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ANTISENSE EXPRESSION OF HETEROLOGOUS *MUSA ACUMINATA* ACC OXIDASE cDNA IN TOMATO (*SOLANUM LYCOPERSICUM* L.) FOR DELAYED RIPENING

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ABSTRACT

Over-ripening causes post-harvest losses in various climacteric fruits including tomatoes.. Antisense of banana 1-aminocyclopropane-1-carboxylate *ACC* oxidase (*MaACO*) cDNA was developed and expressed in tomato to silence the expression of its tomato counterpart, thereby to impede ethylene synthesis. Eleven putative transformants events were confirmed by PCR amplification, of which three events were able to bear ripened fruits. PCR confirmed T_1 plants were selected for raising the T_2 population. Reverse transcription-PCR analysis confirmed the expression of the antisense *MaACO* RNA in T_2 tomato plants. Delay in ripening was observed in the transgenic tomato, with 46.3 days required to attain red ripe stage as against 35.7 days in control plants. Higher titratable acidity (mean 0.76%) was observed during ripening in transgenic fruits over control fruits (mean 0.49%), indicating lower ethylene concentration in transgenic tomato. These results suggest the antisense expression of banana *ACO* is effective in delaying fruit ripening in tomato.

INTRODUCTION

Fruit ripening is a process occurring that results in dramatic changes in the fruit qualities. Ripening is desired to certain extent beyond which it becomes nuisance. Typical climacteric fruits undergo rapid ripening by an autocatalytic burst of gaseous hormone ethylene (Sahitya *et al.*, 2015). Such fruits like tomato (*Solanum lycopersicum*) and banana (*Musa acuminata*) are highly perishable resulting in post harvest losses due to surge in ethylene production concomitant with ripening. (Barua *et al.*, 2018). There is a need to develop methods for delaying ripening and extending the shelf life without affecting the fruit quality (Kumari *et al.*, 2016).

Ethylene, the ripening hormone is synthesized in two steps. Initially S-adenosyl methionine gets converted to 1-aminocyclopropane-1-carboxylic acid (ACC) by the action of ACC synthase (ACS), from which ethylene is synthesized by ACC oxidase (ACO). Strategies to increase the shelf life involve either altering the activities of enzymes involved in the ripening process like down regulating either ACC synthase and ACC oxidase, over expression of SAM hydrolase, ACC deaminase and ACC decarboxylase, altered perception by ethylene receptors or manipulating cell wall metabolism by suppressing polygalacturonase activity (Payasi and Sanwal, 2010; Bapat *et al.*, 2010; Liu *et al.*, 2020).

Tomato is the model crop to study climacteric fruit ripening due to ease of genetic manipulation as well to conduct genetic studies (Liu *et al.*, 2020). In tomato, ACO and ACS are encoded by a multigene family of five and nine members, respectively, each having distinct expression patterns (Bapat *et al.*, 2010). In tomato, antisense approach has resulted in ripening inhibition in fruit of ACC synthase and ACC oxidase antisense lines (Grierson, 2016; Liu *et al.*, 2020). Batra *et al.*, (2010) reported antisense suppression of ACC oxidase to prolong tomato shelf life using heterologous banana gene. Bolitho *et al.*, (1997) and Atkinson *et al.*, (1998) used antisense heterologous apple ACC oxidase to delay ripening in tomato fruits.

The present investigation was carried out to down-regulate the ethylene metabolism in tomato through suppressing ACC oxidase. As the *ACC oxidase* genes are highly conserved, antisense RNA based gene silencing by using other climacteric fruit like banana can be used to reduce ethylene production in tomato fruits. Therefore during the present investigation, *Agrobacterium*-mediated transformation in tomato (cultivar Dhanashree) with the antisense *banana ACC oxidase* (*MaACO*) was undertaken by optimizing various parameters with an attempt to develop transgenic tomato with delayed fruit ripening.

MATERIALS AND METHODS

Cloning of *MaACO* cDNA and development of its antisense cassette in binary vector

Total RNA was isolated from ripened banana cv Grand Naine fruit as per protocol by using Qiagen RNeasy Plant minikit.

Banana *ACC oxidase* (*MaACO*) gene specific forward primer (5'AAGAAAGAGCGTGTCATGGATTCCTTTC3') and reverse primer (5'TTGGGGGCTCTCACTTAAGAGGTAGCGAT3') were designed and custom synthesized. *MaACO* cDNA was synthesized as per protocol using by Qiagen One step RT-PCR kit (Reverse Transcription PCR) 10 ng of template RNA samples with 20 picomoles of *MaACO* primers. *MaACO* cDNA was cloned in pDrive PCR cloning vector (Figure 1). Homology searches were performed with searches limited to tomato (taxid: 4081) using the basic local alignment search tool (BLAST) from NCBI (http://www.ncbi.nlm.nih.gov/). Multiple sequence alignment of most similar tomato ACC oxidase accessions with their banana counterpart was carried out.

To develop antisense construct, gene orientation in pDrive PCR cloning vector was determined by using two pairs of primers viz., *MaACO* forward primer in combination with either M13 forward or M13 reverse primers. Out of seven different unique restriction sites available in multiple cloning site of pBinAR vector (Bevan, 1984) only *Xba*I and *Sal*I sites were found to be absent in the *MaACO* cDNA. Therefore new primers were designed with *Xba*I site on *MaACO-Xba*I reverse primer (5' GGCTCTAGATTAAGAGGTAGCGAT 3') and *Sal*I site on *MaACO-Sal*I forward primer (5' AAGGTCGACCGTGTCATGGATTCC 3'), for cloning in pBin-AR in antisense orientation. Recombinant antisense *MaACO*:pBin-AR plasmid (Figure 2) from *Escherichia coli* was further transformed into *Agrobacterium tumefaciens* (strain EHA105).

Plant material

Tomato seeds of the cultivar "Dhanshree" were collected from All India Coordinated Tomato Improvement Project, M.P.K.V., Rahuri. Seeds were surface sterilized in 4% sodium hypochlorite solution followed by washing several times with sterile distilled water. Seeds were germinated on a MS inorganic salt medium (Murashige and Skoog, 1962) containing 30g/l sucrose, pH 5.8 and solidified using 8g/l agar and kept at 25°C with a 16 hour light period and 8 hour dark period. To investigate the influence of explant age on transformation efficiency shoot tips and hypocotyls were aseptically excised from 7, 14 and 21 days old seedlings.

Transformation protocol

The *Agrobacterium* strain was grown for 48 hours in a LB (Luria Bertani) medium containing 50 mg/l kanamycin and 10 mg/l rifampicin at 28°C on a rotary shaker (200 rpm) until an OD_{600} = 1 was obtained. Bacterial suspension was pelleted at 8000 rpm for 10 min. Bacteria were resuspended in LB medium without antibiotics, diluted to OD_{600} = 0.2, 0.4, 0.6, 0.8 and used for co-cultivation. Shoot tips and hypocotyl explants were placed on preculture medium (MSB_5 + 2 mg/1 zeatin, 0.2 mg/1 IAA, 8g/l agar, pH 5.8) and incubated overnight at 27°C in the dark (Pawar *et al.*, 2012). Precultured explants were dipped in an *Agrobacterium* culture for 3 min, blotted dry on blotting paper and co-cultivated on the same medium for three days. Co-cultivated explants were further transferred to selective shooting medium (MSB_5 medium supplemented with 2 mg/l zeatin, 0.2 mg/l IAA, along with different combination of cefotaxime, carbenicillin and kanamycin concentrations). Explants were further sub cultured after every 12 days and allowed to grow for 5-6 weeks. The shoots having length greater than 2 cm were excised and transferred to rooting medium (MSB_5 medium supplemented with 25mg/1 kanamycin, 250 mg/1 cefotaxime and 250 mg/l carbenicillin). One hundred and fifty explants of each type were grown on selective medium in three replications. One set was kept as a control without co-cultivation.

In order to effectively screen transformants, explants were cultured on preculture medium supplemented with four different concentrations of kanamycin (0, 50, 75 and 100 mg/l). Different combinations of cefotaxime and carbenicillin (0, 250 and 500 mg/l) were used in controlling *Agrobacterium* overgrowth and accessing their influence on regeneration and transformation (Table 1 and 2).

Hardening of transformants and raising of further generations till T_2 population

Eleven transformed plantlets (antisense *MaACO* transformants) with well-developed roots were transferred to plastic pots containing cocopeat and kept in transgenic house. Plants were irrigated with half strength MS solution for 7 days and finally transferred to pots containing soil and cow dung (4:1) and irrigated with water at regular intervals. Out of eleven transformation events, only three were able to grow up to maturity and yield ripened fruits. The germinated T_0 seeds were grown under *in vivo* conditions in pot-trays filled with soil rite mix and used for PCR analysis for confirmation of transgene. The PCR confirmed T_1 generation seeds were grown in transgenic house and the T_2 population developed thereof was used for morphological, biochemical characterization and molecular analysis.

PCR assay

The genomic DNA of tomato was extracted from young leaves of putative transgenic plants of T_0, T_1 and T_2 generation. Presence of the introduced antisense *MaACO* was detected by using gene specific primers (*MaACO-Sal*I FP 5' AAGGTCGACCGTGTCATGGATTCC-3' and *MaACO-Xba*I RP-5'5' AAGGTCGACCGTGTCATGGATTCC-3'). PCR was carried out in 25 µl volumes containing 100 µM of dNTP mixture, 50 ng of each oligonucleotides primer, 2 mM $MgCl_2$, 0.33 Unit *Taq DNA polymerase* and 50 ng template DNA. The reaction mixture was subjected to the PCR profile of 35 cycles at (94°C for 45 s, 55°C for 60 s, and 72°C for 90 s) and final extension at 72°C for 10 min. The amplified fragments were electrophoresed on 1.2% (w/v) agarose gel. Recombinant plasmid DNA from *E. coli* served as control.

Antisense RNA Expression analysis of T_2 population of tomato

The pulp of 35-40 days old tomato fruits from T_2 population was used for total RNA isolation as per protocol using Qiagen RNeasy Plant minikit.. The extracted RNA was quantified spectrophotometrically (Nanodrop, NA-1000USA) and diluted to working concentration. Qiagen One step RT-PCR kit (Reverse Transcription PCR) was used for cDNA synthesis from 10 ng of template RNA samples with 20 picomoles of *MaACO* primers. Reverse transcription at 50°C for 30 min, was followed by hot start DNA polymerase activation at 95°C for 15 min. PCR regime involved 40 cycles of denaturation (94°C for1 min), annealing (55°C for 1 min) and elongation (72°C for 1 min). Final extension was allowed at 72°C for 10 min. and

4°C held up to retrieval. Amplified cDNAs were subjected to 1.2 % agarose gel and visualized in gel documentation system (m/s FlorChem™ Alpha Innotech, USA).

Morphological and biochemical characterization of ripened fruits of T_2 population

T_2 population was observed for their individual flowering to fruit ripening characteristics on each plant and compared with those of control non-transgenic plants. Flowers were tagged at anthesis and days were noted for fruit formation to mature green, breaker red (fruits displaying first sign of color change), and red stages as per Cantwell (2015). Quantitative estimation of reducing sugar in ripened fruits (35-40 days from flowering) was undertaken by Somogyi method (Nelson, 1944) and the intensity of blue coloration was measured calorimetrically at 620 nm. Titratable acidity content in ripened fruits (35-40 days from flowering) of both transgenic and control plants was determined by 0.1 N NaOH titration with phenolphthalein reagent (Silva *et al.*, 2004).

RESULTS AND DISCUSSION

Cloning of *MaACO* cDNA and development of its antisense cassette in binary vector

Agrobacterium with antisense *MaACO*:pBinAR construct was used for transformation in the present study. For this 980 bp banana ACC oxidase cDNA (*MaACO*) was initially cloned in PCR cloning pDrive vector (GenBank accession no. EU131109.1) (Figure 1), which had 927bp coding sequence. Its orientation was identified and desired *Xba*I and *Sal*I restriction sites were introduced by PCR re-amplification of cDNA with primers having restriction sites. *MaACO* cDNA was expressed under the control of cauliflower mosaic virus (CAMV35S) promoter and nopaline synthase (*nos*) terminator in pBinAR binary vector, in antisense orientation with initiation codon placed near *nos* terminator and termination codon placed near CaMV35S promoter (Figure 2). In order to check homologies of banana ACC oxidase (*MaACO*) with corresponding tomato counterparts' homology search was carried out. On megablast analysis none of the tomato accession showed high homology with *MaACO*. On discontiguous megablast analysis, 43 accessions showed identical homology upto 74% and query coverage upto 94%. On discontinuous megablast analysis, *MaACO* showed highest homology with LeACO6 represented by two accessions NM_001247709 and EF01822.1 (total score of 446 with 86% coverage and 72% identity), followed by *LeACO*4 (NM_001246999.1), *LeACO*2 (NM_001329913.1) and *LeACO*1 (NM_001247095.2). However, *LeACO*5 and *LeACO*3 showed very little homology with banana ACO.

It is consistent with previous results, except for *LeACO*3 gene. As per Jafari *et al.*, (2013) amongst the six *LeACO*s family members, *LeACO5* is the most divergent tomato *ACO* gene. *LeACO1, LeACO2 and LeACO3* proteins that shared a high degree of homology belonged to a group, whereas *LeACO4 and LeACO6* together were classified into another group. *LeACO1* showed highest identity with the sequence of *LeACO3* (93% identity). However, a comparison of all six *LeACO*s with *LeACO5* showed lower identity values of 48–50%.

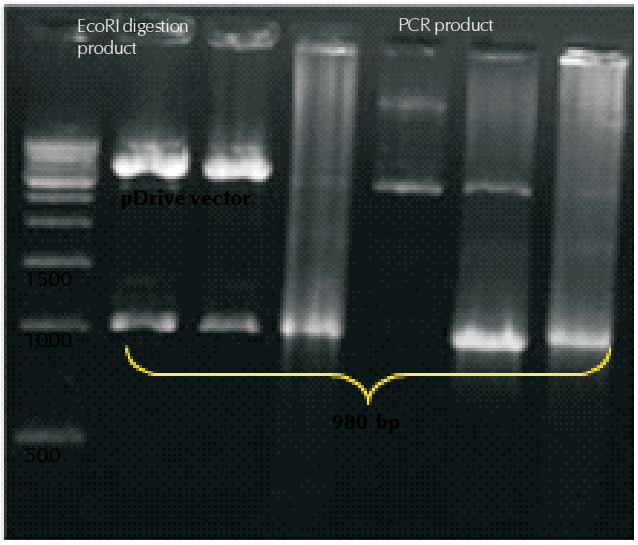
Multiple sequence alignment between tomato ACC oxidase accessions (excluding *LeACO*3 and *LeACO*5) and their banana counterpart (accession no. EU131109.1) is shown in Figure 3b. Homology between banana and tomato ACC oxidase genes suggest that antisense *MaACO* can be used for down-regulation of their tomato counterparts. Bolitho *et al.*, (1997) had observed that the antisense effect is not dependent on complete sequence homology, as divergence of more than 25% between the antisense apple gene and a tomato 'homologue' still produced an effect.

Czarny *et al.*, (2006) reported that due to higher conservation among *ACC oxidase* genes, use of an antisense *ACC oxidase* gene to reduce ethylene production in tomato fruits was successful. Among the five *LeACO* genes encountered in tomato, three of them were expressed in ripening fruit (Pech *et al.*, 2011). *LeACO1* and *LeACO4* gene display ethylene-dependent up-regulation and are expressed throughout fruit development with strongest up-regulation at the breaker-red ripe stage; while *LeACO3* is induced only during pre-climacteric matured green stage.

Transformation protocol

In the present study the MS medium supplemented with 2.0 mg/L zeatin + 0.2 mg/L IAA was successfully used for preculturing and shoot induction.In our previous study (Pawar *et al.*, 2012) the same media was found to be the best regeneration medium in tomato. Borgohain *et al.*, (2018) reported that the frequency of shoot initiation and shoot numbers were more prominent on zeatin supplemented media (2-5 micromolar zeatin with 0.1 μM IBA); while lowering the zeatin dose was not favourable for shoot initiation and elongation. Recently Park *et al.* (2020) also reported enhanced shoot production on media supplemented with 1.0 mg/L zeatin and 0.1 mg/L IAA.

The explants grew very well in control medium (without kanamycin) and their survival decreased with increase in kanamycin concentration as well as duration of culture.



Lane1: StepUp 500 bp DNA Ladder; Lane 2-3 *EcoRI* digested *MaACO*:pDrive; Lane 4-7: PCR amplification product of *MaACO*:pDrive

Figure 1: Confirmation of *MaACO* cDNA cloning into pDrive vector

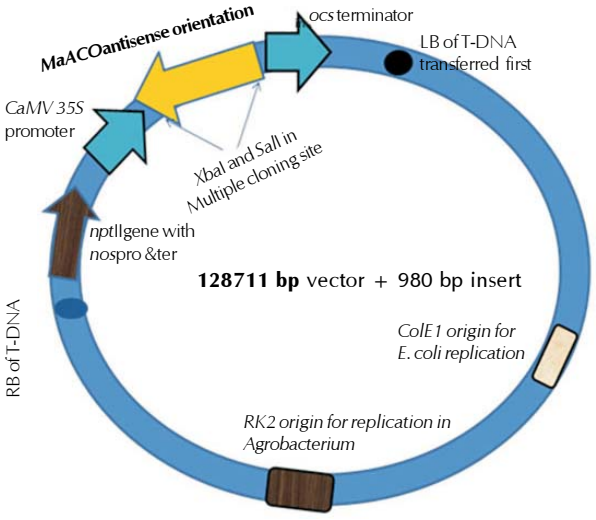


Figure 2: Schematic diagram of Antisense *MaACO*:pBinAR transformation construct

Kanamycin at a concentration of 50 mg/l caused total inhibition of uninfected explants after three weeks; while at higher concentrations (100 mg/l and 75 mg/l) they did not survive beyond two weeks. Thus, minimum lethal concentration of 50 mg/l kanamycin for three weeks was found optimum to screen putative transformants.

Agrobacterium concentration with OD_{600} = 0.2 yielded highest transformation efficiency (11.47 % and 9.33 %) with shoot tip and hypocotyl explants, respectively (Figure 4). Transformation efficiency decreased with increase in concentration of *Agrobacterium*. Gao *et al.*,(2009) also reported that *Agrobacterium* cell density during infection was found to influence transformation efficiency and obtained highest transformation efficiency using *Agrobacterium* density of OD_{600}0.1.

Hu and Phillips, (2001) identified overgrowth-control antibiotics as the most important variable which influenced both the regeneration and transformation efficiency.In control (without antibiotics), uninfected shoot tip and hypocotyl explants gave highest regeneration frequencies (89.6 and 88.3 %), respectively (Table 1 and 2). Limited reduction in regeneration frequency was observed on medium supplemented with 250 mg/ml cefotaxime and 250 mg/ml carbenicillin, while at 500 mg/l concentration, the regeneration frequency reduced to 71.2 %.

In co-cultivated explants, *Agrobacterium* overgrowth was high when cefotaxime/carbenicillin was used individually. However, their combination (250 mg/ml each) was effective in controlling bacterial overgrowth without significantly influencing regeneration. Pawar *et al.,* (2013) also observed that the combination of cefotaxime and carbenicillin was effective in eliminating *Agrobacterium*.

Shoot tip explants from 7 days old seedling exhibited higher transformation efficiency (11.47%), while those from 14 and 21 days old seedlings had lower transformation efficiency (7.20% and 4.53%, respectively) (Figure 5). Similar trend was observed with hypocotyl explants. Gao *et al.*,(2009) used explants excised from 6-10 days old tomato seedlings for efficient genetic transformation. Borgohain *et al.*, (2018) found that cotyledonary leaf explants from 8 day old tomato seedling gave efficient transformation.

PCR analysis of T_0, T_1 and T_2 transgenic tomato plants

Eleven transgenic lines at T_0 stage exhibiting rooting on kanamycin supplemented medium were analyzed for the presence of antisense *MaACO*. Oligonucleotide primers specific to *MaACO* gene amplified the expected size band of 980 bp in all eleven putative T_0 transformants (Figure 6). These T_0 transformants were subjected to hardening and grown up to maturity in transgenic house. Out of eleven only three events were able to yield ripened fruits and produce viable progenies. Out of twenty T_1 plants seventeen plants showed PCR amplification and the seeds from these individual PCR positive plants were harvested separately. Out of seventeen T_2 plants fourteen plants showed PCR amplification.

Expression analysis of T_2 population of tomato

The antisense *MaACO* expression was studied by synthesizing cDNA using total RNA from fourteen transgenic plants. Gene expression was observed with thirteen of fourteen transgenic T_2 tomato plants yielding desired 980bp cDNA (Figure 7). This confirmed that the antisense *MaACO* was successfully transformed and expressed in transgenic plants. Grierson (2016) reviewed various reports on application of antisense technique for silencing ripening genes in tomato. Batra *et al.*, (2010) also used banana antisense ACC oxidase for suppression of its tomato counterpart to conclude that heterologous gene provide can prolong on-vine and off-vine shelf life of tomato. Ye *et al.*, (2018) recorded that ethylene production was reduced against expectation in tomato flowers, leaves, and mature fruits on expression of the non climacteric grapes *ACS1* gene; while altering balance between roots and shoots; .

Morphological and biochemical characterization of ripened fruits of T_2 population

Individual T_2 transgenic tomato plants as well as control plants were observed for their individual fruit ripening characteristics. Variable delay in ripening was observed in the transgenic tomato lines studied. In most of the transgenic plants fruits matured till red ripe stage. On an average transgenic fruits required 21.3, ~31.7 and 46.3 days after flowering to develop into mature green, breaker red and red ripe stages, respectively. On the contrary control non-transgenic fruits required 20.7, 29.0 and 35.7 days to develop into mature green, breaker red and red ripe stages, respectively. However, fruits from transgenic plant exhibiting slowest ripening with delay in the developmental characteristics like fruit colour and rigidity are presented in Figure 8. Most of the fruits of this transgenic plant exhibited cracking and continued to remain in Pink to light Red stage even up to terminal stage of plant growth.

Similarly, Bolitho *et al.*, (1997) had reported variable levels of reduction in ethylene production (by 3.5 to 92 %) in antisense apple ACC-oxidase transgenic tomato fruits. Fruits of the transgenic plants were more firm than non-transgenic counterparts during ripening. Gupta *et al.*,(2013) had suggested that the RNAi based down-regulation of ACS homologs can be an effective approach for obtaining delayed

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EU131109.1__MaACO_mRNA              ATGGATTCCTTTCCGGTTATCGACATGGAGAAGCTTTTGGGAAGGGAGAGAGGAGCAGCC
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NM_001329913.1_LeACO2_mRNA         ATGGAGAATTTCCCAATTATCAACTTGGAAAAACTCAATGGTGCTGAGAGAGTAGCCACA
NM_001247095.2_LeACO1_mRNA         ATGGAGAACTTCCCAATTATTAACTTGGAAAAGCTCAATGGAGATGAGAGAGCCAACACC
NM_001246999.1_LeACO4_mRNA         ATGGAGAACTTCCCAATTATCAACTTGGAAAATCTTAATGGAGATGAGAGAGCCAAAACC
                                                        **** *  **          * ** *      *

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NM_001329913.1_LeACO2_mRNA         ATGGAAAAGATTAATGATGCTTGTGAAAATTGGGGCTTCTTTGAGTTGGTGAACCATGGA
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NM_001246999.1_LeACO4_mRNA         ATGGAAATGATCAAAGATGCATGTGAGAATTGGGGCTTCTTTGAGTTGGTGAACCATGGG
                                    **** *   *     ***** ** ** ** ***** ********* *  * ** *****

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NM_001246999.1_LeACO4_mRNA         ATGGAACAGAGGTTTAAGGAATTGGTAGCAAGTAAGGGACTTGAAGCTGTGCAAGCTGAG
                                   * *** ** *  ** ** **  * *     *  ** *  ** **    *   *    **

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NM_001246999.1_LeACO4_mRNA         TTGGAGAAGTTGGCTGAGGAGTTACTTGACTTACTCTGTGAAAATCTTGGACTTGAAAAA
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NM_001247095.2_LeACO1_mRNA         GGTTACTTGAAAAATGCCT—TTTATGGATCAAAAGGTCCCAACTTTGGTACTAAAGTT
NM_001246999.1_LeACO4_mRNA         GGTTATTTGAAAAATGCCT—TTTATGGATCAAAAGGTCCCAATTTCGGTACTAAAGTT
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NM_001247095.2_LeACO1_mRNA         AGCAACTATCCACCATGTCCTAAGCCCGATTTGATCAAGGGACTCCGCGCTCATACAGAC
NM_001246999.1_LeACO4_mRNA         AGCAACTATCCACCATGTCCTAAGCCCGATTTGATCAAGGGACTCCGCGCTCATACAG
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Figure 3: Multiple sequence alignment between *MaACO* (accession no. EU131109.1) and corresponding *tomato ACC oxidases (LeACO1/2/4/6)*

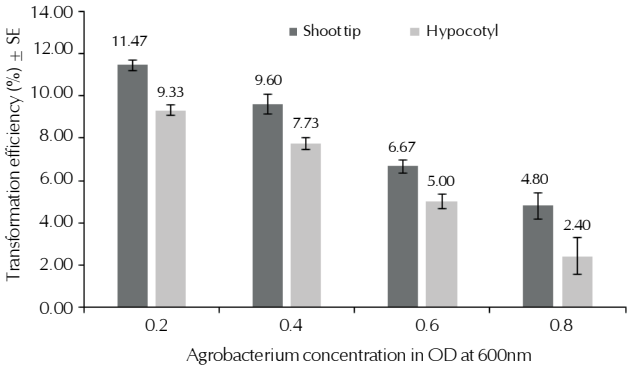

Figure 4: Effect of *Agrobacterium* concentration on the transformation efficiency in tomato

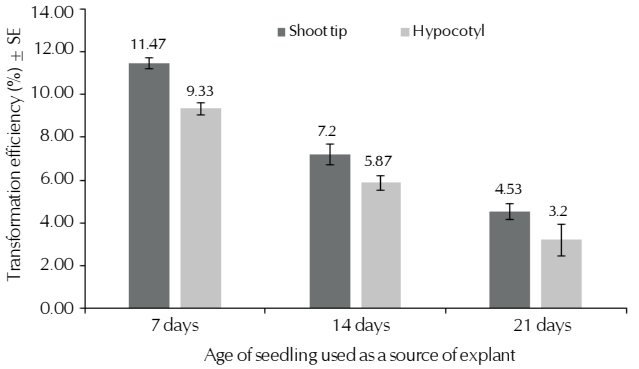

Figure 5: Effect of explant age and type on transformation efficiency in tomato

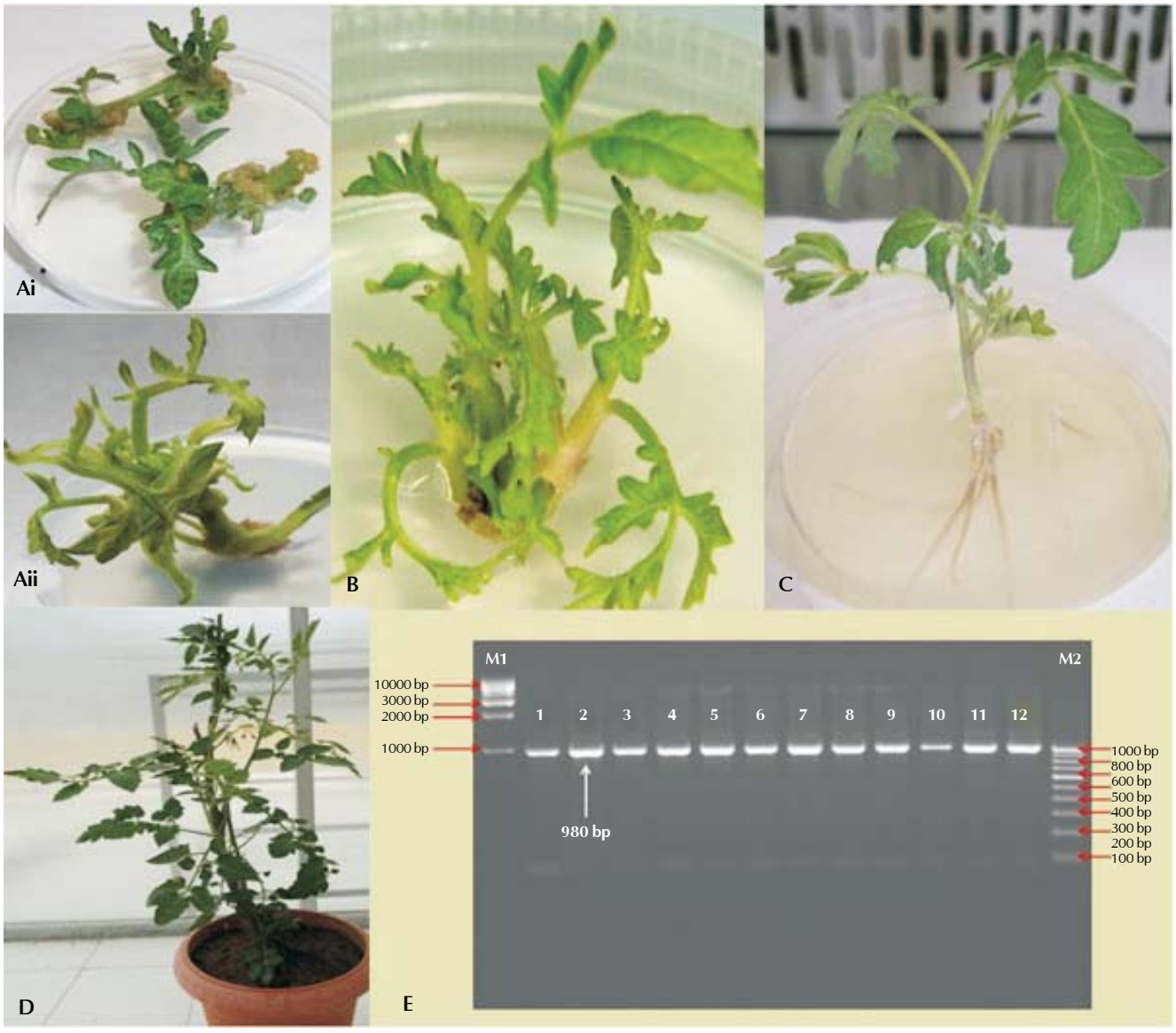

Figure 6 - (A) Shoot initiation on selective shooting medium (B) Multiple shoot formation (C) Root formation from regenerated shoot (D) Hardening of regenerated plantlets (E) Confirmation of presence of transgene in T_0 generation using gene specific primers

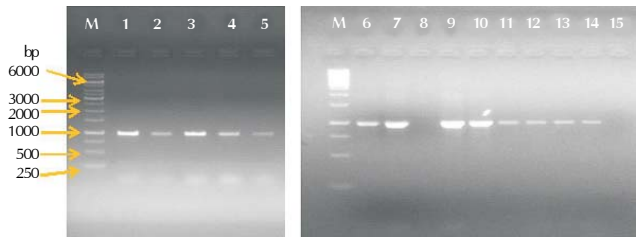

Figure 7: RT-PCR analysis showing antisense *MaACO* expression in T_2 transgenictomatoes (M: 1kb ladder; Lane 1-14: T_2 transgenics; Lane 15: Control non transgenic tomato)

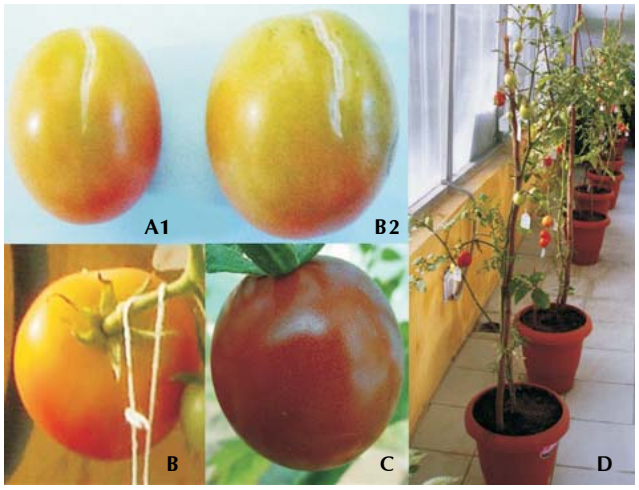

Figure 8: Antisense *MaACO* transgenic fruits showing variation in ripening on 35 days after flowering. (A1 to B: Fruits of transgenic plants; C: Fruits of control, non transgenic plant; D: Transgenic plants raised in containment house)

ripening with extended shelf life for ~45 days. As per Jafari *et al.*, (2013) amongst the six *LeACO*s family members, only three were expressed differentially in fruit tissues. *LeACO1* and *LeACO4* expressions sustained during ripening, while, *LeACO3* expression was low and transitory. Further the accumulation level of the *LeACO1* transcript in all cultivars and all stages was high.

During the process of fruit ripening various biochemical changes take place like conversion of starch into sugar, decrease in acidity, production of aroma/volatiles (alcohol esters). The non-transgenic plant showed the total reducing sugar content concentration of (2.9 to 3.3 %), while the transgenic plants of T_2 generation showed similar range of sugar expression (2.5 to 3.1 %) (Figure 9). The titratable acidity remained high in transgenic fruits (mean 0.76 %), as against control fruits (mean 0.49 %). High titratable acidity in transgenic tomato indicates lower concentration of ethylene. Mandal *et al.*, (2016) reported that salicylic acid treated banana fruits with slightly enhanced shelf life under storage had slightly higher titratable acidity and total sugar. Higher respiration rates cause significant loss in fruit quality during the postharvest storage (Sen *et al.*, 2014). Therefore higher accumulation of titratable acidity in transgenic tomatoes is likely due to lower rate of respiration. Gupta *et al.*, (2013) reported that RNAi-ACS tomato fruits with longer shelf life were found to bear

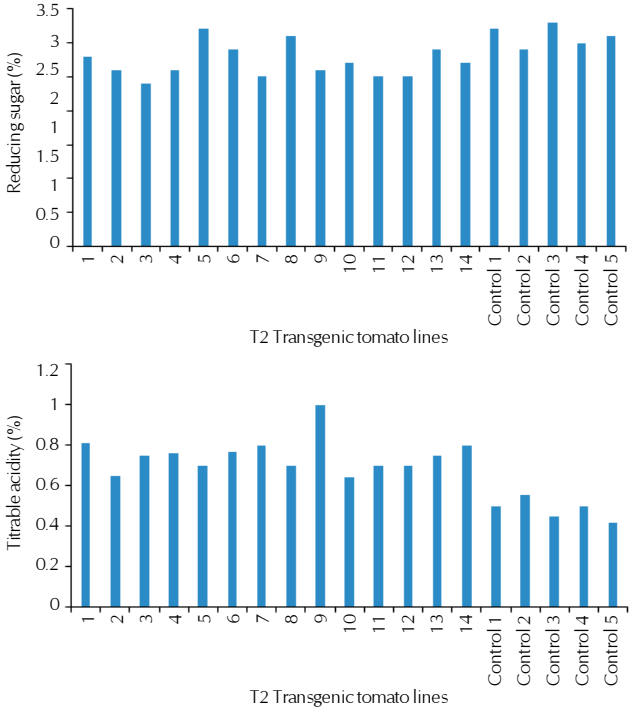

Figure 9: Mean composition of reducing sugar and titratable acidity in transgenic lines and control plants of tomato

~1.5–2.0 fold increase in titratable acidity over controls. They suggested that simultaneous increase in levels of total soluble solids and titratable acidity might provide a characteristic flavor for transgenic fruits. Similarly, Silva *et al.*, (2004) reported a sharp decrease in titratable acidity after 25 DAA, but the titratable acidity remained high in AS3 transgenic melon fruits. The titratable acidity and ethylene concentration show an inverse relationship.

CONCLUSIONS

In the present experiment, antisense of *MaACO* cDNA from ripened banana fruit pulp was expressed in tomato. *Agrobacterium* mediated transformation yielded antisense *MaACO* transformed tomato plants that were confirmed by PCR analysis in T_0, T_1 and T_2 generations, while RT-PCR based expression analysis of antisense *MaACO* was confirmed in T_2 population . Antisense *MaACO* transgenic tomatoes exhibited slower ripening process due to down regulation of ethylene synthesis, which could be of used for improving shelf life. Delayed harvests without any losses in fruit quality can prolong harvesting and marketing duration.

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