dl-ERS2* were isolated from Delphinium flowers by Tanase and Ichimura, (2006).[94] However, the most common ethylene receptor *ETR1* seems to be missing from Delphinium. The flower parts showed higher *Dl-ERS1-3* transcript levels than leaves and stems whereas, *Dl-ERS2* transcript levels in leaves and flowers were found to be equal but greater than that of stem. These observations suggest differential regulation of receptor genes (*Dl-ERS1-3* and *Dl-ERS2*) and their involvement in ethylene signaling of Delphinium flower senescence.

Considering the fact that ethylene receptors are not specifically expressed in the abscission zone,[95] it was concluded by Tanase and Ichimura, (2006)[94] that *Dl-ERS1-3* and *Dl-ERS2* are not expected to determine the ethylene response of the abscission zone. Therefore, ethylene induced sepal abscission is controlled both by ethylene signaling components and *Dl-ERS1-3* and *Dl-ERS2* proteins.

Kuroda et al. (2003)[93] also concluded that *Dl-ESR1* expression is more or less proportional to the endogenous ethylene production implying that ethylene evolved by the florets is perceived by elevated levels of *Dl-ERS1* leading to flower senescence.

Dendrobium. Dendrobium "Pompadour", one of the most important commercial orchids in Thailand is an ethylene sensitive variety and has relatively short vase life. A subfamily one ethylene receptor, ERS1, lacking a receiver domain was found in Dendrobium with a single copy in the genome by Thongkum et al. (2009).[96] However, the possibility of presence of anther ethylene receptor gene cannot be ruled out.

Transcript analysis of *Den-ERS1* was also performed in various vegetative tissues like psuedobulb, young and mature leaves, root, peduncle, bud and pen floret. The transcript expression remains

unperturbed with the development of leaves. During the flower development the expression of Den-ERS1 declined as the senescence progressed and the ethylene production was enhanced suggesting the receptors to be negative regulators of ethylene signaling during natural course of senescence.[96]

Geranium. Two ethylene receptors, *PhETR1* and *PhETR2* are deduced till date, though there is scope for more receptors to be decoded.[97] They have also been characterized for their distribution, temporal and spatial regulation in Geranium (*Pelargonium x hortorum* L.H. Bailey). The rapid nature of ethylene response was figured out by Clark et al. (1997)[98] by demonstrating that during the receptive stage of pollination, Geranium florets were observed to undergo complete abscission even in the turgid petals when treated with very low concentrations of exogenous ethylene.[98] Self pollination of Geranium flower causes rapid burst of ethylene production by the gynoecium within 1 h, which leads to petal abscission in 2–4 h. Enhanced sensitivity of Geranium florets to exogenous ethylene at the pollination receptive stage was also reported by Evensen et al. (1991).[99]

PhETR expression analysis revealed that it was expressed least in roots and maximum in pistils. Considering these observations, hypothesis put forth by Dervinis et al. (2000)[97] cannot be ruled out, which states that the control of ethylene sensitivity in Geranium florets may not be determined by the amount of receptor protein present justifying the repression of ethylene signal transduction pathway by the receptors unless ethylene binds the receptors. Abundant expression of ethylene receptor prior to ethylene production might be a factor responsible for allowing non-autocatalytic plants like Geranium to respond quickly to the ethylene generated after pollination.[97] These observations raise a possibility of overlapping functions and genetic redundancy adopted by *PhETR* genes.

Gladiolus. Gladiolus is an ethylene-insensitive flower as exogenous ethylene and ethylene inhibitors have no effect on the petal senescence.[53] Two ethylene receptor paralogous genes, *GgERS1a* and *GgERS1b,* have been isolated from *Gladiolus grandiflora* hort, an ethylene-insensitive flower.[100] The cDNA sequence of the two genes is almost identical except that *GgERS1 b* lacks 636 nucleotides, encoding first and second in the HK motifs present in *GgERS1a.* The analysis of the genomic DNA sequences (4,776-bp nucleotide designated as *GgERS1* long DNA and 3,956-bp nucleotide designated as *GgERS1* short DNA) revealed that both sequences were identical except that *GgERS1* short DNA was devoid of an 820 bp long nucleotide segment in the first intron of *GgERS1* long DNA. These data suggested that each of the *GgERS1* genes was generated by duplication and splicing from different genomic DNA. The *GgERS1b* mRNA level decreased in petals during flower development, whereas the expression of *GgERS1a* mRNA was constitutive, however, with a high accumulation level, suggesting that high expression level of *GgERS1a* conferred the ethylene insensitive nature in petals of Gladiolus. On the other hand, the sensitivity to ethylene might be regulated by *GgERS1b* expression.

Kalanchoe. *Kalanchoe blossfeldiana* was transformed with the mutant ethylene receptor gene *etr1-1* from Arabidopsis under the influence of the flower specific fbp1 promoter from Petunia. The transgenic flowers were reported with reduced ethylene sensitivity and increased flower longevity. However, one of the transgenic lines was found to be infertile and only slightly less ethylene sensitive. Out of the three transgenic lines containing *etr1-1*, one ethylene resistant, fertile line was crossed with ethylene sensitive cultivar to create T1 progeny. The ratio of the ethylene resistant to ethylene sensitive plants was 3:1. The *etr1-1* gene was found to be expressed only in petals and stamens but not in carpels and sepals, as expected from fbp1 promoter.[101]

Lotus. *Lotus japonicus* B-129 "Gifu"[102] was transformed with *Cucumis melo* ethylene receptor gene *ERS1* (*Cm-ERS1*) point mutated at 70th amino acid (histidine to alanine) designated as *Cm-ERS1/H70A*[103] under Cauliflower mosaic virus 35S (CaMV-35S) constitutive promoter and the transgenic lines designated as *Lj-H70A* showed inhibitory ethylene mechanism and nodulation along with reduced ethylene sensitivity.[104] Parallel work in Lotus using a well characterized two component histidine kinase type *Atetr1-1* allele was done by Lohar et al. (2009).[105] A gradient of ethylene insensitivity was observed in transgenic Lotus plants from hyper insensitive to hypo insensitive. The transgenic plants were insensitive to both endogenous and exogenous ethylene and formed more nodule loci compared with wild type plants. Nodulation varied in transgenic lines as per the insensitivity gradient. The more ethylene insensitive the line was, the more the nodulation had occurred. Some prominent work involving the effect of ethylene perception on nodulation has been contributed by other groups as well.[106-108]

Nemesia. *Nemesia strumosa*, an ethylene-sensitive ornamental plant belongs to Scrophalariaceae, the figwort family. On the lines of mutated *Arabidopsis etr1-1* gene, the missense mutation His-69 to Ala (*H69A*) was introduced into *Cm-ETR1* to create the mutant gene *Cm-ETRI/H69A.* Reduced ethylene sensitivity and flower longevity in transgenic plants of Nemesia was demonstrated by introducing this mutated melon ethylene receptor gene *Cm-ETR1/H69A.*[109,110] The *Cm-ETR1/H69A* expression resulted in longer vase life by 1–3 days due to inhibited ethylene response. The root hair formation in transgenic lines was also reduced to a good extent as compared to wild-type non transgenic plants.

Oncidium. The controversy of Oncidium being ethylene sensitive or insensitive was put to rest by Huang, (1998)[111] and it is now known to be a climacteric crop. Having known the problem, Huang et al. (2007)[51] reported an ethylene receptor in Oncidium, *OgERS1* and examined its expression during the flowering process in response to exogenous ethylene and pollinia cap dislodgment. *OgERS1* mRNA levels were piled up in flowers at bud stage and at lower levels with concomitant increase in ethylene production in fully bloomed flowers. However, the expression was always on the rise in the roots signifying, a differential sensitivity acquired by roots for its development. Comparative expression analysis in dislodged and un-dislodged pollinia caps revealed that the expression of *OgERS1* was on decline throughout the course of senescence in dislodged flowers compared with intact capped flowers. Exogenous ethylene application had even more profound effect within an hour and it lasted for 36 h. They also concluded that petal senescence in Oncidium is anchored to *OgERS1* expression. On being exposed

to exogenous ethylene, the *OgERS1* expression upsurge with early advent of senescence.

Petunia. Tomato and Petunia plants transformed with the Arabidopsis mutated dominant ethylene insensitive *etr1-1* cDNA showed that cross species transfer of an ethylene insensitivity phenotype is possible.[112] The creation of ethylene-insensitive tomato and Petunia plants via the introduction of dominant Arabidopsis ethylene receptor alleles depicts the functional conservation for this component of ethylene signaling.[113] This strategy of developing plants without ethylene perception however, does not lead to a normal development of plant. Therefore, in an attempt to improvise the previous work Cobb et al. (2002)[114] transformed Petunia with *etr1-1* under the influence of floral specific promoters FBP1 (floral binding protein) and AP3 (involved in floral organ development) resulted in increased vase life of transgenic flowers up to 5 times compared with control flowers.

Mutated version of BOERS, an Ethylene Response *S*ensor (ERS) gene of broccoli, *Brassica oleracea*, *boers*[115] conferred ethylene insensitivity (because of non functional sensor domain, not able to bind ethylene) when transformed into *petunia x hybrida* Hort. Vilm.-Andr. Transformed petunia flowers retained pigmentation longer and showed longer life span irrespective of either its storage is in water or exposed to exogenous ethylene. These observations were consistent with those of Bovy et al. (1999)[79] and Wilkinson et al. (1997),[112] depicting successful cross species transfer of ethylene receptor gene. The delay in senescence observed in transgenic etr1-1 flowers was longer than in flowers pretreated with chemicals that inhibit either ethylene biosynthesis (amino-oxyacetic acid) or the ethylene response (silver thiosulfate) and blocked ethylene biosynthesis by ACO1 gene co-suppression.[79] The possible reason for this difference might be that ACO1 co-suppressed flowers are still sensitive to basal levels of ethylene, either produced endogenously or by flowers and fruits in the vicinity, whereas etr1-1 transformed flowers are not. This observation is also of great importance to non-climacteric flowers and fruits that can be damaged by ethylene produced in the surrounding. However, the disease resistance was compromised as the plant became more susceptible to fungal disease. Earlier, Clark et al. (1999);[116] Gubrium et al. (2000)[117] and Clevenger et al. (2004)[118] reported reduced adventitious root formation, enhanced ethylene production in pollinated flowers, slightly faster flowering along with delayed senescence in ethylene-insensitive transgenic petunia.

Shibuya et al. (2004)[119] figured out the central role of signaling molecule *EIN2* in petunia plant development. Transgenic petunia plants with reduced *PhEIN2* expression were produced using co suppression (expressing PhEIN2 sense RNA) and RNA-interference (PhEIN2-RNAi) approach. Out of 68 lines produced, two homozygous lines, EIN2s-182 and EIN2r-12, exhibiting the greatest flower longevity after ethylene treatment and pollination in T1 generation were picked from PhEIN-sense and PhEIN2-RNAi lines, respectively. RNAi mediated gene silencing was more efficient than co suppressed lines. These transgenic plants were compared with wild-type Petunia x *hybrida* cv Mitchell Diploid (MD) and two ethylene insensitive petunia plants transformed with *Atetr1* namely etr-44568, (Wilkinson et al., 1997)[112] and etr-56 (Clevenger, 2004).[118] EIN2s-182 and etr-56 showed moderate delay in fruit ripening and flower senescence with no premature death of flowers and fruits compared with EIN2r-12 and etr-44568. Langston et al. (2005)[120] showed that there was delayed induction of senescence specific nuclease and DNA fragmentation ethylene insensitive 35S::etr1–1 transgenic petunia.

Rose. *Rosa hybrida* should have shown decreased expression of ethylene receptors and *CTR1* undergoing senescence in compliance with the negative regulation model of ethylene signaling. However, study on rose tells a different story, *CTR1, CTR2* (*CTR1* homolog) were constitutively expressed during senescence and increased in response to exogenous ethylene. Also, *RhETR3* (one of the four ethylene receptors in rose) expression increased in senescing flowers. *RhEIN3,* a positive regulator of ethylene signaling was also constitutively expressed throughout the process under the influence of both exogenous ethylene and ABA.[121,122] Constitutive expression of *Rh-EIN3-1* and *Rh-EIN3-2* in cut rose is consistent with previous reports in Arabidopsis,[69] tomato,[123] or miniature potted roses,[124] where the expression of *EIN3* remain unchanged even on being exposed to ethylene. A step further, Muller et al. (2000a)[125] demonstrated the cultivar difference in ethylene receptor levels during flower development. The group also demonstrated the ethylene treatment mediated flower sensitivity to ethylene by *RhETR1* regulation. Though the exact mechanism by which ethylene sensitivity is regulated by ethylene receptor dynamism is still not understood fully. An effort has been made to unravel the mechanism to an extent by receptor expression analysis in rose. Higher expression of *RhETR1* was observed in cultivar "Bronze" (having shorter flower life) as compared with "Vanilla" (longer vase life). Maximum expression of *RhETR1* has been observed in the bud and young flower stage both in bronze and vanilla cultivar respectively. As far as the *RhETR3* expression levels were concerned, in "Bronze" the transcript level increased as the senescence approached however, in "Vanilla" flowers it was constitutively expressed at very low levels. Constitutive expression of *RhETR2*, was observed during senescence although the transcript level was different for the two cultivars. Early expression of *RhETR1* i.e., before the ethylene production, along with the increase in *RhETR3* expression in senescencing flowers of "Bronze" prompts that ethylene response system in rose is mediated via overlapped expression of the multiple receptors. This also explains the reason for the multiplicity of ethylene receptors to some extent. Thus, it can be concluded that RhETR1 and RhETR3 are rate limiting for ethylene perception and determinant for flower longevity. These observations also justify Lashbrook et al. (1998)[126] theory which states that increase in ethylene sensitivity is manifested by increase in receptor abundance.

Ma and coworkers (2003)[127] explained ethylene's regulatory role in flower opening, effect of ethylene on flower pre-pollination opening and identified key regulatory components in ethylene biosynthesis and signaling pathways in cut roses. Exogenous ethylene promoted ethylene production in petals, but 1-MCP did not inhibit ethylene biosynthesis. Moreover decrease in

ethylene production was also not observed. This confirmed that ethylene regulates flower opening of roses through signaling pathway and not through biosynthetic pathway. It was also established that continuous ethylene perception is required for flowers to open without the feedback regulation of ethylene biosynthesis, in accordance with Wang and Woodson's (1989)[62] theory of flower opening.

Xue et al. (2008)[128] showed that ethylene enhanced both ethylene production and the expression of *Rh-ACS2* or *Rh-ACS3* in gynoecia, petals, and receptacles, with gynoecia showing the earliest ethylene enhancement. However, 1-MCP did not suppress ethylene production and the expression of the ethylene biosynthetic genes in these three tissues. In stamens, no obvious changes were found in ethylene biosynthesis either by ethylene or 1-MCP treatment. This suggests that gynoecia are the most sensitive tissue to ethylene treatment, and none of the five floral tissues exhibits positive feedback regulation of ethylene biosynthesis. Among the five floral tissues studied, ethylene-induced expression of *Rh-ETR* genes occurred first in gynoecia, and only the expression of the *Rh-ETR3* gene was regulated in a positive feedback manner by ethylene and 1-MCP. These results suggest that transcriptional regulation of the *Rh-ETR3* gene in gynoecia may play an important role in ethylene-enhanced flower opening. These results differ from the findings in carnation, geranium, and Delphinium.

Out of seven signaling component genes studied in rose, including three ethylene receptors (*Rh-ETR1, Rh-ETR3* and *Rh-ETR5*), two *CTR*s (*Rh-CTR1* and *Rh-CTR2*), and two transcription factors (*Rh-EIN3–1* and *Rh-EIN3-2*), transcripts of *Rh-ETR5*, *Rh-EIN3–1* and *Rh-EIN3-2* were accumulated in a constitutive manner and had no or little response to ethylene or 1-MCP, while transcript levels of *Rh-ETR1* and *Rh-CTR1* were substantially elevated by ethylene and those of *Rh-ETR3* and *Rh-CTR2* were greatly enhanced by ethylene. 1-MCP reduced all the four genes to levels much less than those in control flowers. These results show that ethylene triggers physiological responses related to flower opening in cut rose cv Samantha, and that continued ethylene perception results in flower opening. Ethylene may regulate flower opening mainly through expression of two ethylene receptor genes (*Rh-ETR1* and *Rh-ETR3*) and two CTR (*Rh-CTR1* and *Rh-CTR2*) genes.[127]

Ethylene Perception and Receptor Expression in Fruit Crops

Apple. In apple (*Malus domestica),* the expression of ethylene has been observed during fruitlet abscission and early development.[129] The expression of *MdETR1* and *MdERS1* is found to be tissue specific. Although the expression of *MdETR1* is at steady-state but maximum expression has been observed in the peduncle, abscission zone and in seed, than in the cortex of early developing fruits. A possible role of *MdERS1* receptor in abscission is suggested. The expression of gene encoding for specific cell wall hydrolases is regulated by increase in ethylene evolution at abscission zone which results in abscission zone cell separation and fruitlet shedding. Wiersma et al. (2007)[130] has identified two new ethylene receptors *ETR2* and *ETR5* along with ethylene control element in Summer Apple, "Sunrise" (SR) and late season "Golden Delicious" (GD). For analyzing quantitative expression of four ethylene receptors (*ETR1, ETR2, ETR5* and *ERS1*) and *CTR1, EIN2* only minor changes in expression were observed in two apple cultivars. The expression of *ERS1*was in accordance with feedback inhibition model of ripening fruit in GD fruit, however in SR fruit such expressions was not observed. With a difference in both the cultivars a very small expression of *CTR1* was observed at maturity .A decline in expression of *CTR1* in GD was due to increase in ethylene sensitivity in mature tissues. During various stages very little variation was observed in Apple *EIN2*.[131]

Avocado. In avocado *(Persea americana)* the activity of *PA-ERS1* mRNA increased gradually until the climacteric peak where, it is hyper induced. The stimulated induction of *PA-ERS1* at climacteric peak may be due to suppression by 1-MCP; this may be mechanism adapted by avocado fruit to dissipate high levels of autocatalyic ethylene being produced by the plant.[132]

Citrus. Although Citrus (*Citrus sinensis)* fruit is known to be nonclimacteric, exogenous ethylene is able to affect its ripening in. It accelerates respiration, biosynthesis of carotenoids, degradation of chlorophyll and induction of pigment change of peel in citrus. The expression of *CsERS1* has been detected immediately after harvest in young Valencia fruitlets which is induced further in subsequent days.[133] The expression of *CsERS1* has been found to be ethylene independent in young fruitlets. In mature fruit, 1-MCP or propylene treatments has no affect on expression of both *CsERS1* and *CsETR1* genes. *CsERS1*expression modulates the differential sensitivity to ethylene in mature and young fruitlets.

Cucumber. Three ethylene receptor genes *CSETR1, CSETR2* and *CSERS* were isolated from *Cucumis sativus* and the expression of these genes was analyzed to decipher the role of ethylene in inducing femaleness in cucumber. A correlation between ethylene production and ethylene receptor genes was also established. The expression of *CSETR2* and *CSERS* was more profound in gynoecious cucumber as compared to monoecious one. This expression deepened even further in presence of ethrel and declined in presence of AVG (ethylene biosynthesis inhibitor). This observation was complimented by higher ethylene production and better expression of ethylene biosynthetic gene *CSACS2* in gynoecious cucumber than in monoecious.[134]

Cucurbita pepo. To study the mechanism by which ethylene metamorphoses the sex expression and development, two *CTR1* homologs were identified and the transcript levels of the same were also studied during the male and female flower development and when exposed to external ethylene.[135] During development of male and female flowers, the expression of both the CTR1 homologs increased along with ethylene production in flowers indicating that both the genes are upregulated by ethylene in flowers but not in seedlings and leaves. Exogenous ethylene also did not have any impact on the expression of CTR1s in seedlings and leaves but, upregulated the expression in flowers. During earlier days of male flower development higher expression of both the CTR1s was observed when lesser ethylene was

produced within the flowers, indicating lower ethylene sensitivity in male flowers as compared with female flowers. Role of ethylene in imparting femaleness in Cucurbitaceae is well known. This member of the family also abides by the same principle. Manzano et al. (2010)[135] showed that a particular inbred line of *Cucurbita pepo*, vegetable spaghetti, exhibited extreme male phenotype which can be attributed to either mutation in a receptor or response of gene for ethylene which resulted in weaker sensitivity to ethylene, evident from weaker triple response. The more sensitive F2 plants showed increased femaleness.

Grapes. Although grapes (*Vitis vinifera*) are regarded as a non-climacteric fruit 1-MCP has shown to alter the berry ripening process. There are four ethylene receptor genes known in grapes namely *VvETR1*, *VvETR1*, *VvERS1* and *VvEIN4*. Other orthologs of ethylene signaling cascade identified in grapes are *VvRTE1*, *VvCTR1* and *VvEIN3*. Exogenous ethylene application resulted in increased transcript accumulation of *VvETR2* and *VvCTR1*. Ethylene antagonist 1-MCP application on the other hand resulted in increased transcript levels of *VvEIN3* after 9 weeks and VvRTE1 after 7 weeks. However, no significant change was observed in *VvEIN4* transcript accumulation. These responses by cascade molecules against ethylene and its antagonist in grapes also existed in climacteric crop like tomato and in Arabidopsis. Taken together, these observations prompt the prevalence of a common regulatory pathway in climacteric and non-climacteric fruit.[136]

Kiwifruit. Kiwifruit (*Actinidia deliciosa*) is a typical climacteric fruit, extremely sensitive to ethylene. Yin et al. (2008)[137] showed the expression profile of eight signaling molecules in Actinidia, including five ethylene receptors namely *AdERS1a, AdERS1b, AdETR1, AdETR2, AdETR3,* two CTR1 like genes *AdCTR1, AdCTR2* and an EIN3 like gene *AdEIL1*. None of the gene was found to be fruit specific and all were differentially expressed among various kiwifruit tissues. Like in tomato, no ERS2-type receptor is reported in kiwifruit. Except, *AdERS1a* all the receptor genes were expressed in good amounts in roots, stems and fruits implying that *AdERS1a* has a precise role to play in flower development. Strong expression of *AdERS1a* and *AdETR3* in the early developmental stage, when substantial cell division happens is suggestive of ethylene involvement at this growth stage. However, *AdERS1b* and *AdETR2* were expressed after full bloom prompting the association of these genes with ethylene involvement in cell expansion and development. *AdERS1b* exhibited no ethylene response but its expression was explicitly associated with fruit ripening. *AdERS1a*, *AdETR2* and *AdETR3* showed the most promising expression during fruit ripening on exposure to ethylene. Authors have speculated *AdETR2* to play a role in senescence process rather than its involvement in early ripening event. In the light of the negative regulation model of ethylene receptors, the *AdETR1* downregulation during ripening and on exposure to ethylene was justified as making the signaling pathway active and more responsive or it may be because of the degradation of receptor protein on exposure to ethylene. This suggests, less receptor protein cause de-repression of the pathway. Considering the fact that upregulated receptor proteins have better ethylene affinity for ripening to proceed, the upregulated receptors *AdERS1a*, *AdETR3* are perhaps important in activating signaling pathway on ethylene binding and *AdETR1* may act as a principal suppressor of the pathway. *AdCTR1* and *AdCTR2* showed low levels of transcriptional response to ethylene and *AdEIL1* showed no response to ethylene.

Lettuce. After the success of Cm-ERS1/H70A missensed mutated melon receptor gene in inducing stable sterility in transgenic tobacco plants.[110,138,139] Takada et al. (2007)[140] exploited this gene to make transgenic lettuce *(Lactuca sativa)*. This heterologus transformation effectively induced stable sterility like in tobacco. However, the only pleiotropic phenotype observed in lettuce was reduced pollen production. Reduced ethylene sensitivity imparted by this gene has already been reported in *Nemesia strumosa*, *Lotus japonicus* and *Nicotiana tabacum*. Cm-ERS1/H70A acts as an effective tool in the armoury of a genetic engineer to impart sterility in vegetatively propagated plants and if used with an anther/pollen specific promoter can serve the purpose in seed propagated plants.[138] This system demonstrated how a mutated ethylene receptor could contribute in checking the pollen dispersal from transgenic plants.

Mango. Mango (*Mangifera indica*) is an important tropical fruit crop. There are two ethylene receptor genes known in mango namely *MiETR1*[141] and recently discovered *MiERS1*.[142] The *MiETR1* expression increased during ripening and wounding. However, in the abscission zone, the *MiETR1* transcript levels remained unchanged and *MiERS1* expression increased. During fruit ripening, *MiERS1* expression was almost negligible compared with *MiETR1* which increased both in mesocarp and seed tissue.[142] The comparative expression profiling of the two genes suggest that the *ERS1* counterpart might have a role to play in regulation of abscission and *ETR1* in fruit ripening with a possibility of more members to be added in the family to have a better picture.

Melon. Due to distinct anatomical, reproductive and developmental features, the melon fruit developmental stages are categorized into ethylene sensitive and ethylene insensitive stage with the developing fruit having lower affinity to ethylene than the ripening fruit.[143,144] During fruit ripening in muskmelon, *Cm-ERS1* transcript level increased slightly in the pericarp of fruit. Also there was a concomitant increase in Cm-*ETR1* transcripts along with climacteric ethylene production. Elevated levels of *Cm-ERS1* transcript compared with *Cm-ETR1* mRNA at low ethylene levels and ethylene production suggests *Cm-ERS1* to be more sensitive to even lower levels of ethylene, whereas *Cm-ETR1* is thought to be responsive to high level of ethylene.[103] Studies performed to examine the temporal and spatial expression pattern of Cm-ERS1 protein, during fruit development, revealed that a posttranscriptional regulation of *Cm-ERS1* expression affects stage and tissue-specific accumulation of the protein.[144] The melon subfamily II ethylene receptor, *Cm-ETR2* mRNA, exhibits earlier accumulation compared with *Cm-ETR1* during ripening, and its transcript accumulation increased during melon ripening, and declined in parallel with a reduction in ethylene production.

Sex determination is another repercussion of altered ethylene perception in melon as reported by Little et al. (2007).[145] The

transgenic melon with *At-etr1-1* gene resulting in reduced ethylene sensitivity showed change in sex expression. The expression of *etr1-1* was studied under the influence of a constitutive promoter, CaMV35S and tissue specific inflorescence targeted Apetela3 (AP3) and Crab's Claw (CRC) promoters. The 35s:: *etr1-1* melons showed inhibition of carpel bearing and bud production suggesting involvement of ethylene perception in determination of sex. However, AP3 promoter resulted in increased maleness, poor carpel development and hardly any production of bisexual flowers. On the other hand with CRC promoter, predominant femaleness with increased number of carpel bearing buds was observed. This suggested that ethylene perception by stamen has a significant role to play during sex determination period. However, the hormone perception by carpel is important for maturation of carpel-bearing flowers to anthesis.

Significance of endogenous ethylene production for pistillate flower development has been demonstrated in melon by Papadopoulou et al. (2005).[146] Constitutive overexpression of ethylene biosynthetic gene, ACS resulted in enhanced endogenous ethylene accelerating bisexual flower initiation, development of mature bisexual flowers and fruit set in melon.

Passion fruit. During ripening in *Passiflora edulis* no significant change was observed in the expression of two ethylene receptors, *PeETR1* and *PeERS1*. However, high levels of *PeETR1* and *PeERS1* mRNA were detected in arils than in seeds. *PeERS2* transcript levels were elevated in general but a heightened level it was noticed in particular in the arils of ripened fruit. *PeERS2* mRNA is speculated to be involved in repressing ethylene response; this can be inferred when mature green fruits are exposed to ethylene.[147]

Peach. The expression of *Pp-ETR1* peach *(Prunus persica)* appeared to be upregulated by propylene treatment. In peach its expression appeared to be constitutive and ethylene independent during fruit development and ripening.[148] It was seen that application of 1-MCP delays fruit ripening, evolution of ethylene and also downregulates the activity of *Pp-ERS1*. Its activity was abolished on keeping the fruits in air and rapid activity of *Pp-ERS1* mRNAs stimulated ethylene evolution.

Pear. A gradual increase in ethylene production and expression of ethylene receptor mRNA was observed during cold treatment of late season pear *(Pyruscommunis* cv Passe-Crassane) by (El-Sharkawy et al., 2003).[149] Cold treatment upregulated the expression of *Pc-ETR1* mRNA during ripening, whereas the expression of *Pc-ETR1* and *Pc-ETR5* were found to be less affected by cold treatment. During ripening high accumulation of Pc-*ETR1* and *Pc-ERS1* mRNA was observed in the early-season pear cultivar as compared to the gradual increase seen in late-season pear cultivar, Passe-Crassane. A stark difference in accumulation of *Pc-ETR5* transcript was observed both during early-season cultivar and late season cultivar PC for example: in early season pear fruit the level of *Pc-ETR5*transcript reduced sharply before and during the ethylene climacteric whereas it sharply increased in late-season cultivar. Increased ethylene sensitivity during the early ripening phase of early fruit development can be due to decrease in expression of negative regulator.

Persimmon. The expression of three ethylene receptor genes *DkERS1, DkETR1* and *DkETR2* has been determined during the ripening of persimmon *(Diospyros kaki)* fruit.[150] A correlation was found between the levels of *DkERS1*, *DkETR1* mRNA and ethylene production during fruit development and ripening. The expression of *DkETR1* mRNA was found to be ethylene independent and is expressed during all the stages of fruit ripening. Decreased level of DkERS1 protein prior to fruit maturation suggests its involvement in ethylene perception during fruit ripening.

Plum. Japanese plum (*Prunus salicina* L.) falls in the category of climacteric fruit. Unlike other climacteric fruits, stone fruit plum, exhibit a double sigmoid growth pattern during fruit development stages (S1–S4). Two Japanese plum cultivars "Early Golden" ("EG") with normal climacteric pattern and "Shiro" ("SH") with suppressed climacteric pattern were chosen by El-Sharkawy et al. (2007, 2009)[151,152] for isolation and molecular characterization of 11 ethylene signaling elements, which includes two receptors (ETR1 like proteins, Ps-ETR1, Ps-ERS1), one CTR1 like protein (Ps-CTR1), an ethylene responsive element binding factor (*Ps-ERF*). Ps-ETR1 and *Ps-ERF1* were absent in the late cultivar 'SH'. Ps-ERS1 lacks the C-terminal receiver domain, which was present in *Ps-ETR1*. The predicted Ps-CTR1 protein contained both ATP binding site and Ser/Thr kinase activity. The receptors' expression was upregulated post fertilization and then subsided. During S1 and S2 stages all the four molecules were downregulated in an ethylene independent manner. However, *Ps-ETR1* and *Ps-CTR1* transcripts were accumulated during S3 stage in an ethylene independent manner. The threshold level requirement of ethylene in late cultivar like "Shiro" ("SH") and once this requirement is fulfilled, the developmental changes are accelerated. The transcript levels of *Ps-ETR1* and *Ps-CTR1* increased sharply during climacteric, S4 stage. But it was higher in 'EG' cultivar as compared with "SH". *Ps-ETR1* was constitutively expressed during development with further increased expression during fruit ripening. *Ps-ERS1* and *Ps-ERF1* transcript level increased sharply with the climacteric peak of "EG" fruit in an ethylene dependent manner. However, in "SH" cultivar the expression was constitutive and low. A positive correlation was established between the *Ps-ERS1* and *Ps-ERF1* expression.[151] As many as seven Ps-ERFs were characterized during fruit development and ripening.

Strawberry. When compared with climacteric fruits, there is no other growth regulator corresponding to the role played by ethylene in nonclimacteric fruits. Dearth of literature regarding the role of ethylene receptors in nonclimacteric fruits and an observation that they synthesize ethylene adding to postharvest pernicious effects, suggests a need to establish a relationship between ethylene and ripening of fruits. Three ethylene receptors, *FaEtr1, FaErs1* and *FaEtr2,* have been isolated and their expressions were determined in strawberry (*Fragaria ananassa*) fruits. Ethylene receptors were suspected to be involved in ripening of nonclimateric strawberries,[153] as suggested by the expression of *FaErs1* gene in responses to ethylene at red stage, *FaEtr1* and *FaEtr2* genes in white fruits. The expression of *FaEtr1* mRNA was found to be low in flowers but subsequently

increased in small green fruits and then decreased in large green fruits followed by steep increment throughout ripening phase. The expression of *FaETR2* mRNA showed 3-fold increase to reach maximum in white fruits whereas, *FaErs1* mRNA expression was very high in flowers and decreased to minimum in large green fruits.

Ethylene Perception and Role of Ethylene Receptors in Tomato

Tomato is not only an important vegetable crop but has also emerged as a model crop like Arabidopsis and tobacco. Simpler transformation and genetic manipulation with rapid life cycle and distinct anatomical features has made it a crop of choice for genetic studies. Also there is a detailed information about its genomics.[154,155] Transcriptomic, metabolomic and proteomic profiling of *Nr* mutant has revealed several aspects of regulation and metabolism during ripening and development.[156] Klee (2004)[157] has elaborated on the reasons for tomato to be a model system for study and has also postulated on the dynamic behavior of ethylene receptor expression and function. *Nr* is a semi dominant ethylene receptor mutant, has a mutation in ethylene binding domain of the NR receptor, unable to bind ethylene and the first to be identified in tomato.[158] In tomato, there are six ethylene receptors *LeETR1*,[126,159] *LeETR2*,[126,160] *LeETR4*, *LeETR5*,[161] *LeETR6*[162] and *NR*.[158] *Nr,* mutant of *NR*.[163,164] Three receptors have a potential extra N-terminal membrane-spanning domain. Only one receptor, *NR*, lacks the receiver domain. Three receptors (*LeETR4-6*) are missing one or more conserved HK domains, thus resembling the Subfamily II Arabidopsis receptors. *NR* and *LeETR4* expression increases during fruit ripening[126,161,165] whereas *LeETR1* and *LeETR2* are constitutively expressed in all tissues throughout development,[126,159] with *LeETR1* expressed at about 5-fold higher level than *LeETR2*. In contrast, expression patterns of the other four genes are highly regulated. For example, during fruit development, ovaries express high levels of *NR* mRNA at anthesis.[126,158,165] The level then drops approximately 10-fold until the onset of ripening whereupon, it rises approximately 20-fold. *LeETR5* expression was high in reproductive and lower in vegetative tissues.[161]

Ethylene Receptor Regulation in Tomato is Consistent with Receptor Inhibition Model

As per the Arabidopsis negative regulation model less receptor level increases ethylene sensitivity while more receptor reduces ethylene sensitivity i.e., more receptor requires more ethylene to turn on an ethylene response. Receptor inhibition model states that receptors are active and repress ethylene response in the absence of hormone and vice-versa. The first evidence in favor of the negative regulation model of ethylene receptors was put forth by Ciardi et al. (2000).[166] Overexpressed NR receptor in plant reduces the ethylene sensitivity quantitatively when there is a several fold increase in NR protein.

Considering the fact that there are no examples of decreased ethylene receptor gene expression during ethylene response in tomato there is a need to justify the observation of enhanced *LeETR4, LeETR5, LeETR6*[167] and *NR* gene expression in ovaries and ripened fruit with the ripening process and thus with ethylene.[126, 158] This ripening associated rise is an example of developmentally dependent ethylene inducible i.e., the gene is ethylene inducible in ripening but not in mature fruit.[158] Gallie (2010)[168] demonstrated the ability to control ethylene responses temporally and in amount through the control of mutant receptor (etr1-1) expression.

One justification could be that at the onset of fruit ripening ethylene production becomes autocatalytic, resulting in rapid increase in ethylene levels. To minimize the effect of ethylene, ethylene sensitivity is reduced by increased expression of receptors. Another justification could be in accordance with Schaller and Bleecker (1995)[8] findings that the dissociation time of ethylene saturated *ETR1* receptor is 12 h and under such circumstances the only way a plant can opt for a turn off of an ethylene response is by synthesis of new receptors. Substantiating the negative regulation even further, Tieman et al. (2000)[167] developed transgenic plants with reduced *LeETR4* gene expression resulting in enhanced ethylene sensitivity (severely epinastic and almost all flowers senesced before anthesis). This observation is in accordance with the negative regulation model. However, it does defy the Arabidopsis loss of function mutation findings because in tomato only a single gene reduced expression leads to severe ethylene triple response, whereas in Arabidopsis multiple receptor inactivations exhibited such severe responses. When the same strategy (antisense suppression) was used with NR, negligible phenotypic alterations were observed. Now, having known that the expression of *LeETR4* and *NR* under normal circumstances is more or less the same, the question that needs to be answered is why the loss of expression of *LeETR4* leads to severe responses and the same event in *NR* did not lead to any severe responses? The answer lies in the functional compensation exhibited by the receptors in a multigene family. This is revealed by expression analysis of *LeETR4* in Nr suppressed lines. Even the modest reductions in *NR* results in elevated levels of *LeETR4* by almost 3–4 times. However, the other receptor levels did not differ significantly. On the contrary, in *LeETR4* suppressed lines there was no significant increase in other receptor gene. Thus, *LeETR4* takes care of ethylene sensitivity and compensates for the reduction in expression of other receptors, which is not reported in Arabidopsis. Kevany et al. (2007)[169] deciphered the role of *LeETR4* and *LeETR6* in modulating ethylene responses including fruit maturation. Depleted levels of either of these receptors by antisense suppression or ethylene mediated protein degradation resulted in early fruit ripening. Also, the NR protein levels were parallely depleted on ethylene exposure. In contrast to very high rise in mRNA expression of NR, *LeETR4* and *LeETR6* at the onset of ripening, the protein levels of these receptors showed a significant decline and remained low on exposure to ethylene. However, the same proteins were expressed at their maximum in immature fruits. Thus, it was demonstrated that the reduction in the levels of either of the two family members, *LeETR4* or *LeETR6*, causes an early ripening. The authors hypothesized that the ligand

induced conformational change in the receptors could be a probable reason for receptors' subsequent degradation via 26S proteasome apparatus. Unlike the Arabidopsis model of knockouts where only triple and quadruple mutants depicted profound ethylene response, here the *LeETR4* and *LeETR6* suppressed plants showed phenotypes with exaggerated ethylene response including epinastic growth, premature flower senescence and an early fruit ripening. The results are in contradiction with Wang et al. (2003)[170] theory which says subfamily I receptors are more responsive to ethylene effect than the subfamily II receptors.

The success of antisense suppressed ethylene receptors, *LeETR4* and *LeETR6*, leading to desired premature ripening[167,169] was incomplete as it was accompanied by many unwanted effects on growth of plant. It was hypothesized that the fruit specific suppression would result in early ripening without undesirable issues. Kevany et al. (2008)[171] deciphered the role of ethylene during immature fruit development. The depleted *LeETR4* levels resulted in early ripening without compromising on agronomic features, fruit size, yield and flavour related chemical composition were also not penalized. However, this observation of single ethylene receptor suppression resulting in altered phenotype is in contrast with Arabidopsis model, where only multiple loss of function receptor mutants resulted in obvious ethylene effects. It validates Kevany et al. theory (2007)[169] that if receptors are not replaced after ethylene mediated degradation, the fruit becomes more sensitive to ethylene and ripens faster.

Hackett et al. (2000)[172] used antisense inhibition of a mutant tomato ethylene receptor, *Nr* gene to show that the mutant tomato restored the normal ripening suggesting that the mutant *Nr* receptor is unable to bind ethylene and prevents its inactivation, therefore suppressing the ethylene responses. The transgenic plants developed using antisense *Nr* produced three different phenotypes depending upon the transgene dosage in the progeny gave intermediate fruit type if they were hemizygous for both copies, in case of single transgene inheritance in a homozygous state fetched fruits with intermediate (between *Nr* and wild type) phenotype, whereas hemizygotes produced Nr-type fruit. This finding suggests that a threshold level of mutant receptor is needed to suppress the ethylene response pathway and prevent normal ripening. However, antisense inhibition of the wild type NR gene showed no effect on ripening, raising a question about normal function of this gene. Probable reason of the functional NR gene might be to achieve wild type levels of *LeETR4* expression. The Nr mutants are known to affect ethylene responses in tissues other than ripe fruit (Lanahan et al., 1994),[163] including seedlings. Nr seedlings were devoid of ethylene triple response (shortened, swollen hypocotyls, exaggerated apical hook and shortened root) when germinated in the dark on media with ethylene precursor ACC. Similar results were observed in case of homozygous NR antisense transgenic and Nr seedlings when given similar treatment. These data suggests that although downregulation of the mutant Nr gene was sufficient to alleviate its effect on fruit ripening in the transgenic raised, insensitivity of seedlings to ethylene was not altered. Results indicated that wild type NR gene product is not required for normal ripening and antisense inhibition of the mutant *Nr* gene products restores normal ripening. This provides enough evidence in support of receptor inhibition model and negative regulation of ethylene signaling in tomato.

Under pathogen challenge, the *LeETR4* expression is induced because of hypersensitive response triggered by infection. In antisense lines with severely reduced *LeETR4* expression there is a heightened hypersensitive response to pathogen infection manifested by increased ethylene synthesis and pathogenesis related gene expression as a consequence of enhanced defense response. Had the receptor levels not increased in response to infection, the tissue would have undergone a hypersensitive ethylene response state. This observation suggests that plant takes measures to prevent itself from the collateral damage that could be caused as a result of subsequent ethylene response after pathogen infection.[173]

Conclusion and Future Prospects

The varied expression pattern of ethylene receptor in agriculturally important crops gives an insight regarding regulation of ethylene perception in different species of plants. Though, the expression of receptors varies with crops and their developmental stages and environmental condition, the negative regulation theory is never violated. This information helped in designing plants with altered perception to ethylene with delayed senescence and ripening of fruit, vegetables and flowers. Being a multigene family, the expression analysis of each receptor tells about redundancy, compensatory and regulatory role of each member of the family. Structural understanding of the receptor has led to the development of ethylene receptors mutated in the ethylene binding domain leading global ethylene insensitivity in crops even across the species. Different expression of ethylene receptors at various developmental stages in non-climacteric crops, where only basal level of ethylene is produced, for example in Gladiolus, is an indicator of receptor mediated regulation of senescence and a possible reason of imparting insensitivity to ethylene. It would be interesting to see if this can be validated by using ethylene receptor genes in its natural form from such ethylene-insensitive systems and used in different crops and measure the effect on transformed plants.

The central idea of this review is to present every possible detail about the role of ethylene receptor expression in flower senescence and fruit ripening along with the transgenics developed using mutated ethylene receptors and transcription factors involved in the cascade. To date, only the receiver domain of an ethylene receptor protein is crystallized. If structure of other domains of the receptor protein can be modeled then, this would help in more precise targeting of ethylene signaling at the receptor level itself thus, regulating the relay of signals from ethylene binding domain via HK domain and receiver domain to the downstream CTR1 molecule.

With the advent of next generation sequencing, genomes and transcriptomes of several crops are available leading to rapid discovery of genes and transcription factors governing the ethylene signaling and biosynthesis. These transcription factors

can be exploited to control the expression of desired genes, thus regulating the flux of the proteins formed, affecting ripening and senescence. Also, understanding the role of micro RNAs in fruit ripening and development, and flower senescence will help in precisely manipulating the complex process of regulation of ripening and senescence.

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References

1. Abeles FB, Morgan PW, Saltveit ME. Ethylene in Plant Biology. Ed2. Academic Press, San Diego 1992.
2. Knight LI, Rose RC, Crocker W. Effect of various gases and vapors upon etiolated seedlings of the sweet pea. Science 1910; 31:635-6.
3. Rodríguez FI, Esch JJ, Hall AE, Binder BM, Schaller GE, Bleecker AB. A copper cofactor for the ethylene receptor ETR1 from *Arabidopsis*. Science 1999; 283:996-8; PMID:9974395; http://dx.doi.org/10.1126/science.283.5404.996
4. Gamble RL, Coonfield ML, Schaller GE. Histidine kinase activity of the ETR1 ethylene receptor from *Arabidopsis*. Proc Natl Acad Sci U S A 1998; 95:7825-9; PMID:9636235; http://dx.doi.org/10.1073/pnas.95.13.7825
5. Moussatche P, Klee HJ. Autophosphorylation activity of the *Arabidopsis* ethylene receptor multigene family. J Biol Chem 2004; 279:48734-41; PMID:15358768; http://dx.doi.org/10.1074/jbc.M403100200
6. Dong CH, Jang M, Scharein B, Malach A, Rivarola M, Liesch J, et al. Molecular association of the *Arabidopsis* ETR1 ethylene receptor and a regulator of ethylene signaling, RTE1. J Biol Chem 2010; 285:40706-13; PMID:20952388; http://dx.doi.org/10.1074/jbc.M110.146605
7. Kim H, Helmbrecht EE, Stalans MB, Schmitt C, Patel N, Wen C-K, et al. Ethylene receptor ETHYLENE RECEPTOR1 domain requirements for ethylene responses in *Arabidopsis* seedlings. Plant Physiol 2011; 156:417-29; PMID:21386032; http://dx.doi.org/10.1104/pp.110.170621
8. Schaller GE, Bleecker AB. Ethylene-binding sites generated in yeast expressing the *Arabidopsis ETR1* gene. Science 1995; 270:1809-11; PMID:8525372; http://dx.doi.org/10.1126/science.270.5243.1809
9. Chen YF, Randlett MD, Findell JL, Schaller GE. Localization of the ethylene receptor ETR1 to the endoplasmic reticulum of *Arabidopsis*. J Biol Chem 2002; 277:19861-6; PMID:11916973; http://dx.doi.org/10.1074/jbc.M201286200
10. Clark KL, Larsen PB, Wang X, Chang C. Association of the *Arabidopsis* CTR1 Raf-like kinase with the ETR1 and ERS ethylene receptors. Proc Natl Acad Sci U S A 1998; 95:5401-6; PMID:9560288; http://dx.doi.org/10.1073/pnas.95.9.5401
11. Hall AE, Findell JL, Schaller GE, Sisler EC, Bleecker AB. Ethylene perception by the ERS1 protein in *Arabidopsis*. Plant Physiol 2000; 123:1449-58; PMID:10938361; http://dx.doi.org/10.1104/pp.123.4.1449
12. Binder BM. The ethylene receptors: complex perception for a simple gas. Plant Sci 2008; 175:8-17; http://dx.doi.org/10.1016/j.plantsci.2007.12.001
13. Hua J, Chang C, Sun Q, Meyerowitz EM. Ethylene insensitivity conferred by *Arabidopsis* ERS gene. Science 1995; 269:1712-4; PMID:7569898; http://dx.doi.org/10.1126/science.7569898
14. Roman G, Lubarsky B, Kieber JJ, Rothenberg M, Ecker JR. Genetic analysis of ethylene signal transduction in *Arabidopsis* thaliana: five novel mutant loci integrated into a stress response pathway. Genetics 1995; 139:1393-409; PMID:7768447
15. Solano R, Ecker JR. Ethylene gas: perception, signaling and response. Curr Opin Plant Biol 1998; 1:393-8; PMID:10066624; http://dx.doi.org/10.1016/S1369-5266(98)80262-8
16. Gao Z, Chen YF, Randlett MD, Zhao XC, Findell JL, Kieber JJ, et al. Localization of the Raf-like kinase CTR1 to the endoplasmic reticulum of Arabidopsis through participation in ethylene receptor signaling complexes. J Biol Chem 2003; 278:34725-32; PMID:12821658; http://dx.doi.org/10.1074/jbc.M305548200
17. Sisler EC, Serek M, Dupille E. Comparison of cyclopropene, 1-methylcyclopropene, and 3,3-dimethylcyclopropene as ethylene antagonists in plants. Plant Growth Regul 1996; 18:169-74; http://dx.doi.org/10.1007/BF00024378
18. Serek M, Tamari G, Sisler EC, Borochov A. Inhibition of ethylene-induced senescence symptoms by 1-methyl cyclopropene, a new inhibitor of ethylene action. Physiol Plant 1995; 94:229-32; http://dx.doi.org/10.1111/j.1399-3054.1995.tb05305.x
19. Sisler EC, Serek M. Inhibitors of ethylene responses in plants at the receptor level: recent developments. Physiol Plant 1997; 100:577-82; http://dx.doi.org/10.1111/j.1399-3054.1997.tb03063.x
20. Sisler EC, Serek M. Compounds interacting with the ethylene receptor in plants. Plant Biol 2003; 5:473-80; http://dx.doi.org/10.1055/s-2003-44782
21. Serek M, Woltering EJ, Sisler EC, Frello S, Sriskandarajah S. Controlling ethylene responses in flowers at the receptor level. Biotechnol Adv 2006; 24:368-81; PMID:16584864; http://dx.doi.org/10.1016/j.biotechadv.2006.01.007
22. Kumar V, Parvatam G, Ravishankar GA. $AgNO_3$—a potential regulator of ethylene activity and plant growth modulator. eJ Biotechnology. 2009; 12: DOI: 10.2225.
23. Pirrung MC, Bleecker AB, Inoue Y, Rodríguez FI, Sugawara N, Wada T, et al. Ethylene receptor antagonists: strained alkenes are necessary but not sufficient. Chem Biol 2008; 15:313-21; PMID:18420138; http://dx.doi.org/10.1016/j.chembiol.2008.02.018
24. Kazemi M, Zamani S, Aran M. Effect of Some Treatment Chemicals on Keeping Quality and Vase-life of Gebrera Cut Flowers. American J Plant Physiol 2011; 6:99-105; http://dx.doi.org/10.3923/ajpp.2011.99.105
25. Chen JF, Gallie DR. Analysis of the functional conservation of ethylene receptors between maize and *Arabidopsis*. Plant Mol Biol 2010; 74:405-21; PMID:20835883; http://dx.doi.org/10.1007/s11103-010-9686-4
26. Liu Q, Wen C-K. *Arabidopsis ETR1* and *ERS1* differentially 1 repress the ethylene response in 2 combination with other ethylene receptor genes. Plant Physiol 2012; 158; http://dx.doi.org/10.1104/pp.111.187757
27. Csukasi F, Merchante C, Valpuesta V. Modification of plant hormone levels and signaling as a tool in plant biotechnology. Biotechnol J 2009; 4:1293-304; PMID:19585532; http://dx.doi.org/10.1002/biot.200800286
28. Mattoo AK, Suttle JC. The Plant Hormone Ethylene. CRC Press, Boca Raton, FL1991.
29. Shaharoona B, Muhammad IM, Arshad M, Khalid A. Manipulation of ethylene synthesis in roots through bacterial ACC deaminase for improving nodulation in legumes. Crit Rev Plant Sci 2011; 30:279-91; http://dx.doi.org/10.1080/07352689.2011.572058
30. Binder BM, Patterson SE. Ethylene-dependent and -independent regulation of abscission. Stewart Postharvest Review 2009; 5:1-10; http://dx.doi.org/10.2212/spr.2009.1.1
31. Lin Z, Zhong S, Grierson D. Recent advances in ethylene research. J Exp Bot 2009; 60:3311-36; PMID:19567479; http://dx.doi.org/10.1093/jxb/erp204
32. Little HA, Gurmet R, Hancock JF. Modified ethylene signaling as an example of engineering for complex Traits: secondary effects and implications for environmental risk assessment. HortScience 2009; 44:94-101.
33. Stearns JC, Glick BR. Transgenic plants with altered ethylene biosynthesis or perception. Biotechnol Adv 2003; 21:193-210; PMID:14499129; http://dx.doi.org/10.1016/S0734-9750(03)00024-7
34. Czarny JC, Grichko VP, Glick BR. Genetic modulation of ethylene biosynthesis and signaling in plants. Biotechnol Adv 2006; 24:410-9; PMID:16524685; http://dx.doi.org/10.1016/j.biotechadv.2006.01.003
35. Dahmani-Mardas F, Troadec C, Boualem A, Lévêque S, Alsadon AA, Aldoss AA, et al. Engineering melon plants with improved fruit shelf life using the TILLING approach. PLoS One 2010; 5:e15776; PMID:21209891; http://dx.doi.org/10.1371/journal.pone.0015776
36. Theologis A, Zarembinski TI, Oeller PW, Liang X, Abel S. Modification of fruit ripening by suppressing gene expression. Plant Physiol 1992; 100:549-51; PMID:16653026; http://dx.doi.org/10.1104/pp.100.2.549
37. Arshad M, Frankenberger WT. Ethylene: Agricultural Sources and Applications. New York, NY: Kluwer Academic/Plenum Publishers 2002; 342.
38. Andersson-Gunneråsa S, Hellgren JM, Björklund S, Regan S, Moritz T, Sundberg B. Asymmetric expression of a poplar ACC oxidase controls ethylene production during gravitational induction of tension wood. Plant J 2003; 34:339-49; PMID:12713540; http://dx.doi.org/10.1046/j.1365-313X.2003.01727.x
39. Wang NN, Shih MC, Li N. The GUS reporter-aided analysis of the promoter activities of *Arabidopsis* ACC synthase genes AtACS4, AtACS5, and AtACS7 induced by hormones and stresses. J Exp Bot 2005; 56:909-20; PMID:15699063; http://dx.doi.org/10.1093/jxb/eri083
40. Oeller PW, Lu MW, Taylor LP, Pike DA, Theologis A. Reversible inhibition of tomato fruit senescence by antisense RNA. Science 1991; 254:437-9; PMID:1925603; http://dx.doi.org/10.1126/science.1925603
41. Fluhr R, Mattoo AK. Ethylene: biosynthesis and perception. Crit Rev Plant Sci 1996; 15:479-23.
42. Savin KW, Baudinette SC, Graham MW, Michael MZ, Nugent GD, Lu C. Antisense ACC oxidase RNA delays carnation petal senescence. Hort Sci 1995; 30:970-2.
43. Einset JW, Kopperud C. Antisense ethylene genes for *Begonia* flowers. Acta Hortic 1995; 405:190-6.
44. Good X, Kellogg JA, Wagoner W, Langhoff D, Matsumura W, Bestwick RK. Reduced ethylene synthesis by transgenic tomatoes expressing *S*-adenosylmethionine hydrolase. Plant Mol Biol 1994; 26:781-90; PMID:7999994; http://dx.doi.org/10.1007/BF00028848

45. Kumar A, Taylor MA, Mad ASA, Davies HV. Potato plants expressing antisense and sense S-adenosylmethione decarboxylase (SAMDC) transgenes show altered levels of polyamines and ethylene: antisense plants display abnormal phenotypes. Plant J 1996; 9:147-58; http://dx.doi.org/10.1046/j.1365-313X.1996.09020147.x
46. van Doorn WG. Categories of petal senescence and abscission: a reevaluation. Ann Bot (Lond) 2001; 87:447-56; http://dx.doi.org/10.1006/anbo.2000.1357
47. Jones ML. Ethylene signalling is required for pollination-accelerated corolla senescence in petunias. Plant Sci 2008; 175:190-6; http://dx.doi.org/10.1016/j.plantsci.2008.03.011
48. Hoffman T, Schmidt JS, Zheng X, Bent AF. Isolation of ethylene-insensitive soybean mutants that are altered in pathogen susceptibility and gene-for-gene disease resistance. Plant Physiol 1999; 119:935-50; PMID:10069832; http://dx.doi.org/10.1104/pp.119.3.935
49. Einset JW. Differential expression of antisense in regenerated tobacco plants transformed with an antisense version of a tomato ACC oxidase gene. Plant Cell Tissue Organ Cult 1996; 46:137-41; http://dx.doi.org/10.1007/BF00034847
50. Picton S, Barton SL, Bouzayen M, Hamilton AJ, Grierson D. Altered fruit ripening and leaf senescence in tomatoes expressing an antisense ethylene-forming enzyme transgene. Plant J 1993; 3:469-81; http://dx.doi.org/10.1111/j.1365-313X.1993.tb00167.x
51. Huang WF, Huang PL, Do YY. Ethylene receptor transcript accumulation patterns during flower senescence in *Oncidium* 'Gower Ramsey' as affected by exogenous ethylene and pollinia cap dislodgement. Post Biol Tech 2007; 44:87-94; http://dx.doi.org/10.1016/j.postharvbio.2006.12.012
52. Dandekari AM, Teo G, Defilippi BG, Uratsu SL, Passey AJ, Kader AA, et al. Effect of down-regulation of ethylene biosynthesis on fruit flavor complex in apple fruit. Transgenic Res 2004; 13:373-84; PMID:15517996; http://dx.doi.org/10.1023/B:TRAG.0000040037.90435.45
53. Arora A. Programmed cell death during petal senescence. Postharvest Biology and Technology of fruits, vegetables and flowers. Paliyath G, Murr DP, Handa AK, Lurie S. (Eds). John Wiley and Sons Ltd. 2008; 86-124.
54. Patterson SE, Bleecker AB. Ethylene-dependent and -independent processes associated with floral organ abscission in *Arabidopsis*. Plant Physiol 2004; 134:194-203; PMID:14701913; http://dx.doi.org/10.1104/pp.103.028027
55. Palma JM, Corpas FJ, del Río LA. Proteomics as an approach to the understanding of the molecular physiology of fruit development and ripening. J Proteomics 2011; 74:1230-43; PMID:21524723; http://dx.doi.org/10.1016/j.jprot.2011.04.010
56. Bapat VA, Trivedi PK, Ghosh A, Sane VA, Ganapathi TR, Nath P. Ripening of fleshy fruit: molecular insight and the role of ethylene. Biotechnol Adv 2010; 28:94-107; PMID:19850118; http://dx.doi.org/10.1016/j.biotechadv.2009.10.002
57. Sriskandarajah S, Mibus H, Serek M. Transgenic Campanula carpatica plants with reduced ethylene sensitivity. Plant Cell Rep 2007; 26:805-13; PMID:17221226; http://dx.doi.org/10.1007/s00299-006-0291-6
58. ten Have A, Woltering EJ. Ethylene biosynthetic genes are differentially expressed during carnation (Dianthus caryophyllus L.) flower senescence. Plant Mol Biol 1997; 34:89-97; PMID:9177315; http://dx.doi.org/10.1023/A:1005894703444
59. Jones ML, Woodson WR. Differential expression of three members of the 1-aminocyclopropane-1-carboxylate synthase gene family in carnation. Plant Physiol 1999; 119:755-64; PMID:9952472; http://dx.doi.org/10.1104/pp.119.2.755
60. Shibuya K, Yoshioka T, Hashiba T, Satoh S. Role of the gynoecium in natural senescence of carnation (Dianthus caryophyllus L.) flowers. J Exp Bot 2000; 51:2067-73; PMID:11141180; http://dx.doi.org/10.1093/jexbot/51.353.2067
61. Borochov A, Woodson WR. Physiology and biochemistry of flower petal senescence. Hortic Rev (Am Soc Hortic Sci) 1989; 11:15-43.
62. Wang H, Woodson WR. Reversible inhibition of ethylene action and interruption of petal senescence in carnation flowers by norbornadiene. Plant Physiol 1989; 89:434-8; PMID:16666561; http://dx.doi.org/10.1104/pp.89.2.434
63. Charng YY, Sun CW, Yan SL, Chou SJ, Chen YR, Yang SF. cDNA sequence of a putative ethylene receptor from carnation petals (Accession No. AF016250) (PGR97-144). Plant Physiol 1997; 115:863; PMID:9411456
64. Shibuya K, Satoh S, Yoshioka T. A cDNA encoding a putative ethylene receptor related to petal senescence in carnation (*Dianthus caryophyllus* L.) flowers (Accession No. AF034770) (PGR 98-019). Plant Physiol 1998; 116:67.
65. Nagata M, Tanikawa N, Onazaki T, Mori H. Ethylene receptor gene (*ETR*) homolog from carnation. J Japanese Soc Hort Sci 2000; 69:407.
66. Shibuya K, Nagata M, Tanikawa N, Yoshioka T, Hashiba T, Satoh S. Comparison of mRNA levels of three ethylene receptors in senescing flowers of carnation (*Dianthus caryophyllus* L.). J Exp Bot 2002; 53:399-406; PMID:11847237; http://dx.doi.org/10.1093/jexbot/53.368.399
67. Onoue T, Mikami M, Yoshioka T, Hashiba T, Satoh S. Characteristics of the inhibitory action of 1,1-dimethyl-4-(phenylsulfonyl) semicarbazide (DPSS) on ethylene production in carnation (*Dianthus caryophyllus* L.) flowers. Plant Growth Regul 2000; 30:201-7; http://dx.doi.org/10.1023/A:1006324715438
68. Waki K, Shibuya K, Yoshioka T, Hashiba T, Satoh S. Cloning of a cDNA encoding EIN3-like protein (DC-EIL1) and decrease in its mRNA level during senescence in carnation flower tissues. J Exp Bot 2001; 52:377-9; PMID:11283184; http://dx.doi.org/10.1093/jexbot/52.355.377
69. Chao Q, Rothenberg M, Solano R, Roman G, Terzaghi W, Ecker JR. Activation of the ethylene gas response pathway in *Arabidopsis* by the nuclear protein ETHYLENE-INSENSITIVE3 and related proteins. Cell 1997; 89:1133-44; PMID:9215635; http://dx.doi.org/10.1016/S0092-8674(00)80300-1
70. Kosugi S, Ohashi Y. Cloning and DNA-binding properties of a tobacco Ethylene-Insensitive3 (EIN3) homolog. Nucleic Acids Res 2000; 28:960-7; PMID:10648789; http://dx.doi.org/10.1093/nar/28.4.960
71. Wang KL, Li H, Ecker JR. Ethylene biosynthesis and signaling networks. Plant Cell 2002; 14(Suppl):S131-51; PMID:12045274
72. Guo H, Ecker JR. Plant responses to ethylene gas are mediated by SCF(EBF1/EBF2)-dependent proteolysis of EIN3 transcription factor. Cell 2003; 115:667-77; PMID:14675532; http://dx.doi.org/10.1016/S0092-8674(03)00969-3
73. Potuschak T, Lechner E, Parmentier Y, Yanagisawa S, Grava S, Koncz C, et al. EIN3-dependent regulation of plant ethylene hormone signaling by two *arabidopsis* F box proteins: EBF1 and EBF2. Cell 2003; 115:679-89; PMID:14675533; http://dx.doi.org/10.1016/S0092-8674(03)00968-1
74. Yanagisawa S, Yoo SD, Sheen J. Differential regulation of EIN3 stability by glucose and ethylene signalling in plants. Nature 2003; 425:521-5; PMID:14523448; http://dx.doi.org/10.1038/nature01984
75. Iordachescu M, Verlinden S. Transcriptional regulation of three EIN3-like genes of carnation (Dianthus caryophyllus L. cv. Improved White Sim) during flower development and upon wounding, pollination, and ethylene exposure. J Exp Bot 2005; 56:2011-8; PMID:15983019; http://dx.doi.org/10.1093/jxb/eri199
76. van Doorn WG, Woltering EJ. Senescence and programmed cell death: substance or semantics? J Exp Bot 2004; 55:2147-53; PMID:15361529; http://dx.doi.org/10.1093/jxb/erh264
77. Verlinden S, Garcia JJ. Sucrose loading decreases ethylene responsiveness in carnation (*Dianthus caryophyllus* cv. White Sim) petals. Post Biol Tech 2004; 31:305-12; http://dx.doi.org/10.1016/j.postharvbio.2003.09.010
78. Jones ML, Woodson WR. Pollination-induced ethylene in carnation: role of stylar ethylene in corolla senescence. Plant Physiol 1997; 115:205-12; PMID:12223801
79. Bovy AG, Angenent GC, Dons HJM, Van Altvorst AC. Heterologous expression of the *Arabidopsis* etr1-1 allele inhibits the senescence of carnation flowers. Mol Breed 1999; 5:301-8; http://dx.doi.org/10.1023/A:1009617804359
80. Baudinette SC, Stevenson TW, Savin KW. Isolation and characterisation of the carnation floral-specific MADS box gene, CMB2. Plant Sci 2000; 155:123-31; PMID:10814815; http://dx.doi.org/10.1016/S0168-9452(00)00198-9
81. Ohtsubo N, Mitsuhara I, Koga M, Seo S, Ohashi Y. Ethylene promotes the necrotic lesion formation and basic PR gene expression in TMV-infected tobacco. Plant Cell Physiol 1999; 40:808-17.
82. Angenent GC, Franken J, Busscher M, Colombo L, van Tunen AJ. Petal and stamen formation in petunia is regulated by the homeotic gene fbp1. Plant J 1993; 4:101-12; PMID:8106081; http://dx.doi.org/10.1046/j.1365-313X.1993.04010101.x
83. Papon N, Senoussi MM, Andreu F, Rideau M, Chenieux JC, Creche J. Cloning of a gene encoding a putative ethylene receptor in *Catharanthus roseus* and its expression in plant and cell cultures. Biol Plant 2004; 48:345-50; http://dx.doi.org/10.1023/B:BIOP.0000041085.82296.9c
84. Narumi T, Kanno Y, Suzuki M, Kishimoto S, Ohmiya A, Satoh S. Cloning of a cDNA encoding an ethylene receptor (*DG-ERS1*) from *Chrysanthemum* and comparison of its mRNA level in ethylene-sensitive and -insensitive cultivars. Postharvest Biol Technol 2005. a36:21-30; http://dx.doi.org/10.1016/j.postharvbio.2004.11.001
85. Narumi T, Aida R, Ohmiya A, Satoh S. Transformation of *Chrysanthemum* with mutated ethylene receptor genes: mDGERS1 transgenes conferring reduced ethylene sensitivity and characterization of the transformants. Postharvest Biol Technol 2005. b37:101-10; http://dx.doi.org/10.1016/j.postharvbio.2005.04.008
86. Sumitomo K, Narumi T, Satoh S, Hisamatsu T. Involvement of the ethylene response pathway in dormancy induction in chrysanthemum. J Exp Bot 2008; 59:4075-82; PMID:18952907; http://dx.doi.org/10.1093/jxb/ern247
87. Satoh S, Watanabe M, Chisaka K, Narumi T. Suppressed leaf senescence in *Chrysanthemum* transformed with a mutated ethylene receptor gene *mDG-ERS1*(*etr1-4*). J Plant Biol 2008; 51:424-7; http://dx.doi.org/10.1007/BF03036064
88. Satoh S, Narumi T, Watanabe M, Aida R, Ohmiya A. Reduced leaf senescence in *Chrysanthemum* transformed with a mutated ethylene receptor gene: toward generation of compositae leafy vegetables with a longer shelf-life. Acta Hortic 2006; 721:467-71.

89. Satoh S, Narumi T, Watanabe M, Aida R, Ohmiya A. Reduced leaf senescence in *Chrysanthemum* transformed with mutated ethylene receptor genes. Acta Hortic 2007; 764:89-94.
90. Shinoyama H, Sano T, Saito M, Ezura H, Aida R, Nomura Y, et al. Induction of male sterility in transgenic chrysanthemums (*Chrysanthemum morifolium* Ramat.) by expression of a mutated ethylene receptor gene, *Cm-ETR1/H69A*, and the stability of this sterility at varying growth temperatures. Mol Breed 2012; 29:285-95; http://dx.doi.org/10.1007/s11032-010-9546-6
91. Wang Y, Kumar PP. Heterologous expression of *Arabidopsis* ERS1 causes delayed senescence in coriander. Plant Cell Rep 2004; 22:678-83; PMID:14624308; http://dx.doi.org/10.1007/s00299-003-0738-y
92. Ichimura K, Kohata K, Goto R. Soluble carbohydrates in *Delphinium* and their influence on sepal abscission in cut flowers. Physiol Plant 2000; 108:307-13; http://dx.doi.org/10.1034/j.1399-3054.2000.108003307.x
93. Kuroda S, Hakata M, Hirose Y, Shiraishi M, Abe S. Ethylene production and enhanced transcription of an ethylene receptor gene, ERS1 in *Delphinium* during abscission of florets. Plant Physiol Biochem 2003; 41:812-20; http://dx.doi.org/10.1016/S0981-9428(03)00115-3
94. Tanase K, Ichimura K. Expression of ethylene receptors *Dl-ERS1-3* and *Dl-ERS2*, and ethylene response during flower senescence in *Delphinium*. J Plant Physiol 2006; 163:1159-66; PMID:16500725; http://dx.doi.org/10.1016/j.jplph.2005.12.003
95. Hua J, Meyerowitz EM. Ethylene responses are negatively regulated by a receptor gene family in *Arabidopsis thaliana*. Cell 1998; 94:261-71; PMID:9695954; http://dx.doi.org/10.1016/S0092-8674(00)81425-7
96. Thongkum M, Bhunchoth A, Warin N, Chatchawankanphanich O, Burns P. Cloning and expression of ethylene response sensor 1 (*Den-ERS1*) gene of dendrobium 'pompadour' flower during development and senescence. Thai J Agri Sci 2009; 42:227-36.
97. Dervinis C, Clark DG, Barrett JE, Nell TA. Effect of pollination and exogenous ethylene on accumulation of ETR1 homologue transcripts during flower petal abscission in geranium (*Pelargonium x hortorum* L.H. Bailey). Plant Mol Biol 2000; 42:847-56; PMID:10890532; http://dx.doi.org/10.1023/A:1006409827860
98. Clark DG, Richards C, Hilioti Z, Lind-Iversen S, Brown K. Effect of pollination on accumulation of ACC synthase and ACC oxidase transcripts, ethylene production and flower petal abscission in geranium (Pelargonium x hortorum L.H. Bailey). Plant Mol Biol 1997; 34:855-65; PMID:9290638; http://dx.doi.org/10.1023/A:1005877809905
99. Evensen KB, Page AM, Stead AD. Anatomy of ethylene-induced petal abscission in *Pelargonium xhortorum*. Ann Bot (Lond) 1991; 71:559-66; http://dx.doi.org/10.1006/anbo.1993.1072
100. Arora A, Watanabe S, Ma B, Takada K, Ezura H. A novel ethylene receptor homolog gene isolated from ethylene-insensitive flowers of gladiolus (*Gladiolus grandiflora* hort.). Biochem Biophys Res Commun 2006; 351:739-44; PMID:17084812; http://dx.doi.org/10.1016/j.bbrc.2006.10.111
101. Sanikhani M, Mibus H, Stummann BM, Serek M. Kalanchoe blossfeldiana plants expressing the Arabidopsis etr1-1 allele show reduced ethylene sensitivity. Plant Cell Rep 2008; 27:729-37; PMID:18080125; http://dx.doi.org/10.1007/s00299-007-0493-6
102. Handberg K, Stougaard J. Lotus japonicus, an autogamous, diploid legume species for classical and molecular genetics. Plant J 1992; 2:487-96; http://dx.doi.org/10.1111/j.1365-313X.1992.00487.x
103. Sato-Nara K, Yuhashi KI, Higashi K, Hosoya K, Kubota MH, Ezura H. Stage- and tissue-specific expression of ethylene receptor homolog genes during fruit development in muskmelon. Plant Physiol 1999; 120:321-30; PMID:10318709; http://dx.doi.org/10.1104/pp.120.1.321
104. Nukui N, Ezura H, Minamisawa K. Transgenic Lotus japonicus with an ethylene receptor gene Cm-ERS1/H70A enhances formation of infection threads and nodule primordia. Plant Cell Physiol 2004; 45:427-35; PMID:15111717; http://dx.doi.org/10.1093/pcp/pch046
105. Lohar D, Stiller J, Kam J, Stacey G, Gresshoff PM. Ethylene insensitivity conferred by a mutated Arabidopsis ethylene receptor gene alters nodulation in transgenic Lotus japonicas. Ann Bot 2009; 104:277-85.106.
106. Heidstra R, Yang WC, Yalcin Y, Peck S, Emons AM, van Kammen A, et al. Ethylene provides positional information on cortical cell division but is not involved in Nod factor-induced root hair tip growth in Rhizobium-legume interaction. Development 1997; 124:1781-7; PMID:9165125
107. Penmetsa RV, Frugoli JA, Smith LS, Long SR, Cook DR. A dual mechanism of nodule control in Medicago truncatula. Plant Physiol 2003; 131:1-11; http://dx.doi.org/10.1104/pp.900061
108. Penmetsa RV, Uribe P, Anderson J. The Medicago truncatula of the Arabidopsis EIN2 gene, sickle, is a negative regulator of symbiotic and pathogenic microbial interactions. Plant J 2008; 55:580-95; PMID:18435823; http://dx.doi.org/10.1111/j.1365-313X.2008.03531.x
109. Cui ML, Takada K, Ma B, Ezura H. Over expression of a mutated melon ethylene receptor gene Cm-ETR1/H69A confers reduced ethylene sensitivity in a heterologous plant, Nemesia strumosa. Plant Sci 2004; 167:253-8; http://dx.doi.org/10.1016/j.plantsci.2004.03.024
110. Takada K, Ishimaru K, Minamisawa K, Kamada H, Ezura H. Expression of a mutated melon ethylene receptor gene Cm-ETR1/H69A affects stamen development in Nicotiana tabacum. Plant Sci 2005; 169:935-42; http://dx.doi.org/10.1016/j.plantsci.2005.06.012
111. Huang CC. Ethylene production of Oncidium flower and the change of flower quality affected by ethylene treatment and pollinia cap removal. J Agric Res China 1998; 47:125-34.
112. Wilkinson JQ, Lanahan MB, Clark DG, Bleecker AB, Chang C, Meyerowitz EM. A dominant mutant receptor from Arabidopsis confers ethylene insensitivity in heterologous plants. Nat Biotech 1997; 15: 444-47.113. Giovannoni JJ. Genetic regulation of fruit development and ripening. Plant Cell 2004; 16:170-80.
113. Giovannoni JJ. Genetic regulation of fruit development and ripening. Plant Cell 2004; 16(Suppl):S170-80; PMID:15010516; http://dx.doi.org/10.1105/tpc.019158
114. Cobb D, Schneiter N, Guo S, Humiston GA, Harriman B, Bolar J. Flower specific expression of an ethylene receptor (ETR1-1) confers ethylene insensitivity in transgenic Petunia. In: XXVIth international horticultural congress, Toronto, Canada. Book of Abstracts 2002; 83.
115. Chen HH, Charng YY, Yang SF, Shaw JF. Isolation and characterization of a broccoli cDNA (Accession No. AF047477) encoding an ERS-type ethylene receptor. PGR98-123. Plant Physiol 1998; 117:26.
116. Clark DG, Gubrium EK, Barrett JE, Nell TA, Klee HJ. Root formation in ethylene-insensitive plants. Plant Physiol 1999; 121:53-60; PMID:10482660; http://dx.doi.org/10.1104/pp.121.1.53
117. Gubrium EK, Clevenger DJ, Clark DG, Barrett JE, Nell TA. Reproduction and horticultural performance of transgenic ethylene-insensitive petunias. J Am Soc Hortic Sci 2000; 125:277-81.
118. Clevenger DJ, Barrett JE, Klee HJ, Clark DG. Factors affecting seed production in transgenic ethylene-insensitive petunias. J Am Soc Hortic Sci 2004; 129:401-6.
119. Shibuya K, Barry KG, Ciardi JA, Loucas HM, Underwood BA, Nourizadeh S, et al. The central role of PhEIN2 in ethylene responses throughout plant development in petunia. Plant Physiol 2004; 136:2900-12; PMID:15466231; http://dx.doi.org/10.1104/pp.104.046979
120. Langston BJ, Bai S, Jones ML. Increases in DNA fragmentation and induction of a senescence-specific nuclease are delayed during corolla senescence in ethylene-insensitive (etr1-1) transgenic petunias. J Exp Bot 2005; 56:15-23; PMID:15475372
121. Müller R, Stummann BM, Serek M. Characterization of an ethylene receptor family with differential expression in rose (Rosa hybrida L.) flowers. Plant Cell Rep 2000. b19:1232-9; http://dx.doi.org/10.1007/s002990000251
122. Müller R, Owen CA, Xue ZT, Welander M, Stummann BM. Characterization of two CTR-like protein kinases in Rosa hybrida and their expression during flower senescence and in response to ethylene. J Exp Bot 2002; 53:1223-5; PMID:11971934; http://dx.doi.org/10.1093/jexbot/53.371.1223
123. Tieman DM, Ciardi JA, Taylor MG, Klee HJ. Members of the tomato LeEIL (EIN3-like) gene family are functionally redundant and regulate ethylene responses throughout plant development. Plant J 2001; 26:47-58; PMID:11359609; http://dx.doi.org/10.1046/j.1365-313x.2001.01006.x
124. Müller R, Owen CA, Xue ZT, Welander M, Stummann B. The transcription factor EIN3 is constitutively expressed in miniature roses with differences in postharvest life. J Hort Sci Biotech 2003; 78:10-14.125.
125. Müller R, Lind-Iversen S, Stummann BM, Serek M. Expression of genes for ethylene biosynthetic enzymes and an ethylene receptor in senescing flowers of miniature potted roses. J Hortic Sci Biotechnol 2000. a75:12-8.
126. Lashbrook CC, Tieman DM, Klee HJ. Differential regulation of the tomato ETR gene family throughout plant development. Plant J 1998; 15:243-52; PMID:9721682; http://dx.doi.org/10.1046/j.1365-313X.1998.00202.x
127. Ma QH, Wang XM. Characterization of an ethylene receptor homologue from wheat and its expression during leaf senescence. J Exp Bot 2003; 54:1489-90; PMID:12709495; http://dx.doi.org/10.1093/jxb/erg142
128. Xue J, Li Y, Tan H, Yang F, Ma N, Gao J. Expression of ethylene biosynthetic and receptor genes in rose floral tissues during ethylene-enhanced flower opening. J Exp Bot 2008; 59:2161-9; PMID:18535299; http://dx.doi.org/10.1093/jxb/ern078
129. Cin VD, Danesin M, Boschetti A, Dorigoni A, Ramina A. Ethylene biosynthesis and perception in apple fruitlet abscission (Malus domestica L. Borck). J Exp Bot 2005; 56:2995-3005; PMID:16203755; http://dx.doi.org/10.1093/jxb/eri296
130. Wiersma PA, Zhang H, Lu C, Quail A, Toivonen PMA. Survey of the expression of genes for ethylene synthesis and perception during maturation and ripening of 'Sunrise' and 'Golden Delicious' apple fruit. Postharvest Biol Technol 2007; 44:204-11; http://dx.doi.org/10.1016/j.postharvbio.2006.12.016

131. Wang A, Tan D, Takahashi A, Li TZ, Harada T. MdERFs, two ethylene-response factors involved in apple fruit ripening. J Exp Bot 2007; 58:3743-8; PMID:18057044; http://dx.doi.org/10.1093/jxb/erm224
132. Owino W, Nakano R, Kubo Y, Inaba A. Differential regulation of genes encoding ethylene biosynthesis enzymes and ethylene response sensor ortholog during ripening and in response to wounding in avocados. J Am Soc Hortic Sci 2002; 127:520-7.
133. Katz E, Lagunes PM, Riov J, Weiss D, Goldschmidt EE. Molecular and physiological evidence suggests the existence of a system II-like pathway of ethylene production in non-climacteric Citrus fruit. Planta 2004; 219:243-52; PMID:15014996; http://dx.doi.org/10.1007/s00425-004-1228-3
134. Yamasaki S, Fujii N, Takahashi H. The ethylene-regulated expression of CS-ETR2 and CS-ERS genes in cucumber plants and their possible involvement with sex expression in flowers. Plant Cell Physiol 2000; 41:608-16; PMID:10929944; http://dx.doi.org/10.1093/pcp/41.5.608
135. Manzano S, Martínez C, Gómez P, Garrido D, Jamilena M. Cloning and characterisation of two CTR1-like genes in Cucurbita pepo: regulation of their expression during male and female flower development. Sex Plant Reprod 2010; 23:301-13; PMID:20390430; http://dx.doi.org/10.1007/s00497-010-0140-1
136. Chervin C, Deluc L. Ethylene signalling receptors and transcription factors over the grape berry development: gene expression profiling. Vitis 2010; 49: 129-36.
137. Yin XR, Chen KS, Allan AC, Wu RM, Zhang B, Lallu N, et al. Ethylene-induced modulation of genes associated with the ethylene signalling pathway in ripening kiwifruit. J Exp Bot 2008; 59:2097-108; PMID: 18535296; http://dx.doi.org/10.1093/jxb/ern067
138. Takada K, Ishimaru K, Kamada H, Ezura H. Anther-specific expression of mutated melon ethylene receptor gene Cm-ERS1/H70A affected tapetum degeneration and pollen grain production in transgenic tobacco plants. Plant Cell Rep 2006; 25:936-41; PMID: 16552596; http://dx.doi.org/10.1007/s00299-006-0147-0
139. Ishimaru K, Takada K, Kamada H, Ezura H. Stable male sterility induced by the expression of a mutated melon ethylene receptor genes in Nicotiana tabacum. Plant Sci 2006; 71:355-9; http://dx.doi.org/10.1016/j.plantsci.2006.04.006
140. Takada K, Watanabe S, Sano T, Ma B, Kamada H, Ezura H. Heterologous expression of the mutated melon ethylene receptor gene Cm-ERS1/H70A produces stable sterility in transgenic lettuce (Lactuca sativa). J Plant Physiol 2007; 164:514-20; PMID: 17207555; http://dx.doi.org/10.1016/j.jplph.2006.10.003
141. Martinez PG, Gomez RL, Gomez-Lim LA. Identification of an ETR1-homologue from mango fruit expressing during fruit ripening and wounding. J Plant Physiol 2001; 158:101-8; http://dx.doi.org/10.1078/0176-1617-00238
142. Ish-Shalom M, Dahan Y, Maayan I, Irihimovitch V. Cloning and molecular characterization of an ethylene receptor gene, MiERS1, expressed during mango fruitlet abscission and fruit ripening. Plant Physiol Biochem 2011; 49:931-6; PMID:21676621; http://dx.doi.org/10.1016/j.plaphy.2011.05.010
143. Gillaspy G, Ben-David H, Gruissem W. Fruits: A Developmental Perspective. Plant Cell 1993; 5:1439-51; PMID:12271039; http://dx.doi.org/10.1105/tpc.5.10.1439
144. Takahashi H, Kobayashi T, Sato-Nara K, Tomita KO, Ezura H. Detection of ethylene receptor protein Cm-ERS1 during fruit development in melon (Cucumis melo L.). J Exp Bot 2002; 53:415-22; PMID: 11847239; http://dx.doi.org/10.1093/jexbot/53.368.415
145. Little HA, Papadopoulou E, Hammar SA, Grumet R. The influence of ethylene perception on sex expression in melon (Cucumis melo L.) as assessed by expression of the mutant ethylene receptor, At-etr1-1, under the control of constitutive and floral targeted promoters. Sex Plant Reprod 2007; 20: 123-36.146.
146. Papadopoulou E, Little HA, Hammar SA, Grumet R. Effect of modified endogenous ethylene production on sex expression, bisexual flower development and fruit production in melon (*Cucumis melo* L.). Sex Plant Reprod 2005; 18:131-42; http://dx.doi.org/10.1007/s00497-005-0006-0
147. Mita S, Kawamura S, Asai T. Regulation of the expression of a putative ethylene receptor, PeERS2, during the development of passion fruit (Passiflora edulis). Physiol Plant 2002; 114:271-80; PMID: 11903974; http://dx.doi.org/10.1034/j.1399-3054.2002.1140213.x
148. Rasori A, Ruperti B, Bonghi C, Tonutti P, Ramina A. Characterization of two putative ethylene receptor genes expressed during peach fruit development and abscission. J Exp Bot 2002; 53:2333-9; PMID: 12432026
149. El-Sharkawy I, Jones B, Li ZG, Lelièvre JM, Pech JC, Latché A. Isolation and characterization of four ethylene perception elements and their expression during ripening in pears (Pyrus communis L) with/without cold requirement. J Exp Bot 2003; 54:1615-25; PMID:12730273; http://dx.doi.org/10.1093/jxb/erg158
150. Pang JH, Ma B, Sun HJ, Guinevere I, Ortiz Imanishi S, Sugaya S, et al. Identification and characterization of ethylene receptor homologs expressed during fruit development and ripening in persimmon (Diospyros kaki Thumb). Post Biol Tech 2007; 44:195-03; http://dx.doi.org/10.1016/j.postharvbio.2006.12.017
151. El-Sharkawy I, Kim WS, El-Kereamy A, Jayasankar S, Svircev AM, Brown DCW. Isolation and characterization of four ethylene signal transduction elements in plums (Prunus salicina L.). J Exp Bot 2007; 58:3631-43; PMID:18057041; http://dx.doi.org/10.1093/jxb/erm213
152. El-Sharkawy I, Sherif S, Mila I, Bouzayen M, Jayasankar S. Molecular characterization of seven genes encoding ethylene-responsive transcriptional factors during plum fruit development and ripening. J Exp Bot 2009; 60:907-22; PMID:19213809; http://dx.doi.org/10.1093/jxb/ern354
153. Trainotti L, Pavanello A, Casadoro G. Different ethylene receptors show an increased expression during the ripening of strawberries: does such an increment imply a role for ethylene in the ripening of these non-climacteric fruits? J Exp Bot 2005; 56:2037-46; PMID:15955790; http://dx.doi.org/10.1093/jxb/eri202
154. Barry CS, Giovannoni JJ. Ripening in the tomato Green-ripe mutant is inhibited by ectopic expression of a protein that disrupts ethylene signaling. Proc Natl Acad Sci U S A 2006; 103:7923-8; PMID:16682641; http://dx.doi.org/10.1073/pnas.0602319103
155. Klee H. Highly conserved proteins that modify plant ethylene responses. Proc Natl Acad Sci U S A 2006; 103:7537-8; PMID:16682619; http://dx.doi.org/10.1073/pnas.0602599103
156. Osorio S, Alba R, Damascenob CMB, Lopez-Casado G. Marc, Lohse, Zanorc MI, Tohge T, Usadel B, Rose JKC, Fei Z, Giovannoni JJ, Fernie AR. Systems biology of tomato fruit development: combined transcript, protein and metabolite analysi s of tomato transcription factor (nor,rin) and ethylene receptor (Nr) mutants reveals novel regulatory interactions. Plant Physiol 2011; 157; PMID:21795583; http://dx.doi.org/10.1104/pp.111.175463
157. Klee HJ. Ethylene signal transduction. Moving beyond Arabidopsis. Plant Physiol 2004; 135:660-7; PMID: 15208412; http://dx.doi.org/10.1104/pp.104.040998
158. Wilkinson JQ, Lanahan MB, Yen HC, Giovannoni JJ, Klee HJ. An ethylene-inducible component of signal transduction encoded by never-ripe. Science 1995; 270:1807-9; PMID:8525371; http://dx.doi.org/10.1126/science.270.5243.1807
159. Zhou D, Mattoo A, Tucker M. Molecular cloning of a tomato cDNA encoding an ethylene receptor. Plant Physiol 1996. a110:1435-6.
160. Zhou D, Kalaitzís P, Mattoo AK, Tucker ML. The mRNA for an ETR1 homologue in tomato is constitutively expressed in vegetative and reproductive tissues. Plant Mol Biol 1996. b30:1331-8; PMID: 8704141; http://dx.doi.org/10.1007/BF00019564
161. Tieman DM, Klee HJ. Differential expression of two novel members of the tomato ethylene-receptor family. Plant Physiol 1999; 120:165-72; PMID:10318694; http://dx.doi.org/10.1104/pp.120.1.165
162. Klee H, Tieman D. The tomato ethylene receptor gene family: Form and function. Physiol Plant 2002; 115:336-41; PMID:12081525; http://dx.doi.org/10.1034/j.1399-3054.2002.1150302.x
163. Lanahan MB, Yen HC, Giovannoni JJ, Klee HJ. The never ripe mutation blocks ethylene perception in tomato. Plant Cell 1994; 6:521-30; PMID:8205003
164. Tucker GA, Schindler CB, Roberts JA. Flower abscission in mutant tomato plants. Planta 1984; 160:164-7; http://dx.doi.org/10.1007/BF00392865
165. Payton S, Fray RG, Brown S, Grierson D. Ethylene receptor expression is regulated during fruit ripening, flower senescence and abscission. Plant Mol Biol 1996; 31: 1227-31.166.
166. Ciardi JA, Tieman DM, Lund ST, Jones JB, Stall RE, Klee HJ. Response to Xanthomonas campestris pv. vesicatoria in tomato involves regulation of ethylene receptor gene expression. Plant Physiol 2000; 123:81-92; PMID:10806227; http://dx.doi.org/10.1104/pp.123.1.81
167. Tieman DM, Taylor MG, Ciardi JA, Klee HJ. The tomato ethylene receptors NR and LeETR4 are negative regulators of ethylene response and exhibit functional compensation within a multigene family. Proc Natl Acad Sci U S A 2000; 97:5663-8; PMID: 10792050; http://dx.doi.org/10.1073/pnas.090550597
168. Gallie DR. Regulated ethylene insensitivity through the inducible expression of the Arabidopsis etr1-1 mutant ethylene receptor in tomato. Plant Physiol 2010; 152:1928-39; PMID:20181754; http://dx.doi.org/10.1104/pp.109.151688
169. Kevany BM, Tieman DM, Taylor MG, Cin VD, Klee HJ. Ethylene receptor degradation controls the timing of ripening in tomato fruit. Plant J 2007; 51:458-67; PMID:17655616; http://dx.doi.org/10.1111/j.1365-313X.2007.03170.x

170. Wang W, Hall AE, O'Malley R, Bleecker AB. Canonical histidine kinase activity of the transmitter domain of the ETR1 ethylene receptor from Arabidopsis is not required for signal transmission. Proc Natl Acad Sci U S A 2003; 100:352-7; PMID:12509505; http://dx.doi.org/10.1073/pnas.0237085100
171. Kevany BM, Taylor MG, Klee HJ. Fruit-specific suppression of the ethylene receptor LeETR4 results in early-ripening tomato fruit. Plant Biotechnol J 2008; 6:295-300; PMID:18086233; http://dx.doi.org/10.1111/j.1467-7652.2007.00319.x
172. Hackett RM, Ho CW, Lin Z, Foote HC, Fray RG, Grierson D. Antisense inhibition of the Nr gene restores normal ripening to the tomato Never-ripe mutant, consistent with the ethylene receptor-inhibition model. Plant Physiol 2000; 124: 1079-86.174.
173. Ciardi JA, Tieman DM, Jones JB, Klee HJ. Reduced expression of the tomato ethylene receptor gene LeETR4 enhances the hypersensitive response to Xanthomonas campestris pv. vesicatoria. Mol Plant Microbe Interact 2001; 14:487-95; PMID:11310736; http://dx.doi.org/10.1094/MPMI.2001.14.4.487
174. Zhu H, Zhu B, Shao Y, Wang X, Lin X, Xie Y, et al. Tomato fruit development and ripening are altered by the silencing of LeEIN2 gene. J Integr Plant Biol 2006; 48:1478-85; http://dx.doi.org/10.1111/j.1744-7909.2006.00366.x
175. Chen G, Alexander L, Grierson D. Constitutive expression of EIL-like transcription factor partially restores ripening in the ethylene-insensitive Nr tomato mutant. J Exp Bot 2004; 55:1491-7; PMID:15181103; http://dx.doi.org/10.1093/jxb/erh168
176. Whitelaw CA, Lyssenko NN, Chen L, Zhou D, Mattoo AK, Tucker ML. Delayed abscission and shorter Internodes correlate with a reduction in the ethylene receptor LeETR1 transcript in transgenic tomato. Plant Physiol 2002; 128:978-87; PMID:11891253; http://dx.doi.org/10.1104/pp.010782
177. Shaw JF, Chen HH, Tsai MF, Kuo CI, Huang LC. Extended flower longevity of *Petunia hybrida* plants transformed with *boers*, a mutated ERS gene of *Brassica oleracea*. Mol Breed 2002; 9:211-6; http://dx.doi.org/10.1023/A:1019703627019