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Deciphering the effect of mutations on fruit ripening quality associated gene expression pattern in spontaneous monogenic tomato mutants

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ABSTRACT

To decipher the effect of spontaneous monogenic mutations on fruit ripening associated gene expression pattern, an integrated effort has been made to investigate tomato colored mutants *high pigment-1*(*hp-1*), *high pigment-2^{dark} green* (*hp-2^{dg}*), *old gold crimson*(*og*)^c and ripening impaired mutant *ripening inhibitor* (*rin*), during fruit ripening. Quantitative real time polymerase chain reaction was performed to assess relative transcript accumulation for carotenogenic pathway genes, ethylene anabolic genes, cell wall modifying genes and MADS-BOX transcription factor gene *LeMADS-RIN*. The non photomorphogenic high pigment mutant *og^c* was studied at the transcriptional level for the first time which provided the basis to select the mutant *og^c* as a suitable candidate of non-transgenic source for pigment enrichment by future development of hybrids. Regression analysis found that *LeZDS* and *CYC-B* were the sole positive contributors for lycopene and β-carotene content, respectively, and fruit firmness was negatively correlated to *LeEXP*, *LePG*, *LeTBG-4*, *PME*. Highly similar fruit firmness was observed in *hp-2^{dg}* with the mutant *rin*, which is desirable for extended shelf life. Expression of the major ripening regulator gene *LeMADS-RIN* was also induced in high pigment mutants. Future characterization of the promoter region of candidate genes may decipher unknown regulatory aspects of these mutants.

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1. Introduction

Fruit ripening is a complex developmental phenomenon involving the coordinated regulation of numerous metabolic pathways that influence color, flavor, aroma, texture and ethylene production (Giovannoni, 2004). Insight into the genetic mechanisms that mediate fruit ripening related processes, such as cell wall metabolism, pigment synthesis and ethylene biosynthesis, have resulted from the studies that collectively span a wide range of plant species and indicate that they are broadly conserved (Saravanan and Rose, 2004; Carrari and Fernie, 2006; Faurobert et al., 2007; Page et al., 2010; Zhang et al., 2010; Moing et al., 2011). Tomato (*Solanum lycopersicum*) has emerged as the primary experimental model to study the ripening of fleshy fruits (Giovannoni, 2004). On a more comprehensive scale, tomato fruit development has been examined at the levels of transcriptome (Alba et al., 2005; Lemaire-Chamley et al., 2005; Vriezen et al., 2008), proteome (Saravanan and Rose, 2004; Faurobert et al., 2007; Page et al., 2010) and metabolome (Roessner-Tunali et al., 2003; Fraser et al., 2007; Moco et al., 2007). A number of important advances in understanding of the mechanisms that regulate ripening have come from the characterization of monogenic tomato mutants, including the *ripening-inhibitor* (*rin*), *non-ripening* (*nor*), *colorless ripening* (*Cnr*), *green-ripe* (*Gr*), *green-flesh* (*gf*) and *never-ripe* (*Nr*) (Lanahan et al., 1994; Mustilli et al., 1999; Vrebalov et al., 2002; Liu et al., 2004; Barry et al., 2008). Investigations on a MADS-box gene, *RIN* (*LeMADS-RIN*), belonging to the SEPALLATA (SEP) subfamily have unraveled its indispensible role during fruit ripening in tomato (Alvarez-Buylla et al., 2000; Causier et al., 2002; Vrebalov et al., 2002; Ito et al., 2008).The *rin* mutation has been

Abbreviations: hp-1, *high pigment-1*; *hp-2^{dg}*, *high pigment-2^{dark} green*; *og^c*, *old gold crimson*; *rin*, *ripening inhibitor*; AL, Ailsa Craig; PR, Pusa Ruby; *nor*, *non-ripening*; *Cnr*, *colorless ripening*; *Gr*, *green-ripe*; *gf*, *green-flesh*; *Nr*, *never-ripe*; *DDB1*, UV DAMAGED DNA BINDING protein 1; *DET1*, DEETIOLATED1; *SEP*, SEPALLATA; LTR, Lycopene Rich Tomatoes; *B*, *Beta*; BCKV, Bidhan Chandra KrishiViswavidyalaya; WB, West Bengal; RH, Relative humidity; DAP, days after pollination; MGS, mature green stage; BRS, breaker stage; RS, ripe stage; RT, reverse transcription; NCBI, National Centre for Biotechnological Information; Ct, cycle threshold; DMRT, Duncan Multiple Range Test; MEP, Methyl Erythritol Phosphat; ACC, 1-Amino Cyclopropane-1-Carboxylic acid; qRT-PCR, quantitative real time polymerase chain reaction; *LeACS2*, ACC synthase 2; *ACS4*, ACC synthase 4; *LePDS1*, Phytoene Desaturase 1; *LeEXP1*, Expansin 1; *PME*, Pectin methyl exterase; *LeTBG4*, Thiogalactosidase; *LePG*, Polygalacturonase; *LeACO-1*, *ACC oxidase*; *ZDS*, Zeta carotene desaturase; *LeMADS-RIN*, *MADS BOX transcription factor gene RIN*; *LeIPI*, isopentenyl diphosphate isomerase; *CYC-B*, chromoplast specific lycopene β-cyclase; DOXP, 1-deoxy-d-xylulose 5 phosphate; *LeDXS*, DOXP synthase; *LeDXR*, DOXP reducto-isomerase; *LeGGPPS2*, Geranyl geranyl diphosphate synthase; *LePSY1*, phytoene synthase; *UBQ*, *Ubiquitin*; CIMMYT, International Maize and Wheat Improvement Centre; CGIAR, Consultative group for international agricultural research; IISER-Kolkata, Indian Institute of Science Education and Research.

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Table 1
Genetic material of tomato employed in the present study.

Sl. No.	Tomato genotype	Source/reference
1.	Ailsa Craig (Wild Type)(AL)	The Institute of Genetics, Bulgarian Academy of Science, Sofia, Bulgaria
2.	PusaRuby (IndianCultivatedType)(PR) (Line:BCT-6)	Bidhan Chandra KrishiViswavidyalaya,WB,India
3.	Δ *hp-2*^{dg} (Line:BCT-115 possessing high pigment-2 dark green mutation)	United States Department of Agriculture, U.S.A.
4.	Δ*hp-1* (An isogenic line of Alisa Craig harbouring high pigment-1 gene mutation)	The Institute of Genetics, Bulgarian Academy of Science, Sofia, Bulgaria
5.	Δ *og*^{c} (An isogenic line of Alisa Craig harbouring old gold crimson gene mutation)	The Institute of Genetics, Bulgarian Academy of Science, Sofia, Bulgaria
6.	Δ*rin* (Line:BCT-111, possessing ripening inhibitor gene mutation)	Dr. M.K. Banerjee, Dept. of Vegetable Science, CCS Haryana Agriculture University, Hisar

utilized to develop many commercial tomato varieties to delay ripening and extend the shelf-life of the fruit (Giovannoni, 2007).The *rin* mutant also shows inhibition of carotenoid biosynthesis, production of flavor compounds and softening (Tigchelaar et al., 1978; Knapp et al., 1989; Vrebalov et al., 2002). In addition, several photomorphogenic mutants carrying the monogenic recessive high pigment (*hp-1*, *hp-1w*, *hp-2*, *hp-2*^{j} and *hp-2*^{dg}) mutations have been intensively studied in tomato (de Levin et al., 2006). It was suggested that *hp-2*, *hp-2*^{j} and *hp-2dg* represent different mutations in the gene encoding tomato homologue of the *Arabidopsis thaliana* DEETIOLATED1 (*DET1*), a negative nuclear regulator of photo-morphogenesis (Chory et al., 1989; Pepper et al., 1994; Mustilli et al., 1999; Benvenuto et al., 2002; Schroeder et al., 2002; Levin et al., 2003). *hp-1*, *hp-1*^{w} represent two different mutations in the gene encoding the tomato homologue of *A. thaliana* UV DAMAGED DNA BINDING protein 1 (*DDB1*), a protein which interacts, both genetically and biochemically, with *DET1* (Schroeder et al., 2002; Lieberman et al., 2004; Liu et al., 2004).The increased pigmentation of fruits of these mutants was attributed to significantly increased levels of carotenoids, primarily lycopene, in ripe red fruits. Because of their enhanced fruit lycopene content, *hp* mutations have been introgressed into several tomato cultivars, cultivated as novel 'Lycopene Rich Tomatoes' (LTR) (Wann, 1997) that enable cost-efficient lycopene extraction for industrial usage as a food colorant, and for cosmetics and nutraceuticals. This overproduction of carotenoids is associated with plastid biogenesis i.e. increased plastid number and compartment size in leaf and fruit cells (Liu et al., 2004; Bino et al., 2005; Kolotilin et al., 2007) and light signal transduction pathway genes, as direct regulation of phytoene synthase gene expression by phytochrome interacting factors has been reported (Toledo-Ortiz et al., 2010). Further, *Beta* (*B*) is a single dominant gene that increases β-carotene, and encodes lycopene β-cyclase in the fruit. The mutant *og*^{c} developed due to frame-shift mutation in the *B* gene that abolishes β carotene and increases lycopene (Ronen et al., 2000). Thus, *og*^{c} is a non-photomophogenic lycopene rich mutant.

Our current research focuses on completing a comparative profile on ripening linked genes of high pigment mutants along with a shelf-life enhancing mutant for future designing of hybrid/transgenic lines with nutritionally rich extended shelf-life fruit of high commercial value. Transcript abundance for carotenoid biogenesis genes in *hp-2*^{dg} (Kolotilin et al., 2007) and *hp-1* mutants (Kilambi et al., 2013) have been deciphered in plants grown under controlled conditions but the genes responsible for cell wall softening and ethylene production remain understudied. Moreover, the comparative expression study of fruit quality linked genes in these ripening mutants remains poorly explained. Again, the influences of non-photomorphogenic *og*^{c} mutation on expression level of genes linked to fruit ripening have not been determined previously. Henceforth, this lycopene rich mutant has also been included in the present study for characterization along with the other two photomorphogenic high pigment mutants. The effects of these spontaneous mutations on the expression of the major ripening regulator, the MADS-box transcription factor gene, *LeMADS-RIN* also warrants investigation. Additionally, expression levels of ripening genes in contrast to the non-ripening mutant *rin* also remain unaddressed.

We hypothesized that characterization of ripening related gene expression in carotenoid enriched and non-ripening mutants may provide a molecular foundation for formulating future hybridization programs, manipulating gene expression or utilization of this non-transgenic nutritional source to fortify with other fruit quality traits.

The current study has been designed with the motivation to understand kinetics of ripening-linked gene expression in high pigment (*hp-1*, *hp-2*^{dg}, *og*^{c}) and ripening inhibited (*rin*) mutants as compared to wild and Indian cultivated types of tomato lines. Moreover, presuming that the level of ripening linked gene expression may modify biochemical (concentration of β-carotene and lycopene content) and physical characters (pericarp firmness) of the fruits in these mutants, we unraveled the correlation of ripening linked genes with their respective traits.

2. Materials and methods

2.1. Plant material

Seeds of the experimental genotypes were obtained from a collection maintained at the Department of Vegetable Crops, Bidhan Chandra KrishiViswavidyalaya (BCKV), West Bengal, India, received from various

Table 2
Gene source and primer sequences (5′-3′) used in present study.

Sl No.	Gene	Genomic location	Forward primer	Reverse primer
1	*LeDXS*	Solyc01g067890.2.1	CCA GGT AGT GCA TGA CGT TGA	CTG CTC TGT CCA TTG CAA ACC
2	*LeDXR*	Solyc03g114340.2.1	CGC TGA GAA TCC GGA CAA GT	GCA AGA AGA GTC ACA TTT GAA CCA
3	*LeGGPPS2*	Solyc04g079960.1.1	CGC CGG CGG GAA GA	AAC AAG TTC ACA GGC AGC AAG A
4	*LeIPI*	Solyc05g055760.2.1	GCT GAA GAT GTC CCG GTT GA	TCA GAT GGA GCC TTG TAA AGC A
5	*LePSY1*	Solyc03g031860.2.1	TGT TAT GGG TTG TTT CTC CTT GTG	CTC CCG GAC TGA TTC CAT GA
6	*LePDS1*	Solyc03g123760.2.1	TGA GGA TGG AAG TGT CAA GAG TTT TA	ACA CAA AAG CAT CTC CCT CGA T
7	*LeZDS*	Solyc01g097810.2.1	GGT TTT TGG TGG TAC CCG TTT	CAG CCC GAA TAA CCG ACT TTC
8	*CYC-B*	NM_001247516.1	TTA TGG CAT TTT GGC TGA AGT G	GCC AAT CCA TGA AAA CCA TCT T
9	*LeACS2*	Solyc01g095080.2.1	GCC ATT GCC AAC TTT CAA GAT TA	CAT AAA TTT CGC AAT CGC TTT TCT
10	*ACS4*	NM_001247351.1	CTC CCC TGG ATC TTC GTT CA	AAG TGC GAT CTC CAT TGT TTG A
11	*LeACO1*	Solyc07g049530.2.1	CAC CAT GTC CTA AGC CCG ATT	GAT GCC TCC TGC GTC TGT ATG
12	*LePG*	Solyc10g080210.1.1	TCA AGA CTT GGC AGG GAG GAT	CTT AAC GTC TTG CAT TTC CAC ATT
13	*LeTBG4*	Solyc12g008840.1.1	TGG CCT GGA TAC ATA GCA CAA G	TCT CGT TGA ACG TTC CAG CAT
14	*LeEXP1*	Solyc06g051800.2.1	TGG GAA ACT GCA CAT GCT ACA	CAC CGC CCA TTG TTC CA
15	*PME*	GenBank: S66607.1	TAC TGA TCC CGC TAA AGC TAT GC	AAC CAT GAT CCG CCC TGA A
16	*LeMADS-RIN*	Solyc05g012020.2.1	GTC GTG GCA AGC TTT ATG AAT TTT	TGT ATC TGT GGT ATC TCT CCA ATG TCT
17	*UBQ3*	Genebank X58253.1	GCC GAC TAC AAC ATC CAG AAG G	TGC AAC ACA GCG AGC TTA ACC

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