



MASTER'S THESIS

Analysis of changes in carotenoid biosynthesis in micro-tom tomatoes introduced with citrus *CCD1A* gene.

Dong-sik Jeong

Department of Biomaterials Science and Technology

GRADUATE SCHOOL

JEJU NATIONAL UNIVERSITY

August, 2021



Analysis of changes in carotenoid biosynthesis in micro-tom tomatoes introduced with citrus *CuCCD1A* gene.

지도교수 김 인 중

정동식

이 논문을 이학석사 학위논문으로 제출함

2021 년 8 월

정동식의 이학 석사학위 논문을 인준함



제주대학교 대학원 2021 년 8월



Contents

2. Materials and Methods ······ 5 2-1. Plant materials 2-2. Genomic DNA extraction from seed. 2-3. Genomic DNA extraction from leaves. 2-4. Total RNA extraction and DNase treatment 2-4-1. RNA extraction 2-4-2. DNase treatment 2-5. PCR (Polymerase Chain Reaction) & Electrophoresis 2-5-1. Genotyping of T3 S-CuCCDA1 / T3 AS-CCDA1 lines from single seed 2-5-2. Genotyping of T3-S-CuCCDA1 / T3-AS-CuCCDA1 lines from leaf 2-5-3. cDNA synthesis 2-6. Anti-oxidant activity analysis 2-6-1. Total carotenoid content 2-6-2. Total Flavonoid content 2-6-3. Total Phenolic Content 2-6-4. DPPH radical scavenging 2-7. HPLC(High Performance Liquid Chromatography) 3-1. Genotyping analysis of T3-S/AS-CuCCDA1 transformant lines using genomic DNA from single seed 3-2. Genotyping analysis of T3-S/AS-CuCCDA1 transformant lines using genomic DNA from single seed 3-3. Total RNA extraction and DNase treatment 3-4. RT-PCR 3-5. Anti-oxidant activity analysis 3-5-1. Total carotenoid content 3-5-1. Total Flavonoid content 3-5-2. Total Phenolic Content 3-5-3. DPPH radical scavenging

3-6. HPLC(High Performance Liquid Chromatography)

4. Summary and Conclusions ······60



List of Figure

Fig 1. carotenoid biosynthesis pathway
Fig 2. Transformation vector pCAMBIA 2300·····5
Fig 3. Sampling and color difference measurement by leaf, flower and fruit development
Fig 4. Genomic DNA extraction from Seed. A. Sense lines 3-5-12, 6-13-29 and 20-9-20, B. Anti-Sense lines 9-6-8, 13-2-17, 5A-6-720
Fig 5. Genomic DNA extraction from Leaf. A. Sense lines 3-5-12, 6-13-29 and 20-9-20, B. Anti-Sense lines 9-6-8, 13-2-17, 5A-6-721
Fig 6. RNA extraction and DNase treatment. ·····24
Fig 7. Homology of <i>CuCCD1A</i> and <i>SLCCD1A</i> 27
Fig 8. Electrophoresis of CuCCD1A······28
Fig 9. Electrophoresis of <i>SLCCD1A</i> ······ 28
Fig 10. Homology of <i>CuCCD1A</i> and <i>SLCCD1B</i> 29
Fig 11. Electrophoresis of <i>SLCCD1B</i>
Fig 12. Homology of <i>CuCCD1A</i> , <i>SLCCD4</i> , <i>SLCCD4–1</i> ······31
Fig 13. Electrophoresis of <i>SLCCD4</i> and <i>SLCCD4-1</i> 32
Fig 14. Homology of CuCCD1A, SLCCD7, SLCCD8
Fig 15. Electrophoresis of <i>SLCCD7</i> and <i>SLCCD8-2</i> 35
Fig 16. Homology of CuCCD1A, SLCCD(9' 10')like, SLCCD(9' 10')-1 like, SLCCD(9' 10')-1 like like2 37
Fig 17. Electrophoresis of <i>SLCCD</i> (9'10') Like, <i>SLCCD</i> (9'10')-1 Like and , <i>SLCCD</i> (9'10')-1 Like2.
Fig 18. Gene expression analysis from LCYB1 to VDE in carotenoid biosynthesis.
Fig 19. Electrophoresis results from SLLCYB1 to SLVDE



Fig 20. Total carotenoid content of control MT, Sense line, Anti sense line
Fig 21. Total flavonoid content of control MT, Sense line, Anti sense line
Fig 22. Total Phenolic content of control MT, Sense line, Anti sense line
Fig 23. DPPH radical scavenging activity of control MT, Sense line, Anti sense line
Fig 24. HPLC on leaves among MT, Sense, and Anti sense lines
Fig 25. HPLC on Flower among MT, Sense, and Anti sense lines
Fig 26. HPLC on Immature green among MT, Sense, and Anti sense lines
Fig 27. HPLC on Mature green among MT, Sense, and Anti sense lines
Fig 28. HPLC on Turning among MT, Sense, and Anti sense lines
Fig 29. HPLC on Orange among MT, Sense, and Anti sense lines
Fig 30. HPLC on Orange-Red among MT, Sense, and Anti sense lines
Fig 31. HPLC on Red among MT, Sense, and Anti sense lines



List of Table

Table 1. PCR condition of T3-S-CuCCDA1 seed. 12
Table 2. PCR condition of T3-AS-CuCCDA1 seed.
Table 3. PCR condition of T3-S-CuCCDA1 leaf······14
Table 4. PCR condition of T3-AS-CuCCDA1 leaf······14
Table 5. RT-PCR Primer·····16
Table 6. RT-PCR condition 17
Table 7. Nucleic acid concentration of MT and Sense. 25
Table 8. Nucleic acid concentration of Anti-sense 26
Table 9. HPLC condition 51



Abstract

During the ripening process of citrus fruits, the orange color is caused by the accumulation of carotenoid pigments. Through differentially expressed gene(s) screening, the carotenoid cleavage dioxygenase (CCD) gene was isolated as a pigment-related gene with higher expression than the control group through gene analysis in the citrus eggplant mutant red fruit gene and in the citrus control group. CCD, which decomposes carotenoid metabolites, is an enzyme that catalyzes the reaction of bonding two oxygen atoms to a substrate by cleaving the double bond of C40 carotenoid. Four types have been reported in plants so far. CCD1 and CCD4 have broad substrate specificities ranging from phytoene to neoxanthin, CCD7 and CCD8 are involved in the synthesis of stringolactone from β -carotene, and 9-cis-epoxycarotenoid dioxygenases NCED2, NCED3, NCED5, NCED6, and NCED9 produce abscisic acid.

In this study, the expression changes of carotenoid biosynthesis genes were analyzed in transgenic Micro-Tom tomatoes isolated from citrus fruits introduced with CCD1A gene, total carotenoid content, flavonoid content, and phenolic compound content were analyzed, and antioxidant activity was analyzed to analyze CCDA1 The influence by genes was investigate.



1. Introduction

Citrus fruits are one of the plants growing worldwide, including Brazil, the United States, China, Mexico, Spain, India, and Asia. About 150 million tons of citrus fruits were cultivated in the 2000s, playing an important role in the diet (Liu, Heying et al. 2012).

Citrus fruits are composed of many compounds, such as vitamins A, C, E, carotenoids, and pectins, which have antioxidant properties. The reactive oxygen species is partially reduced as one electron e- is transferred to the oxygen molecule O2 to form a superoxide radical, and the superoxide radical thus formed can generate different reactive oxygen species, hydrogen peroxide and hydroxyl radical, respectively (Halliwell 1991). Free radicals accumulate in the body, causing cancer, (Liou and Storz 2010), inflammation and tissue damage (Mittal, Siddiqui et al. 2014), Aging (Stadtman 2004). According to the results of a recent study, it has been reported that antioxidant activity in citrus varieties depending on the degree of coloration of the fruit (Zacarías-García, Rey et al. 2021).

In the methylerythritol phosphate (MEP) pathway among carotenoid biosynthesis pathways, 1-deoxy-D-xylulose 5-phosphate synthase (DXS) utilizes isoprenoids formed through the reaction of DA3P with pyruvate to form isopentenyl pyrophosphate (IPP) and allylic isomer dimethylallyl pyrophosphate (DMAPP) and mak- 1 -e other plastids as well as substrates for carotenoid biosynthesis (Hermanns, Zhou et al. 2020).

In the first step of carotenoid biosynthesis, phytoene synthase (PSY) produces



- 1 -

phytoene, a C40 carotene, and lycopene is produced through several desaturation and isomerization steps catalyzed by phytoene desaturase (PDS) and Z-carotene desaturase (ZDS) (Yuan, Zhang et al. 2015).

In All-trans-lycopene, it is divided into LCYE and LCYB. In LCYE, Lutein is formed through α -carotene and δ -carotene, and in LCYB, γ -carotene and β -carotene are produced. β -Cryptoxanthin and zeaxanthin are catalyzed by β -carotene hydroxylase (CHYB), zeaxanthin epoxidase (ZEP) acts on zeaxanthin and antheraxanthin to produce violaxanthin, and in the case of violaxanthin de-epoxydase (VDE), it acts in the reverse direction.(Yuan, Zhang et al. 2015, Hermanns, Zhou et al. 2020). Violaxanthin to Neoxanthin conversion occurs by Neoxanthin synthase (NXS), and Violaxanthin and Neoxanthin form abscisic acid (ABA) by 9-cis-epoxycarotenoid dioxygenase (Fraser, Enfissi et al. 2009).





Fig 3. carotenoid biosynthesis pathway.

MEP, methylerythritol phosphate; GA3P, Glyceraldehyde 3-phosphate; IPP, isopentenyl diphosphate;



DMAPP, dimethylallyl diphosphate: GGPP, geranylgeranyl diphosphate; DXS, 1-deoxy- D -xylulose 5phosphate synthase; DXR, 1-deoxy- D -xylulose 5-phosphate reductoisomerase; GGPPS, GGPP synthase; PSY, phytoene synthase; PDS, phytoene desaturase; Z-ISO, ζ - carotene isomerase; ZDS, ζ -carotene desaturase; CrtISO, carotenoid isomerase; LCYE, lycopene ε -cyclase; LCYB, lycopene β -cyclase; BCH, β -carotene hydroxylase; CYP97A, cytochrome P450 carotene β -hydroxylase; CYP97C, cytochrome P450 carotene ε -hydroxylase; ZEP, zeaxanthin epoxidase; VDE, violaxanthin de-epoxidase; NXS, neoxanthin synthase; CCD, carotenoid cleavage dioxygenase; NCED, 9- *cis* epoxycarotenoid dioxygenase; ABA, abscisic acid.

CCD (carotenoid cleavage dioxygenase) family CCD1 and CCD4 have broad substrate specificities ranging from phytoene to neoxanthin, and in the case of CCD7 and CCD8, they are involved in the synthesis of stringolactone from β carotene. 9-cis-epoxycarotenoid dioxygenase NCED2, NCED3, NCED5, NCED6, and NCED9 are involved in the production of the plant hormone Abscisic acid. As a result of isolating only the pigment-related genes with high expression in the control group through gene analysis of the red fruit gene from citrus eggplant mutation and the citrus control group, ZDS (Zeta-carotene desaturase), CCD1A (carotenoid cleavage dioxygenase), F3M (flavonoid 3) '-monooxygenase), MDDX decarboxylase), C4H2 (mevalonate diphosphate and (trans-cinnamate 4monooxygenase) were selected. In this study, the expression changes of carotenoid biosynthesis genes in transgenic Micro-Tom tomatoes into which the CCD1A gene isolated from citrus was introduced. was analyzed, the total carotenoid content, flavonoid content, and phenolic compound content were analyzed, and the effect of CCDA1 gene was investigated by analyzing antioxidant activity.



- 4 -

2. Materials and Methods

2-1. plant materials

Plant selection genes in the pCAMBIA2300 vector are driven by a double-enhancer version of the CaMV35S promoter and terminated by the CaMV35S polyA signal. This vector contains minimal heterologous sequences for plant transformation and selection of transformants(Fig 2).



Fig 4. Transformation vector pCAMBIA 2300

CaMV35S : cauliflower mosaic virus(*CaMV*) 35S promoter npt II : Neomycin phosphotransferase





Fig 3. Sampling and color difference measurement by leaf, flower and fruit development. For each line, flowers, leaves, and fruits were sampled in the order of immature green, mature green, turning, orange, orange-red, and red according to the development process.

The results of color difference by fruit development analyzed by Konica minolta's color difference machine are as follows (Fig. 3). Control-immature green showed the values of L(65.13 ± 1.25), A(-12.08 ± 1.58), and B(20.74 ± 2.19), and mature green showed L(65.23 ± 2.13), A(-9.03 ± 1.85), It showed a value of B (18.59 ± 2.67). In the



- 6 -

case of turning, the values of L(61.81±4.42), A(-5.90±2.9), and B(24.73±3.03) were shown, and Orange was L(49.08±1.91), A(14.41±1.86), B(26.18±1.26) was shown. Orange-red showed color differences of L(40.81±1.88), A(24.77±5.41), and B(20.90±1.68), and in case of red, L(37.96±0.48), A(30.34±1.16), B(18.31± 0.34) was shown. As a result of showing the average color difference values of three lines 3-5-12, 6-13-29, and 20-9-20 for the sense line, the sense line-Immmature green is L(64.52±0.29), A(-11.58± 0.93) and B(20.12±0.24), mature green showed L(63.52±1.23), A(-10.48±0.23), and B(18.89±0.56) color differences. Turning showed $L(58.79\pm2.19)$, A(-6.09±1.16), B(24.67±2.45) color difference, and for Orange, L(47.71±0.58), A(11.32±1.98), B(25.31±0.29) seemed. For Orange-red, L(40.46±2.20), $A(18.31\pm0.66), B(20.17\pm1.84), \text{ for Red, } L(35.86\pm1.31), A(22.28\pm1.06), B(16.61\pm1.26)$ value was indicated. In the case of anti-sense, as a result of obtaining the average color difference values of 3 lines 9-6-8, 13-2-17, 5A-6-7, anti-senseimmature green is $L(65.42\pm0.71)$, $A(-11.89\pm0.14)$ and $B(20.11\pm0.12)$, and for mature green, L(64.97±1.77), A(-9.51±0.17), and B(24.96±0.69) values were shown. Turning showed color differences of L(59.99±1.91), A(-5.27±0.99), and B(24.96±0.69), and Orange showed L(46.56±1.34), A(11.34±2.74), B(24.00±0.77). For orange-red, L(40.6±1.96), A(18.14±1.79), B(19.98±1.72), for red, L(36.31±1.59),



- 7 -

A(22.83±1.82), B(17.05±1.28) value was indicated.

2-2. Genomic DNA extraction from seed.

DNA extraction from seeds was performed using the method of DiaStarTM Direct Multiplex/Fast PCR for plant (Solgent company). Lysis buffer is composed with a 60% PEG8000 and 20mM NaOH (pH 13.3). The seeds of the T2 generation are put into a new tube and the seeds are crushed so that the seeds are broken well. Then, $200 \,\mu$ L of lysis buffer is added to the tubes and vortexed for about 5 seconds. Samples are precipitated by spin down and incubate at room temperature for 3 to 5 minutes, followed by centrifugation at 13000 rpm for 2 minutes. Transfer 100 μ L of the supernatant into a fresh tube.

2–3. Genomic DNA extraction from leaves.

DNA extraction from leaves was used by modifying the method of DNeasy plant mini kit (QIAGENE company). Place the leaves into a 1.5mL fresh tube with 1-2 stainless steel beads, and than added 400 μ L DNA extraction buffer, AP1 buffer(aka Lysis buffer) : 1mM EDTA (ethylene diamine tetraacetic acid, pH 8.0), 0.1% SDS solution (Sodium Dodecyl Sulfate, C₁₂H₂₅SO₄Na), 0.1M NaCl and 10mM Tris-HCl (pH 8.0) are required. After vortexing, incubate mixture for 10min at 65°C. Mix 2~3 times during incubation by inverting tube. Add 130 μ L of buffer AP2(aka Protein precipitation buffer) ; 3M potassium/5Macetate made by adding 60mL of 5M potassium acetate (98.14g in 200mL D.W.), 11.5mL of glacial acetic



- 8 -

acid, 28.5mL D.W. to the lysate, mix and incubate for 10min on ice. Centrifuge for 6min at 13000rpm and then, trans fer flow-through fraction to a fresh tube, without disturbing the pellet(About 300 μ L of lysate is typically recovered). Add 1.5x volumes Buffer AP3/E(aka binding buffer) 1M of ; Guanidine Hydrochloride(4.78g in 50mL 100%EtOH), apply 700 μ L of the mixture including any precipitate to the spin column sitting in a 2mL collection tube. Centrifuge for 1min at 8000rpm. Discard flow-through and reuse the collection tube. Add 500 μ L Buffer AW; 70% EtOH to the spin column and centrifuge 1min at 8000rpm, discard flow through and reuse the collection tube. Add 500 μ L 100% EtOH to spin column and centrifuge for 2min at 13000rpm, discard flow through and centrifuge again at 13000rpm for 1min to dry. Carefully remove the spin column from the collection tube and transfer to 1.5mL tube and 50 μ L of TE buffer directly onto the membrane. Incubate for 5min at room temperature and then centrifuge 6000rpm for 1min.

2-4. Total RNA extraction and DNase treatment

2-4-1 RNA extraction

The sample was stored at -80°C, and 0.2 g of powder was used by grinding the sample with a mortar. RNA추출은 Sabzevari, A. G., & RNA extraction was performed by Hosseini, R.(Sabzevari and Hosseini 2014) RNA extraction was modified from the method of Sabzevari, A. G. et al. Put 0.2g of sample powder into 2mL fresh tube, RNA Extraction Buffer; 100mM Tris-HCl pH 8.0, 10mM EDTA

- 9 -



(ethylene diamine tetraacetic acid, pH 8.0), 0.1M LiCl, 1% (w = v) SDS (Sodium Dodecyl Sulfate, C12H25SO4Na) 600µL was injected and vortexed. 600 µL of PCI (Phenole; Chlorofom; Isoamylalcohol/25:24:1) was added to the mixed solution and vortexed, followed by centrifugation at 14,620 rpm (20,000 g) for 10 minutes. The supernatant was transferred to a 1.5mL fresh tube, mixed with 500 µL of CI (Chloroform: Isoamylalcohol/24:1), vortexed, and centrifuged at 14,620 rpm (20,000g) for 10 minutes. The supernatant was transferred to a 1.5mL fresh tube, and 270 µL of Ice-Cold 8M LiCl was added, inverted, and stored at -80°C for 30 minutes, and then centrifuged at 14,620rpm (20,000g) for 30 minutes. After removing all of the centrifuged solution, 200 µL of DEPC, 0.1 volume of 3M sodium acetate (pH 5.2), and 2 volume of ice-cold absolute ethanol were mixed and stored at -80° C for 30 minutes, they were washed with ice-cold 70% ethanol, dried to evaporate ethanol, and dissolved in 30 µL of DEPC (Diethyl pyrocarbonate).

2-4-2. DNase treatment

For DNase treatment, Invitrogen TURBO DNA freeTM Kit (Thermo Fisher company) was used. Dilute the sample to $10 \,\mu$ g nucleic acid/50 μ L of total sample if possible. After Add 0.1volume 10X DNaseTMBuffer to the RNA, then mix gently that TURBO DNaseTM Enzyme $1 \,\mu$ L to the RNA. Incubate samples at 37°C for 30min and then, resuspend the DNase Inactivation Reagent by flicking or vortexing the tube before use. Add 0.2 volumes of resuspended DNase Inactivation reagent then mix well. Inactivate the sample for 5minutes at room temperature. Flick the tube 2~3times during the incubation period to redisperse the DNase

제주대학교 중앙도서 JEJU NATIONAL UNIVERSITY LIBR

- 10 -

Inactivation Reagent. Centrifuge the samples, then carefully transfer the supernatant containing the RNA to a fresh tube, But do not disturb the pellet of DNase Inactivation Reagent.

2-5. PCR (Polymerase Chain Reaction) & Electrophoresis

2-5-1. Genotyping of T3 S-CuCCDA1 / T3 AS-CCDA1 lines from single seed

Genomic DNA was isolated from single seed of T2 *3-CuCCDA1* lines (3-5-12, 6-13-29 and 20-9-20) and carried out PCR to obtain T3 homo lines.

To perform PCR, $2 \mu L$ of genomic DNA from each lines, $0.5 \mu L$ of forward primer CCDA1 23582F(5'-GCAAACACTTGGTATGCTTG-3'), $0.5 \mu L$ of reverse primer t-NOSR(5'-AATTCCCGATCTAGTAACATAGA-3'), $5 \mu L$ of Prime Star(Dia starTMDirect Mutiplex/Fast PCR, Solgent company), and $2 \mu L$ of deionized water (D.W.) were added in a 0.2 mL PCR tube.

The PCR conditions were as follows. Initial denaturation at 95°C for 5 min, additional denaturation at 95°C for 20 sec, annealing at 58–60°C for 30 sec, extension at 72°C for 30 sec, and final extension at 72°C for 5 min. Repeat 35 cycles from denaturation to extension(Table 1). After the PCR reaction, 8 μ L of PCR product and 2 μ L of 6 × dye were mixed and electrophoresed at 100 V for 25 minutes on 1.2% agarose gel. The conditions of electrophoresis was electrophoresis on 1.2% agarose gel.

Genomic DNA was isolated from single seed of T3 AS-CuCCDA1 lines (9-6-8, 13-2-17 and 5A-



- 11 -

6-7) and carried out PCR to obtain T3 homo lines and carried out PCR to obtain T3 homo lines, To perform the PCR, 2μ L of genomic DNA from each lines, 0.5μ L of forward primer CCDA1 2358R(5' -TCTTCCAGAACTTTGACGGC-3'), 0.5μ L of reverse primer t-NOSR(5' -AATTCCCGATCTAGTAACATAGA-3'), 5μ L of Prime Star, and 2μ L of deionized water(D.W.) were added in a 0.2 mL PCR tube.

The PCR conditions were as follows. Initial denaturation at 95°C for 5 min, additional denaturation at 95°C for 20 sec, annealing at 60°C for 30 sec, extension at 72°C for 30 sec, and final extension at 72°C for 5 min. Repeat 35 cycles from denaturation to extension (Table 2). After the PCR reaction, $8 \,\mu$ L of PCR product and $2 \,\mu$ L of 6 × dye were mixed and electrophoresed at 100 V for 25 minutes.

DNA	2 µ L	95℃	5min		
CCDA1 2382F	0.5 μ L	95℃	20sec		
tNOSR	0.5 μ L	58~60℃	30sec	35cycles	
Prime Star	5 µ L	72℃	30sec		
D.W.	2 µ L	95℃	5min		
	10 µ L	8°C	8		

Table 6. PCR condition of T3-S-CuCCDA1 seed.

Table 7.	PCR	condition	of	T3-AS-CuCCDA1 seed.
----------	-----	-----------	----	---------------------

DNA	2 µ L	95℃	5min	
CCDA1 23+85R	0.5 µ L	95℃	20sec	35cycles
tNOSR	0.5 μ L	60℃	30sec	



Prime Star	5 µ L	72℃	30sec	
D.W.	2 µ L	72℃	5min	
	10 µ L	8°C	8	

2-5-2. Genotyping of T3-S-CuCCDA1 / T3-AS-CuCCDA1 lines from leaf

The seeds of T3 homo lines obtained from above genotyping PCR were sown on soil and cultivated in the green house. After 50~60 days from sowing, the leaf from each lines were sampled and isolated genomic DNA to reconfirm the genotype by PCR.

 $2 \mu L$ of genomic DNA from each lines, $0.5 \mu L$ of forward primer (CCDA1 23582F, $0.5 \mu L$ of reverse primer (t-NOSR), and $17 \mu L$ of deionized water(D.W.) were added in a 0.2 mL PCR tube of AccuPower® PCR PreMix(Top simpleTM DryMix-nTaq, enzynomics company).

The T3-S-*CuCCDA1* PCR conditions were as follows. Initial denaturation at 95°C for 5 min, additional denaturation at 95°C for 20 sec, annealing at 60°C for 30 sec, extension at 72°C for 30 sec, and final extension at 72°C for 5 min. Repeat 35 cycles from denaturation to extension (Table 3). After the PCR reaction, 10 μ L of PCR product electrophoresed at 100 V for 25 minutes.

To perform PCR in the case of T2-AS-*CCDA1*, $2 \mu L$ of genomic DNA from each lines, $0.5 \mu L$ of forward primer (CCDA1 2358R, $0.5 \mu L$ of reverse primer (t-NOSR), and $17 \mu L$ of deionized water (D.W.) were added in a 0.2 mL PCR tube of AccuPower® PCR PreMix (Table 4). After the PCR reaction, $8 \mu L$ of PCR product and $2 \mu L$ of $6 \times$ dye are mixed and electrophoresed at 100 V for 25 minutes.



DNA	2 µ L	94°C	5min	
CCDA1 2382F	0.5 μ L	94°C	20sec	
tNOSR	0.5 μ L	60°C	30sec	35cycles
D.W.	17 μ L	72℃	30sec	
		72℃	5min	
	20 µ L	8°C	∞	

Table 8. PCR condition of T3-S-CuCCDA1 leaf

Table 9. PCR condition of T3-AS-CuCCDA1 leaf

DNA	2 µ L	95℃	5min	
CCDA1 2358R	0.5 μ L	95℃	20sec	
tNOSR	0.5 μ L	60℃	30sec	35cycle
D.W.	17 μ L	72℃	30sec	
2	2. ~ 2	72℃	5min	
	20 µ L	8℃	∞	

2-5-3. RT-PCR(Reverse Transcriptase Polymerase Chain Reaction)

After cDNA synthesis, microtomato CCD family genes were found in NCBI (National Center for Biotechnology Information) to perform RT-PCR. As a result, SLCCD1A, SLCCD1B, SLCCD4, SLCCD4-1, SLCCD (9'10'). SLCCD(9'10')-1 like and SLCCD('10')-2 like were searched, and primer information is in Table 5. PCR condition of CuCCD1A; Denaturation at 94°C for 30 seconds, annealing at 60°C for 30 seconds, and elongation at 72°C for 30 seconds were amplified in 28 cycles, and PCR conditions for SLCCD1A and SLCCD4; Denaturation at 94°C for 30 seconds, annealing at 52°C for 30 seconds,

- 14 -



and elongation at 72°C for 30 seconds were amplified for 30 cycles. PCR condition of SLCCD1B; Denaturation at 94°C for 30 seconds, annealing at 58°C for 30 seconds, and elongation at 72°C for 30 seconds were amplified in 30 cycles, SLCCD4-1, SLCCD7, CCD9' 10' like, CCD9'10'-1 like2, SLLCYB1, SLCHYB1, SLVDE PCR condition; Denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds and elongation at 72°C for 30 seconds were amplified for 30 cycles. Also, SLCCD8-2 PCR condition; Denaturation at 94°C for 30 seconds, annealing at 59°C for 30 seconds, and elongation at 72°C for 30 seconds were amplified as 30 cycles. SLLCYB2 PCR condition; Denaturation at 94°C for 30 seconds, annealing at 56°C for 30 seconds, and elongation at 72°C for 30 seconds were amplified for 30 cycles. SLLCYB2 PCR condition; Denaturation at 94°C for 30 seconds, annealing at 56°C for 30 seconds, and elongation at 72°C for 30 seconds were amplified for 30 cycles. SLCHYB2 PCR condition; Denaturation at 94°C for 30 seconds, annealing at 57°C for 30 seconds, and elongation at 72°C for 30 seconds were amplified for 30 cycles. SLCHYB2 PCR condition; Denaturation at 94°C for 30 seconds, annealing at 57°C for 30 seconds, and elongation at 72°C for 30 seconds were amplified as 30 cycles. SLZEP PCR condition; May be a second secon



Table 10. RT-PCR Primer

RT-PCR primer						
Gene	Sequence(5' -> 3')					
CuCCD1A F14	AAAAGCTGGAGAACGACGTC					
CuCCD1A R196	GAGTCTCGTCAGGAACTGGA					
SLCCD1A F909	GCTAGCTTTTACCTTTGATGC					
SLCCD1A R1297	TTACCTTAGCAATGCTGTTC					
SLCCD1B F911	CATACAGCTTTGACCCCACA					
SLCCD1B R1136	GTGAAACCGTCACGCTGTTC					
SLCCD4 F136	GAAAGACCACAAACAACCAC					
SLCCD4 R260	GAGGGAAATGATGGTTCAAT					
SLCCD4-1 F793	GTCTGGGGCATGACAGCTCA					
SLCCD4-1 R1036	CGAGTATGAACTTGATCGGG					
SLCCD7 F98	TCTTGCCACCGGCTAAACTG					
SLCCD7 R200	GTTGGGGACGTGGTGATCGT					
SLCCD8-2 F1165	ACCATCCTTGACAAGCTCCG					
SLCCD8-2 R1400	CAAGGCCTCTTAGCACCACA					
CCD9'10'F731	ATGAAGTAGGGTACGAGGTT					
CCD9'10'R972	ATGGGCTGTGAATGATCGAG					
CCD9'10'-1 like F848	AAAATACCAGATACGGGTGAACTT					
CCD9'10'-1 like R1083	CCAAGCTCGTCTACTATAATATCT					
CCD9'10'-1 like2 F544	CCTGGTTTTCTTCCCGCTAT					
CCD9'10'-1 like2 R817	TAACAAGTTCGCTTGCTCCA					
SLLCYB1 F297	ACAGCAAGTTTCTGAAGCAG					
SLLCYB1 R519	CGATTTCAGCTGTTTCCGGT					
SLLCYB2 F651	TGATGCGACGGGATTCTCTA					
SLLCYB2 R919	ATCCAGGACGAGCTACAAGG					
SLCHYB1 F81	ACCTACTTCGACAACCTCAC					
SLCHYB1 R374	ACAGCCATAACAGCCATAGA					
SLCHYB2 F477	TCCTTCAGGTAGGAATGGAG					
SLCHYB2 R697	CAATCCAGCGCCGAAACAGA					
SLZEP F121	AGGAGCTTGGAAAATGGGCA					
SLZEP R402	CTGTATCTGTATTGGACCTC					
SLVDE F435	GAAAGAGTGCAGGATAGAGC					
SLVDE R730	TCAAACCGCGAGTGATGTAC					



Table 6. RT-PCR condition

-	RT-PCR condition							
	Denaturation	time	Anealing	time	Elongation	time	cycles	
CuCCD1A	94℃	30	60°C	30	72℃	30	28	
SLCCD1A	94℃	30	52℃	30	72℃	30	30	
SLCCD4	94 C	50	JZC	50	120	50	50	
SLCCD1B	94℃	30	58℃	30	72℃	30	28	
SLCCD4-1								
SLCCD7								
CCD(9'10') like								
CCD(9'10')-1like 2	94°C	30	55°C	30	72	30	30	
SLLCYB1								
SLCHYB1								
SLVDE								
SLCCD8-2	94℃	30	59℃	30	72℃	30	30	
SLLCYB2	94°C	30	56℃	30	72℃	30	30	
SLCHYB2	94°C	30	57°C	30	72℃	30	30	
SLZEP	94°C	30	51℃	30	72℃	30	30	

2-6. Anti-oxidant activity analysis

2-6-1. Total Carotenoid content

Mix 1 mL of ACN:MeOH:THF (50:45:5, by volume) with 5 mg of lyophilized powder, and shaken at 150 rpm for 2 hours. Centrifuge at 15000 rpm for 5 min at -5 ° C and supernatant was filtered through 0.45-mm. using a microplate reader (Thermo) at 450 nm wavelength read the plates. Carotenoid extraction was performed according to the method of DÓKA, Ottó.(Dóka, Ficzek et al. 2013).

2-6-2. Total Flavonoid content

Total flavonoid content was performed by modifying of Chang et al(Chang, Yang et al. 2002). To $20 \,\mu$ L of sample and standard, $10 \,\mu$ L of 50g/L NaNO₂ was added and stored at room temperature for 6 minutes. After add $15 \,\mu$ L of 100g/L AlCl₃ and incubate for 6min, add $75 \,\mu$ L of 1M NaOH. And then incubate in dark for

- 17 -



30 minutes, using a microplate reader (Thermo) at 510 nm wavelength read the plates. Quercetin was used as a standard at 0, 25, 50, 100, 200, 300 μ g/mL to generate a calibration curve.

2–6–3. Total Phenolic content

Total phenolic content was performed by modifying the Folin-Denis analysis protocol by Rosa M et al(Lamuela-Raventós 2018). Mix $100 \,\mu$ L of 10% folinciocalteu reagent with $20 \,\mu$ L of sample and incubate for 5 mins. Add then $80 \,\mu$ L of 10% sodium carbonate anhydrous saturated solution. After incubated in the dark at 25°C for 60min, using a microplate reader (Thermo) at 750 nm wavelength read the plates. The total phenolic content was expressed as gallic acid equivalent(GAE) through a calibration curve 0, 25, 50, 100, 200, 400 μ g/mL.

2-6-4. DPPH radical scavenging

DPPH radical scavenging activity assay was performed with a modification of the protocol of AOSHIMA et al. (Aoshima, Tsunoue et al. 2004). 160 μ L of 0.2 mM DPPH solution dissolved in methanol and sample 40 μ L were added and incubated in dark for 30 minutes. The plates were then read on a Microplate reader(Thermo) at 517nm wavelength. Used ascorbic acid as positive control.

2-7. HPLC(High Performance Liquid Chromatography)

For HPLC measurement, carotenoid was extracted according to the method of P



- 18 -

Gupta, and HPLC measurement was performed. Add 1.2 mL chloroform:dichloromethane (2:1, v/v) to the lyophilized plant sample and mix for 20 min at 1000 rpm at 4°C using a thermal mixer. 0.4 mL of 1M sodium chloride solution was added, mixed by inversion, and centrifuged at 5000 g for 10 minutes to collect the organic phase. The remaining aqueous phase was re-extracted, and after mixing for 20 minutes at 1000 rpm at 4° C. using a thermal mixer, 0.2 mL of 1M sodium chloride solution was added and mixed by inversion.

After centrifugation at 5000 g for 10 minutes, the organic phase was collected and dried by centrifugal evaporation. It was also dissolved in 0.5 mL of methanol.

3. Result

3-1. Genotyping analysis of T3-S/AS-CuCCDA1 transformant lines using genomic DNA from single seed

- 19 -



and the remained lines were not amplified the expected PCR product in the some of genotyping PCR from each lines (Fig. 2B).



Fig 4. Genomic DNA extraction from Seed. A. Sense lines 3-5-12, 6-13-29 and 20-9-20, B. Anti-Sense lines 9-6-8, 13-2-17, 5A-6-7

3-2. Genotyping analysis of T3-S/AS-CuCCDA1 transformant lines using genomic DNA from single Leaf

As a result of genotyping of the T2-S/AS-CuCCDA1 transformant using genomic DNA from a single seed, three homo lines (3-5-12, 6-1329, 20-9-20) of the T2-S-CuCCDA1 transformant was obtained. and four homo lines

- 20 -



of the T2-AS-CuCCDA1 transformant (9-6-8, 13-2-17 and 5A-6-7).



Fig 5. Genomic DNA extraction from Leaf. A. Sense lines 3-5-12, 6-13-29 and 20-9-20, B. Anti-Sense lines 9-6-8, 13-2-17, 5A-6-7



3-3. Total RNA extraction and DNase treatment

Total RNA was extracted from all samples, treated with DNase and electrophoresed (Fig. 4). In addition, the average values of nucleic acid, A260/280, and A260/230 of 3 lines of MT and 3 sense lines were obtained (Tables 7 and 8). Nucleic acid concentration of MT-Leaf was 296.9ng/µL, A260/280 showed a value of 1.98, A260/230 showed a value of 1.7, and MT-Flow's Nucleic acid concentration was 137.8ng/µL, A260/280 showed a value of 1.99, and A260/230 showed a value of 1.71. Nucleic acid concentration in Sense Leaf was 141.43±15.77 ng/µL, A260/280 was 1.97±0.03, A260/230 was 1.69±0.02, and Nucleic acid concentration in Sense Flower was 125.6 ± 3.44 ng/µL and A260/280 was 1.95. A value of ± 0.03 was shown, and A260/230 showed a value of 1.56±0.99. The nucleic acid concentration of anti-sense flower was 149.7±8.05 ng/µL, A260/280 1.95±0.05, and A260/230 1.65 ± 0.1 , and the nucleic acid concentration of anti-sense flower was 139.43 ± 2.51 , A260/280 showed a value of 1.95±0.01, and A260/230 showed a value of 1.71±0.03. Nucleic acid concentrations, A260/280 and A260/230 values for each fruit development were measured.

The concentration of MT-Immature green nucleic acid was 296.9 ng/ μ L, A260/280 showed a value of 1.93, and A260/230 showed a value of 1.82, and the nucleic acid concentration of mature green was 427.2 ng/ μ L, A260/280 showed a value

- 22 -



of 1.95 and A260/230 showed a value of 1.91. The nucleic acid concentration of Turning showed a value of 535.4 ng/µL, A260/280 showed a value of 1.99, A260/230 showed a value of 1.7, and the nucleic acid concentration of Orange showed a value of 225.9 ng/µL, A260/280 showed a value of 1.8 and A260/230 showed a value of 1.55. The concentration of nucleic acid in orange red was 275.4 ng/µL, A260/280 showed a value of 1.92, and A260/230 showed a value of 1.26, and the concentration of nucleic acid in red was 107.9 ng/µL, A260/ 280 showed 1.83 and A260/230 showed 1.62. Sense-Immature green Nucleic acid concentration was 104.1 ± 34.66 mg/µL, A260/280 showed a value of 1.9 ± 0.14 , A260/230 showed a value of 1.55±0.16. Nucleic acid concentration of mature green was 167.07±155.74ng/µL, 1.9±0.03 for A260/280, and 1.54±0.28 for A260/230. The nucleic acid concentration of Turning was 187.93±184.4ng/µL, A260/280 showed a value of 1.88 ± 0.05 , A260/230 showed a value of 1.49 ± 0.28 , and the nucleic acid concentration of Orange was 254.8±254.8± 283.71ng/µL, A260/280 showed a value of 1.72 ± 0.07 , and A260/230 showed a value of 1.50 ± 0.27 . The concentration of nucleic acid in orange-red was 219.37±244.79ng/µL, A260/280 showed a value of 1.78 ± 0.12 , A260/230 showed a value of 1.51 ± 0.31 , and the concentration of nucleic acid in red was 264.47. ±207.51 ng/µL, A260/280 showed



- 23 -

1.79±0.13, and A260/230 showed 1.62±0.24.

Anti sense-Immature green Nucleic acid concentration was 143.33±7.23ng/µL, A260/280 showed a value of 1.96±0.07, A260/230 showed a value of 1.71±0.08. The mature green Nucleic acid concentration was 138.23±4.51ng/µL, 1.92±0.02 for A260/280, and 1.61±0.09 for A260/230. The nucleic acid concentration of Turning showed a value of 133.4 ± 36.57 ng/ μ L, A260/280 showed a value of 1.9 ± 0.06 , A260/230 showed a value of 1.47±0.08, and the nucleic acid concentration of Orange was $122.93 \pm 60.18 \ln g/\mu L$, A260/280 showed a value of 1.79 ± 0.08 , and A260/230 showed a value of 1.45±0.26. The concentration of nucleic acid in orange-red was 140.13±33.89ng/µL, A260/280 showed a value of 1.79±0.08, A260/230 showed a value of 1.51±0.10, and the concentration of nucleic acid in red was 101.00. ±63.34 ng/µL, A260/280 showed 1.78±0.11, and A260/230 showed 1.28±0.28.



Fig 6. RNA extraction and DNase treatment.

- 24 -

(A) Electrophoresis of DNase-treated RNA by MT fruit development, (B) Electrophoresis of DNase-treated RNA by S 3-5-12 fruit development, (C) Electrophoresis of DNase-treated RNA by S 6-13-29 and 20-9-20 fruit development, (D) Electrophoresis of DNase-treated RNA by AS 9-6-8 fruit development, (E) Electrophoresis of DNase-treated RNA by AS 13-2-17, 5A-6-7 fruit development, (F) Electrophoresis of DNase-treated RNA by MT, Sense and Anti sense Leaf, (G) Electrophoresis of DNase-treated RNA by MT, Sense and Anti sense Leaf, (G) Electrophoresis of DNase-treated RNA by MT, Sense and Anti sense Flower.

		MT	S 3-5-12	S 6-13-29	S 20-9-20	AVER	STD
	Nucelic Acid	142.9	137.8	127.8	158.7	141.43	15.77
Leaf	260/280	1.98	2	1.95	1.97	1.97	0.03
	Nucelic Acid 260/280 260/230 Nucelic Acid 260/230 Red 260/230 Nucelic Acid 260/230 Red 260/230 Nucelic Acid 260/230 Nucelic Acid 260/230 Nucelic Acid 260/230 Nucelic Acid 260/230 Nucelic Acid	1.7	1.7	1.66	1.7	1.69	0.02
	Nucelic Acid	137.8	126.9	128.2	121.7	125.60	3.44
Flower	260/280	1.99	1.97	1.96	1.91	1.95	0.03
	260/230	1.71	1.62	1.61	1.46	1.56	0.09
	Nucelic Acid	296.9	143.8	79.9	88.6	104.10	34.66
Immature Green	260/280	1.93	2.01	1.98	1.76	1.92	0.14
	260/230	1.82	1.73	1.46	1.45	1.55	0.16
	Nucelic Acid	427.2	355.9	86	86.3	176.07	155.74
Mature Green	260/280	1.95	1.87	1.91	1.93	1.90	0.03
Mature Green	260/230	1.91	1.86	1.44	1.32	1.54	0.28
	Nucelic Acid	535.4	400.6	72.4	90.8	187.93	184.40
Turning	260/280	1.66	1.93	1.86	1.84	1.88	0.05
	260/230	1.59	1.8	1.26	1.42	3.7 141.43 97 1.97 1.7 1.25.60 .91 1.95 .46 1.56 3.6 104.10 76 1.92 45 1.55 5.3 176.07 93 1.90 32 1.54 0.8 187.93 84 1.88 42 1.49 1.6 254.80 65 1.72 36 1.50 0.6 219.37 66 1.78 1.4 1.51 0.1 264.47 1.8 1.79	0.28
	Nucelic Acid	225.9	582.4	90.4	91.6	254.80	283.71
Orange	260/280	1.8	1.71	1.79	1.65	1.72	0.07
	260/230	1.55	1.81	1.32	1.36	1.50	0.27
	Nucelic Acid	275.4	502	75.5	80.6	219.37	244.78
Orange Red	260/280	1.92	1.89	1.78	1.66	1.78	0.12
	260/230	1.26	1.86	1.27	1.4	1.51	0.31
	Nucelic Acid	107.9	504	139.3	150.1	264.47	207.51
Red	260/280	1.83	1.91	1.66	1.8	1.79	0.13
	260/230	1.62	1.9	1.49	1.47	1.62	0.24

Table 7. Nucleic acid concentration of MT and Sense.



		AS 9-6-8	AS 13-2-17	AS 5A-6-7	AVER	STD
	Nucelic Acid	150.7	141.2	157.2	149.70	8.05
Leaf	260/280	1.97	1.99	1.89	1.95	0.05
	260/230	1.71	1.71	1.54	1.65	0.10
	Nucelic Acid	142.3	138.4	137.6	139.43	2.51
Flower	260/280	1.96	1.95	1.94	1.95	0.01
	260/230	1.75	1.69	1.7	1.71	0.03
	Nucelic Acid	149.3	145.4	135.3	143.33	7.23
Immature Green	260/280	2	1.88	1.99	1.96	0.07
	260/230	1.78	1.63	1.72	1.71	0.08
	Nucelic Acid	143.4	136.2	135.1	138.23	4.51
Mature Green	260/280	1.94	1.91	1.91	1.92	0.02
	260/230	1.51	1.69	1.64	1.61	0.09
	Nucelic Acid	147.9	91.8	160.5	133.40	36.57
Turning	260/280	1.93	1.95	1.83	1.90	0.06
_	260/230	1.54	1.49	1.38	1.47	0.08
	Nucelic Acid	155.3	53.5	160	122.93	60.18
Orange	260/280	1.83	1.84	1.69	1.79	0.08
	260/230	1.62	1.15	1.58	1.45	0.26
	Nucelic Acid	170	103.3	147.1	140.13	33.89
Orange Red	260/280	1.71	1.79	1.87	1.79	0.08
	260/230	1.51	1.41	1.6	1.51	0.10
Red	Nucelic Acid	36.4	103.6	163	101.00	63.34
	260/280	1.88	1.66	1.79	1.78	0.11
	260/230	1.03	1.22	1.58	1.28	0.28

Table 8. Nucleic acid concentration of Anti-sense

3-4. RT-PCR(Reverse Transcriptase Polymerase Chain Reaction)

As a result of searching for CuCCD1A gene and SLCCD1A homology, 79% homology was found (fig. 4), and 3 μ L of cDNA in 1.5 mL tube, 0.5 μ L of CuCCD1A F14 0.5 μ L-R196 0.5 μ L, D.W. 16 μ L mixing RT-PCR was performed. As a result of electrophoresis of 5 μ L of the PCR product with 2% gel, in the case of MT, it was confirmed that the gene was not amplified as a control in which the CuCCD1A gene was not introduced. It was confirmed that the gene was amplified in plants into which was introduced (Fig. 5).

For SLCCD1A, 3 μL of cDNA, 0.5 μL of SLCCD1A F909-R1297 0.5 $\mu L,$ D.W. After

- 26 -



RT-PCR was performed by mixing 16 μ L, 5 μ L of the PCR product was loaded on a 2% gel and electrophorese. In the case of MT, since only CCD1A of tomato itself was present, it was confirmed that its own gene was amplified properly, and in the case of 3 sense lines and 3 anti-sense lines, electrophoresis was performed and similar patterns were shown (Fig. 6).

							Query	719	CCTTTGGCTATGCACACACACCACCATATATCACATACRGAGTTATTTCAAAGGATGGTT	
ſ	Sequence ID: Query_10669 Length: 1638 Number of Matches: 1					Sbjct	713		772	
	Range 1: 1 to 1638 Graphics V Next Match & Previou			Query Sbjct						
	NW Score 1521	1	Identities 1299/1647(79%)	Gaps 12/1647(0%)	Strand Plus/Plus		Query			898
	Query	1		CTGGAGAACGACGTCGTAGCACGA		58	Sbjct			
SLCCDA1 🗪	Sbjct	1		ATGGAGTA-GAGAGAATTGAAGGA		55	Query Sbjct			
	Query				118	Query				
	Sbjct	56		GAATAACTGCGAAAGCCATAGAC		115	Sbjct			
	Query	119	TAGTGAAGTGGATGTACGATTCTTCACAGCCTCTTCATTATCTCCCG		TCCGGTAACTTCGCTC	178			1078	
	Sbjct	116		ICCTCTAAGCCACTTCACTATCT		175	Sbjct	1013		
	Query	179	CAGTTCCTGACGAGACTCCTC	CCTACTAAAGACCTCCACGTCATC	GGCCATCTCCCCGATT	238	Query	1079	TAGACAAGCCGGATCTAGACATGGTCAATGGGGCTGTCAAAGAAAAGCTAGAAAATTTCT	1138
	Sbjct	176		CCTCTTAATGACCTTGTTGTACAA		232	Sbjct	1073	TIGIGAACCCAGACCTAGACAIGGICAAIGGAGCIGITAAAGAAAAGCIIGAGAAIIICI	1132
	Query	239		AGGGTTGGTCCTAATCCCAAGTTT		298	Query	1139		
	Sbjct	233		AGAGTTGGTCCAAATCCAAAGTTT		292	,			
	Query	299	ACCACTGGTTTGATGGAGATGGCATGATTCATGGTCTGCGCATCAAA		AAAGACGGAAAGGCTA	358	Query	1199	TATCGGCATCCGCTGTTGATTTTCCTAGGGTGAATGAGTGCTACACTGGCAGGAAGCAAA	
	Sbjct	293	ATCACTGGTTTGATGGGGATG	GTTTGATGGGGATGGCATGATTCATGGCTTGCGCATTAAAGAT		352			TATCGGAGTCTGCTGTTGATTTTCCAAGGATCAACGAGAACTACACAGGCAGG	
	Query	359	CATATGTCTCCCGTTTTGTGAGGACTTCACGTCTTAAACAGGAAGAATTTTTCGGAGGI		GAATTTTTCGGAGGTG	418	Query	1259		
I	Sbjct	353		AGGACTTCACGTCTAAAGCAAGAA		412				
	Query	419	CTAAATTTATGAAGATTGGAG	JACCTTAAGGGGGCTATTTGGATTJ	CTCATGGTTAACATGC	478				
I	-	413	CTAAGTTTATGAAGGTTGGAG	ATCTTAAAGGGCTGTTTGGTCTG	III III IIII TTCACAGTTTATATGC	472	Query			
I	Query	479	AAATGCTTAGAGCAAAGTTGA	AAAGTACTGGATGTTTCATATGGA	LAATGGGACAGCTAATA	538	Sbjct			
	Sbjct	473	AAATGCTCAGAACAAAGCTGA	AAGTATTGGACATCTCCTATGGA	AATAGCACAGCTAATA	532	Query	1438		1497
I	Query		CAGCTCTTGTATATCACCATG	5GGAAACTTCTAGCACTCTCAGAG	GCGGACAAACCGTATG	598	Sbjct	1432	CCTGGTACAGAACGTGAAGAGGATGATGGCTACTTGATATTCTTCGTTCATGACGAGAAC	1491
	Sbict	533	CAGCTCTAGTATATCACCATG		GCTGATAAACCTTATG	592	Query	1498		1557
	Query	599	CCGTCAAAGTTCTGGAAGATG	GAGATCTGCAAACACTTGGTATG	CTTGACTATGACAAGA	658	Sbjct	1492	ACCGGAAAGTCAGCTGTGAATGTAATTGATGCAAAAACAATGTCCGCGGAGCCTGTGGCA	1551
	Sbjct 593 CACTTAAAGTTCTAGAGGATGGAGATCTTCAAACACTTGGCATGCTTGGATATG			652						
	Query	659		SCCCATCCAAAGGTTGATCCATAC		718	Sbjct			1611
l	Sbjct	653			 ACTGGTGAGATGTTTA	712		1618 1612	CAACITGAAGAGCAAGCAAAATTGTGA 1644 CAAATTCAAGAACCAAACCGAACTGTGA 1638	

Fig 7. Homology of CuCCD1A and SLCCD1A

Homology was searched through NCBI. The upper part marked in red is the sequence position for CuCCD1A F14-R196, and the lower part marked in red is the sequence position for SLCCD1A F909-R1297.





Fig 8. Electrophoresis of CuCCD1A

Gene expression analysis of CuCCD1A F14-R196. Left is MT, middle is 3-5-12, 6-13-29, 20-9-20 3 sense lines, right is 9-6-8, 13-2-17, 5A-6-7 3 Anti -sense line.

MT SLCCD1A F909-R1297	T3-S-CCDA1 3-5-12 SLCCD1A F909-R1297	T3-AS-CCDA1 9-6-8 SLCCD1A F909-R1297			
MT-CDAA Leaf Flow IM MA TUR O O.R R M	T3-S-CCDA1-3-5-12 ¢DNA	T3-AS-CCDA1 9-6-8 CAND . M Leaf Flow IM MA TUR O O-R R M			
SLCCD1A F909-R1297	BLCCDIA P990-R1297	SLCCD1A F909-R1297			
	T3-S-CCDA1 6-13-29 SLCCD1A F909-R1297	T3-AS-CCDA1 13-2-17 SLCCD1A F909-R1297			
	T3-S-CCDA1 6-13-29-CDNA M Leaf Flow IM MA TUR O O.R R . M	T3-A5-CCDA1 13-2-17-c DNA M Leaf Flow IM MA TUR O O-R R M			
	SLCCD 1A F909-R1297	SLCCD1A F909-R1297			
	T3-S-CCDA1 20-9-20 SLCCD1A F909-R1297	T3-AS-CCDA1 5A-6-7 SLCCD1A F909-R1297			
	Ta-B-CCDA120-9-20-00HA Lead Plow IN MA TUR O O-R M	TE AS COATSA BY CONA +			
	SLCCD1A F909-R1297	SLCCD1A F909-R1297			

Fig 9. Electrophoresis of SLCCD1A.

Gene expression analysis of SLCCD1A F14-R196. Left is MT, middle is 3-5-12, 6-13-29, 20-9-20 3 sense lines, right is 9-6-8, 13-2-17, 5A-6-7 3 Anti -sense line.

As a result of confirming the homology of CuCCD1A and SLCCD1B, 77% of homology was shown (Fig. 7). 3 μ L of cDNA, 0.5 μ L of SLCCD1B F911-0.5 μ L of SLCCD1B R1297, D.W. 16 μ L was mixed and RT-PCR was performed. In addition,


5 μ L of the PCR product was loaded on a 2% gel and electrophoresed (Fig. 8).

	NW Score	Identities	Gaps	Strand	Que	y 839	TTACTGAGAATTATGCTATTTCATGGATCTTCCGCTGTATTTCAGACCAAAGGAAATGG	898
	1354	1266/1649(77%)	16/1649(0%)	Plus/P	Shi			889
CuCCD1A	Query 1		GAACGACGTCGTAGCACGAAATGGAATAG		59			958
SLCCD1B 📥	Sbjct 1	Á TGGGGA TGÁ Á TGÁ Á GÁ Á GA TGGÁ			55			949
	Query 60	GGATCCCAAACCGAAGAATGGAGT TGATCCAAAACCACAAAATGGAGT	AACTTCGAAAGTGATAGACTTTGTGGAGA					1018
	Sbjct 54 Query 120		ACAGCCTCTTCATTATCTCTCCGGTAACT		113 Uue 179 Sbj	,		1009
	Query 120 Sbjct 114				173 Que			1078
	Query 180	AGTICCIGACGAGACTCCICCTAC	TAAAGACCTCCACGTCATCGGCCATCTCC	CCGATTG	239 Sbj			1069
	Sbjct 174	TACC-GATGAAACTCCTCCACT	TAAAAACCTTCCCGTTATAGGCCATCTGC	CGGAGTG	230 Que			1135
	Query 240	CTIGAATGGGGAGTTTGTCAGGGT	TGGTCCTAATCCCAAGTTTGCCCCTGTGG		299 Sbj			1129
	Sbjct 231		TGGTCCAAATCCTAAATTTGCTCCAGTTG		290 Que			1195
	Query 300	CCACTGGTTTGATGGAGATGGCAT	GATTCATGGTCTGCGCATCAAAGACGGAA	AGGCTAC	359 Sbj		0 CACAAATGAGTTGTATGAGATGAGGTTCAATATGAAGAATGGTGTAGCATCACAGAAGA	1189
	Sbjct 291	CCATTGGTTTGATGGAGATGGCAT	GATTCATGGCTTGCAAATTAAGGATGGAA	AAGCAAC	350 Que	y 119	6 GATTATCGGCATCCGCTGTTGATTTTCCTAGGGTGAATGAGTGCTACACTGGCAGGAAGC 1	1255
	Query 360	ATATGTCTCCCGTTTTGTGAGGAC	TTCACGTCTTAAACAGGAAGAATTTTTCG	GAGGTGC	419 Sbj	t 119	D AACTGTCAGAGGCTGCTGTTGATTTTCCACGGATCAACGAGAACTACACTGGAAGGAA	1249
	Sbjct 351		ATCACGTCTTAAGCAAGAAGAGTTCTTTG			y 125	6 AAAGATATGIGTATGGAACAATACTAGATAGCATIGCAAAAGICACAGGGATCATCAAAT 1	1315
	Query 420	111.1111101011000_0		TH T	479 Sbj	t 125	D AACGCTATGTATATGGAACCATTTTAAACAATGTTGCCCAGATCACAGGAGTTGTCAAAT 1	1309
	Sbjct 411 Query 480	TAAGTTTATGAAGATTGGAGATCT AATGCTTAGAGCAAAGTTGAAAGT	TAAAGGGCTGTTCGGGTTGTTCTCAGTAT		470 539 Que	y 131	6 TTGATCTGCATGCTGAACCAGACGAAGAGAAAACAAAGCTTGAAGTTGGAGGAAATGTGA 1	1375
	Sbict 471	CATGCTCAGGGAAAAGCTGAAAGT	ACTGGATGTTTCATATGGAAATGGGACAG TTTGGACACTTCCTATGGAAATGGCACAG		530 ^{Sbj}	t 131	D TTGATTTGCATGCCGAACCAGAAACTGGAAAAACAAAGCTTGAAGTAGGTGGAAATGTTC 1	1369
	Query 540		ACTTCTAGCACTCTCA-GAGGCGGACAAA		598 Que	y 137	6 GAGGCATCTTTGATCTGGGCCCTGGAAGATTTGGTTCAGAGGCTGTTTTTGTTCCTAGAG 1	1435
	Sbjct 531	AGCTATGATATATCACCATGGGAA	GCTTTTGGCTCT-TCATGAGGGTGATAAA	CCATATG	589 Sbj	t 137	O ctagaatttttgaccttagacctggaagatttggatcagaggcaatatttgttccccgtc 1	1429
	Query 599	CCGTCAAAGTTCTGGAAGATGGAG	ATCTGCAAACACTTGGTATGCTTGACTAT	GACAAGA	658 Que	у 143	BE AGCCTGGAACCTCTTCTGAAGAAGATGATGGTTACTTAATATTCTTCTCTCATGATGAGA 1	1495
	Sbjct 590	TAGTTAAGATTCTGGAGGATGGAG	ATCTGCAAACGCTTGGCATGCTGGATTAC	GATAAAA	649 Sbj	t 143	BD AGCCTGGGACTGAATGTGAAGAGGATGACGGCTACTTAATATTGTTTGT	1489
	Query 659	GATTACAACATTCCTTTACTGCCC	ATCCAAAGGTTGATCCATACACTGGCGAG	ATGTTTA	718 Que	y 149	AAACCOGGAAAGTCATCAGTGAACGTGATTGATGCAAAAAACAATGTCAGCTGATCCTGTTG	1555
	Sbjct 650		ACCCGAAGGTTGACCCTGTAACTGGGGAA		709 Sbj		D ACACTGGAAAGTCATCAGTGAATGTAATTGATGCGAAAACAATGTCAGCTGAACCTGTGG 1	1549
	Query 719		CATATATCACATACAGAGTTATTTCAAAG		778 Que			1615
	Sbjct 710		ĊTŤTCGCTÁĊÁŤÁTÁĠÁĠŤCÁŤAŤĊCÁÁĠ		769 Sbj		D CÁGTTGTTGAÁTTÁCCCAÁAÁGAGTTCCÁTTTGGÁTTCCÁTGCCTTCTTTGTCACÁGÁGG 1	1609
	Query 779	TCATGCATGATCCTGTACCAATAA	CCGTATCAGACCCCATCATGATGCACGAC		838 Que			
	Sbjct 770	TCATGCAAGATCCAGTTCCAATAA	ĊGAŤĂĊĊĂĠĊAŦĊŦĠŤŦĂŤĠĂŤĠĊĂĊĠĂŦ	TITIGCTA	⁸²⁹ Sbj	t 161	0 ÁÁCÁÁATTCÁGGÁGCÁÁÁGCCÁÁÁATGTGÁ 1638	

Fig 10. Homology of CuCCD1A and SLCCD1B

Homology was searched through NCBI. The lower part marked in red is the sequence position for SLCCD1B F911-R1136.



Fig 11. Electrophoresis of SLCCD1B.

Gene expression analysis of SLCCD1B F911-R1136. Left is MT, middle is 3-5-12, 6-13-29, 20-9-20 3 sense lines, right is 9-6-8, 13-2-17, 5A-6-7 3 Anti -sense line.



As a result of checking the homology of CuCCD1A, SLCCD4, and SLCCD4-1, the homology was about 50%, and 3 μ L of cDNA, 0.5 μ L of SLCCD4 F136, 0.5 μ L of SLCCD4 R260, D.W. After RT-PCR by mixing 16 μ L, 5 μ L of PCR product was loaded on 2% gel and electrophoresis was performed (Fig. 10A). Homology of LCCD4 and 4-1 was 82% (Fig. 9B). cDNA 3 μ L, SLCCD4-1 F793 0.5 μ L, SLCCD4-1 R1036 0.5 μ L, D.W. After RT-PCR was performed by mixing 16 μ L, 5 μ L of the PCR product was loaded on 2% gel and electrophoresed (Fig. 10B).

In the case of SLCCD4 and SLCCD4-1, it was confirmed that they were amplified in flowers and leaves. This shows that CCD4 is a gene acting on flowers and leaves. In the experiment of HAI, Nguyen Thi Lam, *Lilium brownii var. Colchesteri's* flower color changed from white to yellow during the flowering period. (Hai, Masuda et al. 2012).



A				_	O -1111	015	10. 9.80.999970. 0180.0180. 9180.009
CuCCD1A	NW Score -1057	Identities Gaps 959/1908(50%) 387/1908(20%)	Strand Plus/Plus			917 1083	TATTCACATIGATGATACAAAGAAGCTCGTITIGGTAT 956
SLCCD4	Query 1 Sbjct 1	ATGGTGGAGAAGG 	1 1 1 1 1 1		Query	957	ACTTOCCCGRATATGCARAGAATGRGAGTCGARATGAATGGTTTGRGCT-TCCTARTGCT 101 GATACCACGTTACGCCARAGATGGGTCGGARAATGAGTGGTGGTGGTGGTGATGCAGGTT 115
	Query 23 Sbjct 61	CGTCGTCGTCGTCGTCGTCGTCGTCGTCGTCGTCGTCGTCGTCGT	11 1 1 111 1 1 1	5552		1143 1016	GATACCROGTTACGCCAAAGATGAGTCGGAAATGAGGTGGTTCGATGTATCAGGATT 115 TTATCTTCCATAATGCCAATGCTTGGGAGGAGGAGGAGGAGGAGGAGGATGAAGTGGTTCTGATCACTT 107
	Query 51	AGTGGCGGTGGATC	-сдалассдалда	76	Sbjet		TAATATTGTACAC-GCGATAAACGCGTGGGATGAGGATG-GAGGTGATAC-GAT-AGTG 128
	Sbjct 120 Query 77	TTCTGTTAGAATTGAAGAAGACCACAAACAACCACAACTATAJ	TTGTGGAGAAA-CT	116	Query	1073	GCCGCCTAGA-CAAGCCGGATCTAGACATGGTCRATGGGGCTGTCRA-AGAAAAGCTAGA 113
	Sbjct 180	ATCAACCCCTTCACCACCAAAACCATCTCCAAAAAAGAGAACCAJ	ATACCCTCAAGAAAACC	239	Sbjct Query	1255 1131	İTĞATÖSCÖĞĞAAİRİÄİTATÖSGİĞĞAACATACÁCTÄĞÄQÄSĞAİGĞİ ARATITCTCARARG-AACTGTATGAGATGAGATTCAACTTGAAAACTGGCCTAGCTI 116
	Query 117 Sbjct 240	GATAGTGAAGTGGAT ATTGAACCATCATTTCCCTCTITTATCTTCAATGCATTCGAT	GTACGATTCTT- GATTTCGTAAATACTTT	142 299	Sbjet	1305	CATGATACATGCAAGTGT-TGAGAAAGTGAAAATAAATTTGAAGACAGGAATGGTGA 130
	Query 143 Sbjct 300	TATTGATCCACCTAGGAAATCGTCTGTTGATCCAAGGTATGTT				1187 1361	-CACAAAAGAGATTATCGGCATCCGCTGTTGATTTTCCTAGGGTGAATGAGTGCT-A 124 GCAGACATCCAATT-ICTACAAGAAATCTTGATTTGGASTCA-TCAATCCAGCTTA 141
	Query 177	TCCAGTICCTGCTAGAGAGCCCCCCCCCCCCCCCCCCCCC					CACTGGCAGGAAGCAAAAATATGTGTATGGAACAATACTAGATAGCATTGCAAAAGTCAC 133
	Sbjct 360 Query 237	TCCAGTAGACGAACTTCCTCCTACCGAATGCGAAGTAGTGC TTGCTTGAATGGGGGAGTTTGTCAGGGTTGGTCCTAATCC-CAAC		1.	Sbjct Query		тотторалалаласалсалотасотататоскоссаттороросстатоссалаласаласт 147 лодоратсятсяластторатосторассаларадалаласаларостторарт 136
	Sbjet 417	TTGCCTAGATGGCGCGCGTATATTAGAAATGGACCTAACCCTCAA	TATCTTCCACGTGGACC	476		1476	AGGGATAGCAAAATTAGACGTATCC-GTAGCAGAAATTGATCGTCGCGATTGCA 152
	Query 296 Sbjct 477	GATACCACTGGTTTGATGGAGATGGCATGATTCATGGTCTGCGG IIIII IIIIIIIIIIIIIIIIIIIIIIIIIIIIII	CATCAAAGACG ATTAAAATTTCCCAAG	349 529	Query	1362	TGGAGGAAATGTGAGAGGCATCTTTGATCTGGGCCCTGGAAGATTTGGTTCAGAGG 141 TCCTGCCTGTCGTATATTTGAAAAGATTGCTATGCTGGTGAAC 157
	Query 350 Sbjct 530	GARAGGCTACAT-AIGTCTCCCGTTTIGIGAGGACTTCACGTC 	TTANACAGGAAGAATTT	408 587	Sbjct Query		
	Query 409	TTCGGAGGTGCTAAATTTATGAAGAT-TGGAGACCTTAA	GGGGCTATTTGGATTA-	462		1574	CTGTITIFGTTCCTARGABACCTGCCACCTCTCCTARGABABATGTTGGTTACTTARTAT 147
	Sbjct 588 Query 463	AGCCG-GGTCACCCATTATTCCTAATGTGTTCTCCGGTTTCAAC	TACTGGAT-GTTTCATA	518	Query	1478 1628	TCTTCTCTCATGATGAGAAAACCGGAAAGTCATCAGTGAACGTGATGCAGAAAACAA 153 CATACGTACACAATGAGAAGAACAGGGGAATCAAATTTTTTGGTCATGGATGCAACAATCAC 168
	Sbjct 647 Query 519	cccccccccccccccccccccccccccccccccccccc				1538	TGTCAGCT-GATCCTGTTGCAGTTGTCGAATTACCACAAAGGGTTCCATACGGATTTCAT 155
	Query 519 Sbjct 698	TGG-AAATGGGACAGCTAATACA-GCTCTTGTATATCJ CCGCAAACGGTATAGGCCTTGCGAATACAAGCTTAGCTT	I II IIIIII IGGA-GGTAAACTTITC	756	Sbjet	1688 1597	C-TANTCTTGACATTGTGGCTAATGCCAAATGCCTCGTCGTGTGCCTTACGGTTCCAT GCCTTCTTGTGCCGGGGACAACTGGAGAGCAGCAAGTGTGA 1644
	Query 571 Sbjct 757	GCGCTCTCAGAGGCGGACAAACCATATGCCGTCAAAGTTCTGGJ 	ARGATGGAGATCTGCAA CAAATGGTGACATTTTT	630 816		1747	GGACTITICGIGAGIGAAAAIGAICIT-AIGAAGCTAIAA 1785
	Query 631 Sbjct 817	ACACTTGGTATGCTTGACTATGACA-AGAGATTACAACATTCCT 	TTTACTGCCCATCCAAA	689 875			
	Query 690	GGTTGATCCATACACTGGCGAGATGTTTACCTTTGGCTATGCA	CACACACCACCATATAT	749			
	Sbjct 876 Query 750	AATCGACCACGAAACTAACGAGGCTTTGCTTTCCGTTACGGCC CACATACAGAGTTATTTCAAAGGATGGTTTCATGCATGJ	ATCCTGTACCAATAACC	804			
	Sbjet 936	AACTTACTTTCGGGTCAACCCGGATGGTACAAAAACCCCAGG					
	Query 805 Sbjct 991	GTATCAGACCCCATCATGA-TGCACGACTTTGCTATTA	CIGAGAATIAIGCIA	1047			
	Query 857 Sbjct 1048	TTTTCATGGATCTTCCGCTGTATTTCAGACCAAAGGAAATGGT 		916 1082			
В	NW Score	Identities Gaps	Strand	٦			
SLCCD4	1963 Query 1	1467/1798(82%) 77/1798(4%) ATGGATGCTTTGTCTTCAACTTTCCTTTCTACATTATCACAAAACO	Plus/Plus CTAAATCTCTTCTT 60	Que	ry 1014	TCACG	ACTTOGCARTCACRARARATACGCGRTATTTTCGGACATACARATTGGARTGAR 1073
SLCCD4-1	Sbjct 1	ATGAATGCTTTGTCTTCAACTTTTCTTTCTACATTACCACAACACC		0.14	ct 957		ACATTGCAATCACAAAAAAAAAAAACCCCATAATTICGGACATACAAAATAGGAATGAA TIGATTIACTCACCGOTGCTTCACCGOTGGGTACTGACTGCGGGGAAAATTCC 1130
	Query 61 Sbjct 61	ICTCCTTATAATAATAATAATAATAATAATAATAATAATAA	CTCCTACTCTAAAA 11	o Sbj	ct 1017		ITGATTACTCACOGEGEGTCCACCGEGEGATACTGACTCGGGGAAAAATTCC 1130
	Query 115 Sbjct 121	GTATTTTCTGTTAGAATTGAAGAAAAGACCACAAACAACCACAACTA 	TAACAAAACCACAA 17				
				4 Sbj	ry 1131 ct 1077	CCGAC	TTGGTGGTATACCACGTTACGCCAAAGGATGAGTGGAATGAGGTGGTTCGATGT 1190 TTGGAGTGATTCCACGTTATGCCAAGGGTGAATCGGAAATGAGGTGGTTGATGT 1136
	Query 175	GARARATCARCCCCTTCRCCRCCRARACCATCTCCRARAGAGARC	à 14 CAATACCCTCAAGA 23	4 Sbj	ry 1131 ct 1077	CCGAC	TTGGAGTGATTCCACGTTATGCCAAGGGTGAATCGGAAATGAGGTGGTTTGATGT 1136
	Query 175 Sbjct 144	GAAAAATCAACCCCTTCACCACCAAAACCATCTCCAAAAAGAGAAC IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII		4 Sbj 3 Que 4 Sbj 6 Que	ery 1131 at 1077 ery 1191 at 1137 ery 1251	ATCAGO ATCAGO III GCCAGO AGTGT	HIDBACTARTCCRCCTTATOCOLOGOTALTCOCOLATALGOTATICSTOT 1136 BATTIBATATICIACCCOTORIAGACCOTOCOLIAGORATORIOGITATICONT 1180 BOTTIBATATICIACCCATORIAGORATORIOGITAGATA 1196 TATOCOCCOLARIATITATICOGTOGORACATACATIGARGOTAGACTIGAT 1310
	Query 175	GAAAAATCAACCCCTTCACCACCAAAACCATCTCCAAAAAGAGAAC		4 Sbj 3 Que 4 Sbj 6 Que 4 Sbj	ery 1131 et 1077 ery 1191 et 1137	ATCAG ATCAG III GCCAG AGTGT AGTAT	НОВАЛБАНТСКОНТИЧЕСКАВСТВАЛГОВДАЛБАВСТВАТТСКИТ ТАКТИ ВАЛТТАЛИЛГОГАСОСОПЛАНССОГОВДАЛБАВСЛОВАСТВАЛГОВЛ БОГПТАЛИЛГИСКОСОПЛАНССОГОВДАЛВАВСТВАЛСКИТ (186 ВОГПТАЛИТАТИСКОСОПЛАНССОГОВДАЛВАВСТВАЛСКИТ 1010 ТАИТОСКОСОНАТИЛИТИСТВАТВАНСКИТАКЛАВИВСКИТИКТ (1910) ТАИТОСКОСОНАТИЛИТИСТВАЛВАСТИСКИТАНДВАЛБАТИЗИТАТИТИ 1266
	Query 175 Sbjct 144 Query 235 Sbjct 187 Query 295	GALARATCARCCOCTTCACCACADACCATCTCCALALAGAGAC I IIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIIII	14 CAATACCCTCAAGA 23 IIIIIIIIA AAATACCCTCAAGA 16 ATGATTTCGTAAAT 25 IIIIIIIIII ACGATTTTGTGAAT 23 ITCTCTCTAACAAC 35	4 Sbj 3 Que 4 Sbj 6 Que 4 Sbj 7 Que 54 Sbj	ery 1131 at 1077 ery 1191 at 1137 ery 1251 at 1197 ery 1311 at 1257	ATCAG ATCAG III GCCAG AGTGT AGTGT AGTAT ACATG	НОВАНИАТОЛАСНИТИЧЕСКА ОСОБАНИСКА И ОБЛАНИ КАЛИТИКИ ТОЛИТ ТАЛИТ 136 САЛИТАЛИ ПОТАСООВАТИЛИ ОСОБАНИСКИ ТОЛИТОВИ ОТОЛИТОВИ ТОЛИТОВИ ТОЛИТОВИ ТОЛИТОВИ ТОЛИТОВИ ТОЛИТОВИ ТОЛИТОВИ ТОЛИТ ТОЛИТОВИСТИ ПОТАСООВАТИЛИ ОСОБАНИСКИ ТОЛИТОВИ ТОЛИТОВИ ТОЛИТОВИ ТОЛИТОВИСТИ ТОЛИТИВИ ТОЛИТОВИ ТОЛИТОВИ ТОЛИТОВИ ТОЛИТОВИ ТОЛИТОВИ ТОЛИТОВИСТИ ТОЛИТИВИ ТОЛИТОВИ ТОЛИТОВИ ТОЛИТОВИ ТОЛИТОВИ ТОЛИТОВИ ТОЛИТОВИ ТОЛИТОВИ ТОЛИТОВИ ТОЛИТОВИ ТОЛИТОВИ ТОЛИТОВИСТИ ТОЛИТИВИ ТОЛИТОВИ ТОЛИТОВИ ТОЛИТОВИ ТОЛИТОВИ ТОЛИТОВИ ТОЛИТОВИ ТОЛИТОВИ ТОЛИТОВИ ТОЛИТОВИ ТОЛИТОВИ ПОТОТ ТОЛИТИВИ ТОЛИТОВИ ТОЛИТИ ВИ ТОЛИТОВИ ТОЛИТИ ТОЛИТИ ТОЛИТОВИ ТОЛИТИ ТОЛИТОВИ ТОЛИТИ ТОЛИТИ ТОЛИТИ ТОЛИТИ ТОЛИТИ ТОЛИТИ ТОЛИТИ ТОЛИТИВИ ТОЛИТИ ТОЛИТИ ТОЛИТИ ТОЛИТИ ТОЛИТИ ТОЛИТИ ТОЛИТИ ТОЛИТИ ТОЛИТИ ТОЛИТИ ТОЛИТИ ТОЛИТИ ТОЛИТИ ТОЛИТИ ТОЛИТИ ТОЛИТИ ТОЛИ ТОЛ
	Query 175 Sbjet 144 Query 235 Sbjet 187	алланталассттенскалаласситесалалассия аластикаторосскалаласситесалалассия аластикатарассискаторосскалала аластикатарассискаторосститикаторосскатор аластикатарасскаторосститикаторосскатор аластикатарасскатараласторосскатарасскаласка алтикатарасскатараласторосскатарасскаласка алтикатарасскатараласторосскатарасскаласка алтикатарасскатараласкатараскаласка алтикатараскатараскатараскаласка алтикатараскатараскатараскаласка алтикатараскатараскатараскаласка алтикатараскатараскатараскаласка алтикатараскатараскаласка алтикатараскатараска алтикатараскатараска алтикатараскатараска алтикатараскатараска алтикатараскатараска алтикатараскатараска алтикатараскатараска алтикатараска		4 Sbj 3 Que 4 Sbj 6 Que 4 Sbj 7 Que 4 Sbj 7 Que	ery 1131 at 1077 ery 1191 at 1137 ery 1251 at 1197 ery 1311 at 1257	ATCAG ATCAG III GCCAG AGTGT AGTGT AGTAT ACATG	НОВАЛБАНТСКОНТИЧЕСКАВСТВАЛГОВДАЛБАВСТВАТТСКИТ ТАКТИ ВАЛТТАЛИЛГОГАСОСОПЛАНССОГОВДАЛБАВСЛОВАСТВАЛГОВЛ БОГПТАЛИЛГИСКОСОПЛАНССОГОВДАЛВАВСТВАЛСКИТ (186 ВОГПТАЛИТАТИСКОСОПЛАНССОГОВДАЛВАВСТВАЛСКИТ 1010 ТАИТОСКОСОНАТИЛИТИСТВАТВАНСКИТАКЛАВИВСКИТИКТ (1910) ТАИТОСКОСОНАТИЛИТИСТВАЛВАСТИСКИТАНДВАЛБАТИЗИТАТИТИ 1266
	Query 175 Sbjct 144 Query 235 Sbjct 187 Query 295 Sbjct 238 Query 355 Sbjct 298	ВАМАНТСИАТСЯТ ГСИСОКАВАНСКИТИСТАВАНИКАВИ в — СИАНТСИТИСКИ СОСКАВАНИСИТИСКАВАНИКАВИ АМАСТАНТВИАССА ГСАНТИСКИ ГНАНИКИ СОСКАВАНИИ АМАСТАТИКАВИТИСКИ ГНАНИКИ СОСКАВАНИИ АМАСТАТИКАВИТИСКИ СОСКАВАНИИ СПОЛНИКИ СОСКАВИТИК АКТИТИКА ПОКОТОКИ СПОЛНИКИ СОСКАВИТИСКИ АКТИТИКА ПОКОТОКИ СПОЛНИКИ СОСКАВИТИСКИ АКТИТИКА ПОКОТОКИ СПОЛНИКИ СОСКАВИТИСКИ ПОПОСТОКИ ПЛАНИКИ ПОСКАВИТИСКИ ПОСКАВИТИСКИ ПОСТОСИИ ПЛАНИКИ ПОСКАВИТИСКИ ПОСКАВИТИСКИ ПОСТОСИИ ПЛАНИКИ ПОСКАВИТИСКИ ПОСКАВИТИКИ ПОСТОСИИ ПЛАНИКИ ПОСКАВИТИСКИ ПОСКАВИТИСКИ ПОСТОСИИ ПЛАНИКИ ПОСКАВИТИСКИ ПОСКАВИТИКИ ПОСТОСИИ ПЛАНИКИ ПОСКАВИТИСКИ ПОСКАВИТИСКИ ПОСТОСИИ ПЛАНИКИ ПОСКАВИТИСКИ ПОСКАВИТИКИ ПОСТОСИИ ПЛАНИКИ ПОСКАВИТИСКИ ПОСКАВИТИКИ ПОСТОСИИ ПЛАНИКИ ПОСКАВИТИСКИ ПОСКАВИТИКИ ПОСТОСИИ ПЛАНИКИ ПОСКАВИТИСКИ ПОСКАВИТИСКИ ПОСТОСИИ ПЛАНИКИ ПОСКАВИТИСКИ ПОСКАВИТИКИ ПОСТОСИИ ПЛАНИКИ ПОСКАВИТИСКИ ПОСТОСИИ ПОСКАВИТИСКИ ПОСКАВИТИСКИ ПОСТОСИИ ПЛАНИКИ ПОСКАВИТИСКИ ПОСКАВИТИСКИ ПОСТОСИИ ПЛАНИКИ ПОСКАВИТИСКИ ПОСКАВИТИСКИ ПОСТОСИИ ПЛАНИКИ ПОСКАВИТИСКИ ПОСКАВИТИСКИ ПОСКАВИТИСКИ ПОСТОСИИ ПЛАНИКИ ПОСКАВИТИСКИ ПОСКАВИТИСКИ ПОСТОСИИ ПЛАНИКИ ПОСКАВИТИСКИ ПОСКАВИТИСКИ ПОСТОСИИ ПЛАНИКИ ПОСКАВИТИСКИ ПОСКАВИТИСКИ ПОСКАВИТИСКИ ПОСТОСИИ ПЛАНИКИ ПОСКАВИТИСКИ ПОСКАВИТИСКИ ПОСКАВИТИСКИ ПОСКАВИТИСКИ ПОСКАВИТИИ ПОСКАВИТНИ ПОСКАВИТИ ВИ ПОСКАВИТИ	A 14 CAATACCCTCAAGA 21 AAATACCCTCAAGA 21 AAATACCCTCAAGA 11 AAGATACCCTCAAGA 12 AIGATTICGTAAGAT 21 TTCTCCTAACAAC 31 IIGTTICTAACAAC 31 IIGTTICTAACAAC 31 IIGTTICTAACAAC 31 IIGTTICTAACAAC 31 IIGTTICTAACAAC 31 AAGGCTCCCTTCCA 41 IIGTTICTAACAAC 31 AAGGCTCCCTTCCA 31	4 Sbj 3 Que 4 Sbj 6 Que 4 Sbj 7 Que 5 Sbj 7 Que 4 Sbj 7 Que 5 Sbj 7	ery 1131 et 1077 ery 1191 et 1137 ery 1251 et 1197 ery 1311 et 1257 ery 1371	ATCRG ATCRG GCCRG AGTATI AGTATI ACATG AATTTY ACATG	НОВАНИАТОЛАСНИТИЧЕСКА ОСОБАНИСКА И ОБЛАНИ КАЛИТИКИ ТОЛИТ ТАЛИТ 136 САЛИТАЛИ ПОТАСООВАТИЛИ ОСОБАНИСКИ ТОЛИТОВИ ОТОЛИТОВИ ТОЛИТОВИ ТОЛИТОВИ ТОЛИТОВИ ТОЛИТОВИ ТОЛИТОВИ ТОЛИТОВИ ТОЛИТ ТОЛИТОВИСТИ ПОТАСООВАТИЛИ ОСОБАНИСКИ ТОЛИТОВИ ТОЛИТОВИ ТОЛИТОВИ ТОЛИТОВИСТИ ТОЛИТИВИ ТОЛИТОВИ ТОЛИТОВИ ТОЛИТОВИ ТОЛИТОВИ ТОЛИТОВИ ТАЛИТОВИСТИ ТОЛИТИВИ ТОЛИТОВИ ТОЛИТОВИ ТОЛИТОВИ ТОЛИТОВИ ТОЛИТОВИ ТОЛИТОВИ ТОЛИТОВИ ТОЛИТОВИ ТОЛИТОВИ ТОЛИТОВИ ПОТОТО ТОЛИТИВИ ТОЛИТОВИ ТОЛИТ
	Query 175 Sbjct 144 Query 235 Sbjct 187 Query 295 Sbjct 238 Query 355	алланталассттенскалаласситесалалассия аластикаторосскалаласситесалалассия аластикатарассискаторосскалала аластикатарассискаторосститикаторосскатор аластикатарасскаторосститикаторосскатор аластикатарасскатараласторосскатарасскаласка алтикатарасскатараласторосскатарасскаласка алтикатарасскатараласторосскатарасскаласка алтикатарасскатараласкатараскаласка алтикатараскатараскатараскаласка алтикатараскатараскатараскаласка алтикатараскатараскатараскаласка алтикатараскатараскатараскаласка алтикатараскатараскаласка алтикатараскатараска алтикатараскатараска алтикатараскатараска алтикатараскатараска алтикатараскатараска алтикатараскатараска алтикатараскатараска алтикатараска	A 14 CAATACCCTCAAGA 21 AAATACCCTCAAGA 21 AAATACCCTCAAGA 11 AAGATACCCTCAAGA 12 AIGATTICGTAAGAT 21 TTCTCCTAACAAC 31 IIGTTICTAACAAC 31 IIGTTICTAACAAC 31 IIGTTICTAACAAC 31 IIGTTICTAACAAC 31 IIGTTICTAACAAC 31 AAGGCTCCCTTCCA 41 IIGTTICTAACAAC 31 AAGGCTCCCTTCCA 31	4 Sbj 3 Que 4 Sbj 6 Que 4 Sbj 7 Que 4 Sbj 7 Que 4 Sbj 7 Que 4 Sbj 7 Que 4 Sbj 7 Que	ery 1131 et 1077 ery 1191 et 1137 et 1137 ery 1251 et 1197 ery 1311 et 1257 ery 1371 et 1317 ery 1431 et 1377 ery 1491	ATCAGE ATCAGE AGTGT: AGTGT: AGTGT: AGTATGE AGTATGE AGTATGE AGTATGE AGTATGE AGTATGE AGTATGE AGTATGE AGTAGE AGTAGE AGTAGE AGTAGE	НОВАНИАТ ПОЛОТИТИТИСКИ КОЛТИКАТИ ОКОЛТИСКИ ПОЛОГИ
	Query 175 Sbjet 144 Query 235 Sbjet 187 Query 255 Sbjet 238 Query 355 Sbjet 298 Query 415 Sbjet 388 Query 475	ВАМАНТСКАССОТТ СССОСАВАЛОСЯТСТСКАВАЛОВИСЯ — — СТАЛТОСИТОВОССКАВАЛОСЯТСТСКАВАЛОВИИ АМАСТАНТКАТСКАСТИСКСТИТИТАТТСКАВОТАТСЯ АМАСТАТСКАСТАКОНТОСТОТИТИТАТТСКАВОТАТСЯ АСТИТИАТТСКАТСОССТИОЛАЛАТОГСТОТТОВОТССАВИТАТ АСТИТИАТТСКАТСОСОСТАВЛАЛАТОГСТОВОТОСАВИТАТСЯ АСТИТИАТТСКАТСОСОСТАВЛАЛАТОГСТОВОТОСАВИТАТА ПОСТОСЛОВИТОВОТОСТАВЛАТСЯ СТАВЛАТОВОВИТАТАТА ССТТООСТАВЛАТОВОСТИТИТИТАВАЛ СОДИССТАВЛАТОВОВИТАВИТА СОТТООСТАВЛАТОВОСТИТИТИТИВАЛ СОДИССТАВЛАТОВОВИТАВИТА СОТТООСТАВЛАТОВОСТИТИТИТИВАЛ СОДИСТВАТАСТАВАСТИКАТОВОВИТАВИТАВ СОТТООСТАВЛАТОВОСТИТИТИТИВАЛ СОДИСТВАТАСТАВАСТИСКАТИТОВОВИТАВИТАВИТСЯ СОТТООСТАВЛАТОВОСТИТИТИТИВАЛ СОДИСТВАТАСТАВАСТИКАТОВОВИТАВИТАВИТСЯ СОТТООСТАВЛАТОВОСТИТИТИВАЛАТОВОВИТИВАТОВОСТИТИВАТОВОВИТИВИТАВИТАВИТАВИТСЯ СОТТООСТАВЛАТОВОСТИТИТИТИВАЛАТОВИТСЯ СТАВЛАТОВОТНОВИТОВИТАВИТАВИТОВОВИТИВИТСЯ ВИТОВОТНИИ СОТТООСТАВЛАТОВОСТИТИТИТИВАЛАТОВИТСЯ СТАВЛАТОВОВИТАВИТАВИТАВИТОВОВИТИВИТАВИТИВИСТИ СОДИСОВИТИВИТАВИТАВИТИВИССИ ВИТОВИТИВИТИВИИ СОТТООСТАВЛАТОВОСТИТИТИТИВАЛАТОВИТСЯ СТАВЛАТОВОВИТИВИИ СОТТООСТАВЛАТОВОССИТИВИТИТИВИТОВИТОВИТИВИТИВИИ СОТТООСТАВЛАТОВОСТИТИТИТИВИИ ПОСЛЕДИИ СОДИСОВИТАВИТАВИТИВИИ СОТТООСТАВЛАТОВОСТИТИТИТИВИИ ПОСИТОВИТСЯ ПОСЛЕДИИСТИВИИ СОТТООСТАВЛАТОВОСТИТИИТИВИИ ПОСИТОВИТОВИТИВИИ СОТТООСТАВЛАТОВОСТИТИИТИТИВИИ ПОСИТОВАТОВИТИВИИ СОТТООСТАВЛАТОВОСТИТИИТИТИВИИ ПОСИТОВАТОВИТИВИИ СОТТООСТАВЛАТОВОСТИТИИТИТИВИИ ПОСИТОВИТОВИТИВИИ СОТТООСТАВЛАТОВОСТИТИИТИТИВИИ ПОСИТОВИТОВИТИВИИ ПОСИТОВИТСЯ ПОСИТОВИТОВИТИВИИ СОТТООСТАВЛАТОВОСТИТИИТИТИВИИ ПОСИТОВИТОВИТИВИИ СОТТООСТАВЛАТОВОСТИТИИТИТИВИИ ПОСИТОВИТОВИТИВИИ ПОСИТОВАТОВИТИВИИ ПОСИТОВИТСЯ ВИТОВИТСЯ ПОСИТОВИТИВИИ ПОСИТОВАТИИ СОТТООСТИВЛИТИТИИ СОСТИВИИ ПОСИТОВИТСЯ ПОСИТОВИТСЯ ПОСИТОВИТСЯ ПОСИТОВИТСЯ ПОСИТОВИИ ПОСИТИВИИ ПОСИТИВИИ ПОСИТОВИТИВИИ ПОСИТОВИТСЯ ПОСИТОВИТИВИИ ПОСИТОВИТСЯ ПОСИТОВИТСЯ ПОСИТОВИТСЯ ПОСИТОВИИ ПОСИТОВИИ ПОСИТОВИИ ПОСИТОВИТСЯ ПОСИТОВИИ ПО ВИТОВИТСЯ ПОСИТИВИИ ПОСИТОВИТСЯ ПОСИТИВИИ ПОСИТИВИИ ПОСИТОВИИ ПОСИТИВИИ ПО ВИТОВИТИВИИ ПОСИТИВИИ ПО ВИТИВИИ ПОСИТОВИТСЯ ПОСИТИВИИ ПОСИТИВИИ ПО ВИТИВИИ ПОСИТИТИВИИ ПОСИТИВИИ ПО ВИТИВИИ ПОСИТИВИИ ПО	CATACOCCADADA 21 ANATACOCCADADA 21 ANATACOCCADADA 21 ANGANTOGTADAT 22 ANGANTOGTADAT 22 ANGANTOGTADAT 22 ANGANTOGTADAT 22 ANGANCADADA 21 ANGANCADADAT 23 ANGANCADATACADA 21 ANGANCADATACADAT 23 ANGANCADATACADATA ANGANCADATAGATATA	4 Sbj 3 Qual 14 Sbj 16 Qual 16 Qual 14 Sbj 17 Qual 14 Sbj 14 Sbj 14 Sbj 14 Sbj	ery 1131 et 1077 ery 1191 et 1137 ery 1251 et 1197 ery 1311 et 1257 ery 1311 et 1317 ery 1431 et 1377	AGTAGE AG	НОВАННАЯ ГОЛАСТИТИТОССКАВОННАЯ ГОЛАЛИТИКОВИ ПОЛАГИ ТОЛАГИ 136 САЛТТАЛИТАТОТА СОСОЛГАНИИ ОСОГОЗИ ГОЛАГИЗИТОВОГГОВИТСКИ. ТОЛОГОЗИ ГОЛАГИИ СОСОЛГАНИИ ОСОГОЗИ ГОЛАГИЗИТОВОГГОВИТСКИ. ТОЛОГОЗИ ГОЛАГИИ СОСОЛГАНИИ ОСОГОЗИ ГОЛАГИЗИТОВОГГОВИТСКИ. ТОЛОГОЗИ ГОЛАГИИ СОСОЛГАНИИ ОСОГОЗИ ГОЛАГИЗИТОВОГГОВИТСКИ. 1310 ТОЛОГОЗИ ГОЛАГИИ СОСОЛГАНИИ СОСОЛГАНИИ ОСОГОЗИ ГОЛАГИИ СПОСИМИИ СОСОЛГАНИИ СОСОЛГАНИИ СОСОЛГАНИИ СОСОЛГАНИИ СОСОЛГАНИИ СПОСИМИИ СОСОЛГАНИИ СОСОЛГАНИИ СОСОЛГАНИИ СОСОЛГАНИИ СОСОЛГАНИИ СПОСИМИИ СОСОЛГАНИИ СОСОЛГИИ СОСОЛГАНИИ СОСОЛГАНИИ СОСОЛГАНИИ СОСОЛГАНИИ СОСОЛГАНИИ СОСОЛГИИ СОСОЛГАНИИ СОСОЛГИИ СОСОЛГАНИИ СОСОЛГАНИИ СОСОЛГАНИИ СОСОЛГИ СОСОЛГОНИИ СОСОЛГИИИ СОСОЛГИИ СОСОЛГИИ СОСОЛГИИ СОСОЛГИИ СОСОЛГИИ СОСОЛГИИ СОСОЛГИИ СОСОЛГИИ СОСОЛГИИ СОСОЛИИ СОСОЛГИИ СОСОЛГИИ СОСОЛГИИ СОСОЛГИ
	Query 175 Sbjet 144 Query 235 Sbjet 187 Query 255 Sbjet 238 Query 355 Sbjet 298 Query 415 Sbjet 358	ВАЗАЛТСЬЯ ССОТТ СОСОСАВЛАЮСЯТСТСКАЗАЛИСИИ — — — СПАТОСИТСКИ СОСОБЛАЮСЯТТСКАЗАЛИСИИ МАССТАТСКА СОКТОССТИТИТАТСКАТИРОВЛЕС МАССТАТСКАТОРОССТИТИТАТСКАТИРОВЛЕС АСТИТИАТСКИ СОКТОВЛАТСЯ СТОТИТИТСКАТИРОВЛЕС МАССТАТСКАТИРОВЛЕТИИ СТОТИТИТСКАТИРОВЛЕС МАССТАТСКАТИРОВЛЕТИИ СТОТИТИТИСКАТИРОВЛЕТИИ МАССТАТИРОВЛЕТИИ СТОТИТИТИСКАТИРОВЛЕТИИ МАССТАТИРОВЛЕТИИ СТОТИТИТИСКАТИРОВЛЕТИИ МАССТАТИРОВЛЕТИИ СТОТИТИТИСКАТИРОВЛЕТИИ МАССТАТИРОВЛЕТИИ СТОТИТИСКАТИРОВЛЕТИИ МАССТАТИРОВЛЕТИИ СТОТИТИСКАТИРОВЛЕТИИ МАССТАТИРОВЛЕТИИ СТОТИТИСКАТИРОВЛЕТИИ МАССТАТИРОВЛЕТИИ СТОТИТИСКАТИРОВЛЕТИИ МАССТАТИРОВЛЕТИИ СТОТИТИСКАТИРОВЛЕТИИ МАССТАТИРОВЛЕТИИ СТОТИТИСКАТИРОВЛЕТИИ ПОСТОИРОВЛЕТИИ СТОТИТИСКАТИРОВЛЕТИИ СТОТИСКАТИРОВЛЕТИИ СТОТИТИСКАТИРОВЛЕТИИ СТОТИСКАТИРОВЛЕТИИ СТОТИТИСКАТИРОВЛЕТИИ СТОТИСКАТИРОВЛЕТИИ СТОТИТИСКАТИРОВЛЕТИИ СТОТИСКАТИРОВЛЕТИИ СТОТИТИСКАТИРОВЛЕТИИ СТОТИСКАТИРОВЛЕТИИ СТОТИТИИ СТОТИСКАТИРОВЛЕТИИ СТОТИСКАТИРОВЛЕТИИ СТОТИСКАТИРОВЛЕТИИ СТОТИТИИ СТОТИСКАТИРОВЛЕТИИ СТОТИТИИ СТОТИСКАТИРОВЛЕТИИ СТОТИСКАТИРОВЛЕТИИ СТОТИТИСКАТИРОВЛЕТИИ СТОТИССТИ ПОВЛЕТИИ СТОТИСКАТ	CATACOTCARGA 21 MATACOTCARGA 21 MATACOTCARGA 21 MATACOTCARGA 21 MATACOTCARGA 21 TICTCTCARGANC 21 TICTCTCARGANC 21 TICTCTCARGANC 21 MAGGETOCITICA 21 MAGGETOCITI	4 Sbj 3 Qual 14 Sbj 16 Qual 14 Sbj 14 Sbj 14 Sbj 15 Qual 14 Sbj 17 Qual 16 Qual 17 Qual 16 Sbj 17 Qual 14 Sbj 17 Qual 14 Sbj 17 Qual 14 Sbj 14 Sbj 14 Sbj 14 Sbj 14 Sbj 14 Sbj 15 Sbj 16 Qual 17 Qual 18 Sbj 19 Qual 10 Sbj 11 Sbj 12 Sbj 13 Sbj 14 Sbj	ery 1131 et 1077 ery 1191 et 1137 ery 1251 et 1197 ery 1311 et 1257 ery 1311 et 1257 ery 1371 et 1317 ery 1491 et 1437 ery 1551 et 1497	ATCAG COGACI ACCAG AGTGT III AGTAT ACATG AATTTO ACATG AATTTO CAAGTI CAAGTI CAAGTI CAAGTI AGACG AAAAGI AAAAGI AAAAGI	НОВАННАЯ ПОЛОГИТАТОРОВ СТАЛА
	Query 175 Sbjct 144 Query 285 Sbjct 187 Query 295 Sbjct 288 Query 355 Sbjct 298 Query 415 Sbjct 358 Query 415 Sbjct 418 Query 535 Sbjct 418 Query 535 Sbjct 478	ВАМАНТСЬАСССТ ГСЬССКАМАСНАТСТСКАМАЛЛАВИЛ в — СИАНТСОЛГСКОССКАМАЛ АЛАССАНТСКАТОРОССКАМАЛ АЛАССАНТСКАТОРОССТАТИТАТТСКАМАЛЛАВИТСК АЛАССАНТСКАТОРОССТАТИТАТТСКАЛОВИТСК АЛАССАНТСКАТОРОССКАТИРАЛИСТ АЛСТИТАТСКАТОСАСТИВИАЛАТОСТСКАТОРАТІСК АЛСТИТАТСКАТОСАСТИВИАЛАТОСТСКАТОРАТІСКА АЛСТИТАТСКАТОСАСТИВИАЛАТОСТСКАТОРА АЛСТИТАТСКАТОСАСТИВИАЛАТОСТСКАТОРА АЛСТИТАТСКАТОРАСТИВИТСКАТОРАТІСКАТОРА АЛСТИТАТСКАТОРАТИСТ ГОЛАГОРАТИССКАТИРА АЛСТИТАТСКАТОРАТИСТ ГОЛАГОРАТИССКАТИРА АЛСТИТАТСКАТОРАТИРА СПОСТОКАТИРАТИРА СТИПОСТОКАТОРАТИСКАТОРАТИССТКАТОРА СПОСТОКАТИРАТИРА СПОСТОКАТИРА СПОСТОКАТИРАТИРА СПОСТОКАТИРА СПОСТОКАТИРА СПОСТОКАТИРАТИРА СПОСТОКАТИРА СПОСТОКАТИРА СПОСТОКАТИРАТИРА СПОСТОКАТИРА СПОСТОКАТИРА СПОСТОКАТОРА СПОСТОКАТИРА СПОСТОК	CATACCTCAGE 2 JANTACCTCAGE 2 JANTACCTCAGE 2 JANTACCTCAGE 2 JANTACCTCAGE 2 JANTACCTCAGE 2 JANTACCTCAGE 2 JANTACCAGE 2 JANTA	4 Sbj 33 Qual 44 Sbj 45 Sbj 46 Sbj 47 Qual 48 Sbj 49 Sbj 44 Sbj 45 Sbj 46 Sbj 47 Qual 48 Sbj 49 Sbj 44 Sbj 45 Sbj 46 Sbj 47 Qual 48 Sbj 49 Sbj 40 Sbj 41 Sbj 42 Sbj 43 Sbj 44 Sbj 45 Sbj 46 Sbj 47 Sbj 48 Sbj 49 Qual	ery 1131 et 1077 ery 1191 et 1137 ery 1251 et 1197 ery 1311 et 1257 ery 1311 et 1377 ery 1431 et 1377 ery 1491 et 1437 ery 1551	LILI COGACT ATCRG ATCRG ACTOR AGTOR AGTOR AGTOR ACA	HIGH HIGH THAT AND ADDRESS AND
	Query 175 Sbjet 144 Query 235 Sbjet 187 Query 255 Sbjet 238 Query 355 Sbjet 358 Query 415 Sbjet 358 Query 475	ВАЗАЛТСЬЯ ССОТТ СОСОСАВЛАЮСЯТСТСКАЗАЛИСИИ — — — СПАТОСИТСКИ СОСОБЛАЮСЯТТСКАЗАЛИСИИ МАССТАТСКА СОКТОССТИТИТАТСКАТИРОВЛЕС АПОСТАТСЯ СОКТОВИТСЯ ПО СОСОССТИТИТАТСКАТИРОВЛЕСА АПОСТАТСЯ СОСОССТИСИИ АПОСТАТСЯ СОСОССТИСИИ АПОСТАТСЯ СОСОССТИСИИ ПОСТОСЛЕНИИ СОСОССТИСИИ ПОСТОСЛЕНИИ СОСОССТИСИИ ПОСТОСЛЕНИИ СОСОССТИСИИ СТПОССТСИИ ПОВОССТИТИТИТИСИИ ПОСТОСИИ СПИСОСТИИ ПОВОССТИТИТИТИСИИ ПОСТОСИИ СПИСОСТИИ ПОВОССТИТИТИТИСИИ ПОВОССТИИ СПИСОСТИИ ПОВОССТИТИТИТИСИИ ПОВОССТИИ СПИСОСТИИ ПОВОССТИТИТИТИСИИ ПОВОССТИИ СПИСОСТИИ ПОВОССТИИ ПОВОССТИИ ПОВОССТИИ СПИСОСТИИ ПОВОССТИИ ПОВОССТИИ ПОВОССТИИ СПИСОСТИИ ПОВОССТИИ ПОВОССТИИ ПОВОССТИИ СПИСОСТИИ ПОВОССТИИ ПОВОССТИИ ПОВОССТИИ СПИСОСТИИ ПОВОССТИИ ПОВОССТИИ ПОВОССТИИ СПИСОСТИИ ПОВОССТИИ ПОВОССТИИ СПИСОСТИИ ПОВОССТИИ ПОВОСТИИ СПИСОСТИИ СОСОСТИИ ПОВОССТИИ СПИСОСТИИ ПОВОССТИИ ПОВОССТИИ СПИСОСТИИ ПОВОССТИИ ПОВОССТИИ СПИСОСТИИ ПОВОССТИИ ПОВОССТИИ СПИСОСТИИ ПОВОССТИИ СПИСОСТИИ ПОВОССТИИ ПОВОССТИИ СПИСОСТИИ ПОВОССТИИ СПИСОСТИИ ПОВОССТИИ СПИСОСТИИ ПОВОССТИИ СПИСОСТИИ ПОВОССТИИ СПИСОСТИИ ПОВОССТИИ СПИСОСТИИ ПОВОССТИИ СПИСОСТИИ ПОВОССТИИ СПИСОСТИИ ПОВОССТИИ СПИСОСТИИ ПОВОССТИИ СПИСОСТИИ ПОВОССТИИ СПИСОСТИИ ПОВОССТИИ СПИСОСТИИ ПОВОССТИИ СПИСОСТИИ ПОВОССТИИ СПИСОСТИИ СПИСОСТИИ ПОВОССТИИ СПИСОС	ADDATACCOTCAMAGA 2 CANTACCOTCAMAGA 2 ATOMICTICOTAMAT 2 ATOMICTICOTAMAT 2 ACOMITICOTAMAT 2 ACOMITICOTAMAT 2 ACOMITICOTAMAT 2 ADDATACCAMA 2 ADDATACCAM	4 Sbj 3 Qual 44 Sbj 45 Sbj 46 Sbj 47 Qual 48 Sbj 49 Sbj 44 Sbj 45 Sbj 46 Sbj 47 Qual 48 Sbj 44 Sbj 45 Sbj 46 Sbj 47 Qual 48 Sbj 49 Sbj 44 Qual 45 Qual 46 Qual 47 Sbj 48 Qual	rry 1131 act 1077 rry 1191 act 1137 rry 1251 act 1197 rry 1311 act 1257 rry 1311 act 1317 rry 1431 act 1317 rry 1431 act 1437 rry 1551 act 1497 rry 1608	LILI COGACT ATCRG ATCRG ACTOR AGTOR AGTOR AGTOR ACA	HIGH HIGH THAT AND ADDRESS AND
	Query 175 Sbjct 144 Query 255 Sbjct 237 Query 256 Sbjct 238 Query 355 Sbjct 238 Query 355 Sbjct 415 Sbjct 358 Query 555 Sbjct 418 Query 535 Sbjct 418 Query 535 Sbjct 418 Query 535 Sbjct 548 Query 535 Sbjct 548 Query 535 Sbjct 548 Query 555 Sbjct 545 Query 555	ВАМАЙСЬАСССТ СКОСОСАНАСТ ТСС МАНАСИЛСИ — СПАЛОСИТОСОССАНАНА АМАСТАТ БОЛАССА ССАТООСССАНАНА АМАСТАТ БОЛАССА ССАТООСССТИТИТАТСТ СКАРОНТОВ АМАСТАТОВАНСКАТОВАНИТИТАТСКАРОНТОВ АСТИТИТАТОВАТСА СТАРОНТОВАНИТИТАТСКАРОНТОВ АСТИТИТАТОВАТСЯ СТАТОВАНИТИТАТОВАНИТИТАТОВАНИТИТА АСТИТИТАТОВАТСЯ СТАТОВАНИТИТАТОВАНИТИТАТОВАНИТИТА АСТИТИТАТОВАТСЯ СТАТОВАНИТИТАТОВАНИТИТАТОВАНИТИТА АСТИТИТАТОВАТСЯ СТАТОВАНИТИТАТОВАНИТИТАТОВАНИТИТА ПОСТООСНИКАТОВАНИТИТАТОВАНИ С СТАЛИТОВАНИТИТАТОВАНИТИТАТОВАНИТИТАТОВАНИТИТАТОВАНИТИТАТОВАНИТИТАТОВАНИТИТАТОВАНИТИТАТОВАНИТИТАТОВАНИТИТАТОВАНИТИТАТОВАНИТИТАТОВАНИТИТАТОВАНИТИТАТОВАНИТИТАТОВАНИТИТАТОВАНИТИТАТОВАНИ В С СТАЛИТИТИТОВАНИТИТИТОВАНИТИТИТОВАНИ В С СТАЛИТИТИТИ СТОВАНИТИТАТОВАНИТИТАТОВАНИТИТАТОВАНИТИТАТОВАНИТИТИТОВАНИТИТАТОВАНИТИТАТОВАНИТИТАТОВАНИИ В С С СТАЛИТИТИТОВАНИ В С С С КОЛИТИТАТОВАНИ В С С С КОЛИТИТАТОВАНИ В С С С КОЛИТИТАТОВАНИ В С С С КОЛИТИВАНИТИ В С С С КОЛИТИТОТОВАНИ В С С С КОЛИТИТОВАНИ В С С С КОЛИТИТАТОВАНИ О С С	ADDATACOTOCIANGA 21 ADATACOTOCIANGA 21 ADATA	4 Sbj 3 Sbj 44 Sbj 45 Sbj 46 Sbj 47 Sbj 48 Sbj 49 Sbj 44 Sbj 45 Sbj 46 Sbj 47 Quat 48 Sbj 44 Sbj 44 Sbj 45 Sbj 46 Sbj 47 Quat 48 Sbj 49 Quat 49 Quat 40 Quat 414 Quat 415 Quat 416 Quat 417 Sbj 42 Quat 43 Quat 44 Quat 45 Quat 46 Quat 47 Sbj	rry 1131 at 1077 rry 1191 at 1137 rry 1251 at 1257 rry 1311 at 1257 rry 1311 at 1317 at 1317 at 1317 at 1317 at 1317 rry 1431 at 1437 rry 1608 at 1657 rry 1608 at 1617	ACCASC ACCASC AGTATI AGTATI ACATGK AGTATI ACATGK AATTIX CAAGGI AAATTIX CAAGGI AAATTIX AGAAGG AGAAGI AGAAGI AGAAGGI AGAAGGI AGAAGGI AGAAGGI AGAAGGI AGAAGGI AGAAGGI AGAAGGI AGAAGAAGAAGA AGAAGAAGAAGAAGAAGAAGAAGAAG	HUMANNAN TOLOGONI TANGGALAA GUARANA ALAGA
	Query 175 Sbjct 144 Query 235 Sbjct 238 Query 245 Sbjct 238 Query 355 Sbjct 238 Query 355 Sbjct 158 Query 355 Sbjct 418 Query 535 Sbjct 418 Query 535 Sbjct 538 Sbjct 538	ВАМАНТСЬАССОТТ СК.ССИ. В.С. С. С. К. С. С. С. К. К.С. К. Т. С. С. К. К. К. К. К. К. К. К. К. К. К. К. К.	ADDATACOTOCAGE CAATACOTOCAGE ADATA	4 Sej 3 Que 5 Sej 4 Sej 5 Sej 6 Que 5 Sej 7 Que 4 Sej 7 Que 4 Sej 7 Que 5 Sej 7 Que 4 Sej 7 Que 5 Sej 7 Que 4 Sej 7 Que 5 Sej 7	rry 1131 at 1077 rry 1191 at 1137 rry 1251 at 1257 rry 1311 at 1257 rry 1311 at 1317 at 1317 at 1317 at 1317 at 1317 rry 1431 at 1437 rry 1608 at 1657 rry 1608 at 1617	ACCASC ACCASC AGTATI AGTATI ACATGK AGTATI ACATGK AATTIX CAAGGI AAATTIX CAAGGI AAATTIX AGAAGG AGAAGI AGAAGI AGAAGGI AGAAGGI AGAAGGI AGAAGGI AGAAGGI AGAAGGI AGAAGGI AGAAGGI AGAAGAAGAAGA AGAAGAAGAAGAAGAAGAAGAAGAAG	HIGH HIGH THAT AND ADDRESS AND
	Quary 2155 Bbjct 144 Quary 235 Sbjct 187 Quary 285 Sbjct 298 Sbjct 298 Sbjct 398 Quary 355 Sbjct 416 Sbjct 418 Quary 478 Sbjct 478 Quary 555 Sbjct 478 Quary 555 Sbjct 658 Quary 659 Sbjct 658 Sbjct 658 Sbjct 658 Sbjct 658		ADDATACOTTONAGA 21 ADATACOTTONAGA 21 ADATACOTTONAGA 21 ADATACOTTONAGA 21 ADATACOTTONAGA 21 ADATACOTTONAGA 21 ADATACOTTONA 21 ADATACOTTO	4 Sbj 3 Qué Sbj 4 Sbj 4 Sbj 4 Sbj 7 Qué Sbj 7 Qué Sbj 7 Qué Sbj 7 Qué Sbj 7 Qué Sbj 8 Sbj 7 Qué Sbj 7 Qué Sbj 8 Sbj 7 Qué Sbj 7 Sbj	rry 1131 at 1077 rry 1191 at 1137 rry 1251 at 1257 rry 1311 at 1257 rry 1311 at 1317 at 1317 at 1317 at 1317 at 1317 rry 1431 at 1437 rry 1608 at 1657 rry 1608 at 1617	ACCASC ACCASC AGTATI AGTATI ACATGK AGTATI ACATGK AATTIX CAAGGI AAATTIX CAAGGI AAATTIX AGAAGG AGAAGI AGAAGI AGAAGGI AGAAGGI AGAAGGI AGAAGGI AGAAGGI AGAAGGI AGAAGGI AGAAGGI AGAAGAAGAAGA AGAAGAAGAAGAAGAAGAAGAAGAAG	HUMANNAN TOLOGONI TANGGALAA GUARANA ALAGA
	Query 175 Shyic 184 Query 355 Shyic 187 Query 285 Shyic 283 Query 355 Shyic 289 Query 455 Shyic 288 Query 456 Shyic 188 Query 585 Shyic 188 Query 585 Shyic 588 Query 583 Query 583 Query 585 Shyic 589 Shyic 589 Query 585 Query 585 Shyic 589 Query 571	ВАМАНТСЬАССОТТ СК.ССИ. В.С. С. С. К. С. С. С. К. К.С. К. Т. С. С. К. К. К. К. К. К. К. К. К. К. К. К. К.	CARTACCTCAAGA 2 AAATACCCTCAAGA 2 AAATACCTCAAGA 2 AAATACATTCOTAAGA 2 ACATTCOTAAGA 2 ACATTCOTAAGA 2 ACATTCOTAAGA 2 ACATTCOTAAGA 2 AAATACAACCONTACA 3 AAATACAACCOSCOCCOCCC AAAATACAACCOSCOCCOCCC AAAATACAACCOSCOCCOCCC AAAATACAACCOSCOCCOCCC AAAATACAACCOSCOCCOCCC AAAATACAACCOSCOCCOCCC AAAATACAACCOSCOCCOCCC AAAATACAACCOSCOCCOCCC AAAATACAACCOSCOCCOCCC AAAATACAACCOSCOCCOCCC AAAATACAACCOSCOCCOCCC AAAATACAACCOSCOCCOCCC AAAATACAACCOSCOCCOCCCC AAAATACAACCOSCOCCOCCCC AAAATACAACCOSCOCCOCCCC AAAATACAACCOSCOCCOCCCC AAAATACAACCOSCOCCOCCCC AAAATACAACCOSCOCCOCCCC AAAATACAACCOSCOCCOCCCC AAAATACAACCOSCOCCOCCCC AAAATACAACCOSCOCCOCCCC AAAATACAACCOSCOCCCCCC AAAATACAACCOSCOCCCCCC AAAATACAACCOSCOCCCCCCC AAAATACAACCOSCOCCCCCC AAAATACAACCOSCOCCCCCC AAAATACAACCOSCOCCCCCCCCCCCCCCCCCCCCCCCCC	4 Sb 5 3 Quak Sc 5 4 Sc 5 4 Sc 5 4 Sc 5 7 Quak Sc 5	rry 1131 at 1077 rry 1191 at 1137 rry 1251 at 1257 rry 1311 at 1257 rry 1311 at 1317 at 1317 at 1317 at 1317 at 1317 rry 1431 at 1437 rry 1608 at 1657 rry 1608 at 1617	ACARGE AGTATI ACARGE AGTATI ACARGE AGTATI ACARGE AATTIV CAAGTI AAATTIV CAAGTI AGAAG AGATI AGAAG AGAAG AGAAG AGAAG AGAAG AGAAG AGAAG AGAAG AGAAG	HUMANNAN TOLOGONI TANGGALAA GUARANA ALAGA
	Quary 175 Bhjet 144 Quary 235 Sbjet 107 Quary 285 Sbjet 295 Sbjet 295 Sbjet 385 Sbjet 385 Sbjet 385 Quary 455 Sbjet 478 Quary 555 Sbjet 589 Quary 655 Sbjet 689 Quary 755 Sbjet 689 Quary 756 Sbjet 689 Quary 756 Sbjet 689 Quary 775 Sbjet 785 Quary 755 Sbjet 788 Quary 775 Sbjet 783 Quary 755	SAMANCHARCHETCHERCHENAUGUNETICSMAANCHARCHENAU G	CARTACCTCHAGA 2 CARTACCTCHAGA 2 DATACCTCHAGA	4 Sb j 3 Quak 4 Sc j 4 Sc j 6 Quak 4 Sc j 7 Quak 5 Sc j 7 Sc j 7 Sc j 7 Quak 5 Sc j 7 Quak 5 Sc j 7 Sc j 7 Sc j 7 Z 5	rry 1131 at 1077 rry 1191 at 1137 rry 1251 at 1257 rry 1311 at 1257 rry 1311 at 1317 at 1317 at 1317 at 1317 at 1317 rry 1431 at 1437 rry 1608 at 1657 rry 1608 at 1617	ACARGE AGTATI ACARGE AGTATI ACARGE AGTATI ACARGE AATTIV CAAGTI AAATTIV CAAGTI AGAAG AGATI AGAAG AGAAG AGAAG AGAAG AGAAG AGAAG AGAAG AGAAG AGAAG	HUMANNAN TOLOGONI TANGGALAA GUARANA ALAGA
	Query 175 Sbjct 144 Query 355 Sbjct 235 Sbjct 236 Sbjct 236 Sbjct 238 Query 355 Sbjct 238 Query 355 Sbjct 438 Query 456 Sbjct 438 Query 555 Sbjct 538 Query 716		CARTACCTCAGA 2 CARTACCTCAGA 2 CARTACTCCAGA 2 CARTACTCCAGA 2 CARTACTCCAGA 2 CARTACTCCAGA 2 CARTACTCCAGA 2 CARTACTCAGA 2 CARTACTCCAGA 2	4 Sb 5 3 Quak 5 G 2 6 Quak 5 G 2 6 Quak 5 G 2 7 Quak 5 G 2	rry 1131 at 1077 rry 1191 at 1137 rry 1251 at 1257 rry 1311 at 1257 rry 1311 at 1317 at 1317 at 1317 at 1317 at 1317 rry 1431 at 1437 rry 1608 at 1657 rry 1608 at 1617	ACARGE AGTATI ACARGE AGTATI ACARGE AGTATI ACARGE AATTIV CAAGTI AAATTIV CAAGTI AGAAG AGATI AGAAG AGAAG AGAAG AGAAG AGAAG AGAAG AGAAG AGAAG AGAAG	HUMANNAN TOLOGONI TANGGALAA KANANA KA
	Query 175 Sbjct 134 Query 355 Sbjct 235 Sbjct 236 Sbjct 236 Sbjct 238 Sbjct 238 Sbjct 238 Sbjct 238 Sbjct 358 Sbjct 358 Sbjct 538 Query 555 Sbjct 538 Query 555 Sbjct 538 Query 755 Sbjct 538 Query 555 Sbjct 718 Query 555 Sbjct 738 Query 835 Sbjct 738 Sbjct 738 Sbjct 738 Sbjct 838 Sbjct 838 Sbjct 838 Sbjct 838 Sbjct 838 Sbjct </td <td>ВАЗАЛТСЬЯ СОСТТСКОСОВАНОСТТСКОВАНОСНАТОСОВАНОВЛИКИ В</td> <td></td> <td>4 50- 50- 50- 50- 50- 50- 50- 50-</td> <td>rry 1131 at 1077 rry 1191 at 1137 rry 1251 at 1257 rry 1311 at 1257 rry 1311 at 1317 at 1317 at 1317 at 1317 at 1317 rry 1431 at 1437 rry 1608 at 1657 rry 1608 at 1617</td> <td>ACARGE AGTATI ACARGE AGTATI ACARGE AGTATI ACARGE AATTIV CAAGTI AAATTIV CAAGTI AGAAG AGATI AGAAG AGAAG AGAAG AGAAG AGAAG AGAAG AGAAG AGAAG AGAAG</td> <td>HUMANNAN TOLOGONI TANGGALAA KANANA KA</td>	ВАЗАЛТСЬЯ СОСТТСКОСОВАНОСТТСКОВАНОСНАТОСОВАНОВЛИКИ В		4 50- 50- 50- 50- 50- 50- 50- 50-	rry 1131 at 1077 rry 1191 at 1137 rry 1251 at 1257 rry 1311 at 1257 rry 1311 at 1317 at 1317 at 1317 at 1317 at 1317 rry 1431 at 1437 rry 1608 at 1657 rry 1608 at 1617	ACARGE AGTATI ACARGE AGTATI ACARGE AGTATI ACARGE AATTIV CAAGTI AAATTIV CAAGTI AGAAG AGATI AGAAG AGAAG AGAAG AGAAG AGAAG AGAAG AGAAG AGAAG AGAAG	HUMANNAN TOLOGONI TANGGALAA KANANA KA
	Quary 175 Sbjct 184 Quary 355 Sbjct 285 Sbjct 285 Sbjct 285 Sbjct 285 Sbjct 285 Sbjct 285 Sbjct 385 Sbjct 385 Sbjct 585 Sbjct 585 Sbjct 585 Sbjct 585 Sbjct 598 Sbjct </td <td>ВАМАЛТСЬАСССТТСКСОСАВАЛССИТТССКАВАЛАСІАЛ G</td> <td>ADDRESS AND ADDRESS AD</td> <td>4 50- 50- 50- 50- 50- 50- 50- 50-</td> <td>rry 1131 at 1077 rry 1191 at 1137 rry 1251 at 1257 rry 1311 at 1257 rry 1311 at 1317 at 1317 at 1317 at 1317 at 1317 rry 1431 at 1437 rry 1608 at 1657 rry 1608 at 1617</td> <td>ACARGE AGTATI ACARGE AGTATI ACARGE AGTATI ACARGE AATTIV CAAGTI AAATTIV CAAGTI AGAAG AGATI AGAAG AGAAG AGAAG AGAAG AGAAG AGAAG AGAAG AGAAG AGAAG</td> <td>HUMANNAN TOLOGONI TANGGALAA KANANA KA</td>	ВАМАЛТСЬАСССТТСКСОСАВАЛССИТТССКАВАЛАСІАЛ G	ADDRESS AND ADDRESS AD	4 50- 50- 50- 50- 50- 50- 50- 50-	rry 1131 at 1077 rry 1191 at 1137 rry 1251 at 1257 rry 1311 at 1257 rry 1311 at 1317 at 1317 at 1317 at 1317 at 1317 rry 1431 at 1437 rry 1608 at 1657 rry 1608 at 1617	ACARGE AGTATI ACARGE AGTATI ACARGE AGTATI ACARGE AATTIV CAAGTI AAATTIV CAAGTI AGAAG AGATI AGAAG AGAAG AGAAG AGAAG AGAAG AGAAG AGAAG AGAAG AGAAG	HUMANNAN TOLOGONI TANGGALAA KANANA KA

Fig 12. Homology of CuCCD1A, SLCCD4, SLCCD4-1

Homology was searched through NCBI. (A)The lower part marked in red is the sequence position for SLCCD4 F136-R260, and the lower part marked in red is the sequence position for SLCCD1A F793-R1036.





Fig 13. Electrophoresis of SLCCD4 and SLCCD4-1.

(A) Gene expression analysis of SLCCD4 F136-R260. Left is MT, middle is 3-5-12, 6-13-29, 20-9-20 3 sense lines, right is 9-6-8, 13-2-17, 5A-6-7 3 Anti -sense line. (B) Gene expression analysis of SLCCD4 F793-R1036. Left is MT, middle is 3-5-12, 6-13-29, 20-9-20 3 sense lines, right is 9-6-8, 13-2-17, 5A-6-7 3 Anti -sense line.



As a result of searching for the homology of CuCCD1A, SLCCD7, and SLCCD8-2, the homology of CuCCD1A and SLCCD7 was 49% (Fig. 11A). In a 1.5 mL tube, 3 μ L of cDNA, 0.5 μ L of SLCCD7 F98, 0.5 μ L of SLCCD7 R200, DW After RT-PCR was performed by mixing 16 μ L, 5 μ L of the PCR product was loaded on a 2% gel and electrophoresed (Fig. 12A).

As a result of searching for homology of CuCCD1A and SLCCD8-2, the homology was 50% (Fig. 11B), and 3 μ L of cDNA, 0.5 μ L of SLCCD8-2 F1165, 0.5 μ L of SLCCD8-2 R1400, D.W. After mixing 16 μ L and performing RT-PCR, 5 μ L of the PCR product was loaded on a 2% gel and electrophoresed (Fig. 12B). It was confirmed that amplification was not performed in SLCCD7 and SLCCD8-2, and in the study of jUNWEI Liu, CCD7 and 8 genes appear to affect the root

and stem growth of plants (Liu, Novero et al. 2013).



Cucco 1. Succo 1 Succo 1. S	1048 857 1105 995 1222 990 1222 1047 1335 1105 1333 1162 1450 1209 1505 1319 1505 1319 1562 1319 1616 1374 1637 1713 1431 1713 1431 1713 1545 1818 1593
Cuccol A SLCCOP Cuccol A SLCCOP Cuccol A SLCCOP Cuccol A SLCCOP Cuccol A SLCCOP Cuccol A SLCCOP Cuccol A SLCCOP Cuccol A SLCCOP Cuccol A SLCCOP Cuccol A SLCCOP Cuccol A SLCCOP	1105 895 1162 946 1222 990 1282 1047 1335 1345 1353 1450 1259 1562 1359 1562 1359 1616 1374 1667 1431 1713 1490 1763 1545 1553 1545 1593 1878 1612
Overy 03 FORDERGIANTONA AND PRODUCTATION CONTRACT - CONTRACTOR AND PRODUCTATION CONTRACT - CONTRA	1162 996 1222 990 1282 1047 1335 1105 1393 1162 1450 1209 1505 1259 1562 1319 1616 1374 1667 1431 1713 1490 1763 1545 1818 1593 1878 1612
Bigs 9 COMPARTMENT COMPART	946 1222 990 1282 1047 1335 1105 1393 1162 1450 1209 1505 1259 1562 1319 1616 1374 1667 1713 1431 1713 1490 1763 1545 1818 1593 1878 1612
Barery 16 Anticipation	1222 990 1282 1047 1335 1105 1393 1162 1450 1209 1505 1259 1562 1319 1616 1374 1667 1713 1490 1763 1545 1818 1593 1878 1612
Big to 117 Bit to	990 1282 1047 1335 1393 1162 1450 1209 1562 1319 1616 1374 1667 1431 1713 1490 1763 1545 1818 1593 1878 1612
Bary 130	1282 1047 1335 1105 1393 1162 1450 1209 1562 1319 1616 1374 1667 1431 1713 1490 1763 1545 1818 1593 1878 1612
Bays Bays	1335 1105 1393 1162 1450 1209 1505 1259 1562 1319 1616 1374 1667 1431 1713 1545 1819 1593 1878 1678
Biger 84 APRILETOCIATIONALIZATIONAL	1105 1393 1162 1450 1209 1505 1259 1562 1319 1616 1374 1667 1431 1713 1490 1763 1545 1818 1593 1878 1612
Bigter 294 AccounterActionationControl Accounter Control	1393 1162 1450 1209 1505 1259 1562 1319 1616 1374 1667 1431 1713 1490 1763 1545 1818 1593 1878 1612
Bigter 294 AccounterActionationControl Accounter Control	1162 1450 1209 1505 1259 1616 1374 1667 1431 1713 1490 1763 1545 1818 1593 1878 1672
By:et: 314 HithCHClocoldocolarity Colouring C	1209 1505 1259 1562 1319 1616 1374 1667 1431 1713 1490 1763 1545 1818 1593 1878 1612
Overy 394 CODATACCAT	1505 1259 1562 1319 1616 1374 1667 1431 1713 1490 1763 1545 1818 1593 1878 1612
Big et 41 Conductor Control Alexandre Al	1259 1562 1319 1616 1374 1667 1431 1713 1490 1763 1545 1818 1593 1878 1612
Overy 931 000 - CLOCAL MARKAGE - STATAL	1562 1319 1616 1374 1667 1431 1713 1490 1763 1545 1818 1593 1878 1612
Query 380 Query 380 <t< td=""><td>1616 1374 1667 1431 1713 1490 1763 1545 1818 1593 1878 1612</td></t<>	1616 1374 1667 1431 1713 1490 1763 1545 1818 1593 1878 1612
Bbjet 531 1000000000000000000000000000000000000	1374 1667 1431 1713 1490 1763 1545 1818 1593 1878 1612
Overy 418 Overy 418 Overy 418 Overy 418 Overy 418 Description Big to 1817 Difference	1667 1431 1713 1490 1763 1545 1818 1593 1878 1612
Bages 942 Proceedings	1713 1490 1763 1545 1818 1593 1878 1612
Bojet 660 THOTHUTOCOMPOSITION THAT - AND - THOM	1763 1545 1818 1593 1878 1612
Deary 519	1763 1545 1818 1593 1878 1612
Ouery 569	1545 1818 1593 1878 1612
Duery 617 ATCOMENTATION CONCENTRATION CONTRACTION CONTRACTOR AND CONCENTRATION CONTRACTOR AND CONCENTRATION CONTRACTOR AND CONCENTRATION CONTRACTOR AND CONCENTRATION CONTRACTOR AND CONCENTRATION CONTRACTOR AND CONCENTRATION CONTRACTOR AND CONCENTRATION CONTRACTOR AND CONCENTRATION CONTRACTOR AND CONCENTRATION CONTRACTOR AND CONCENTRATION CONCENTRATICONCENTRATION CONCENTRATION CON	1878 1612
Udery 617 Alcohort - Low Alcohort - Low Alcohort - Although and the although and the	1878 1612
Biget 879 CARTOCTITACISCO-CARCCALACACCULAACCULAACCULAACCULAACCULAACCULAACCULA	
	1938
Bigge 394	
B INW Score Identities Galacy Strand -1191 914/1282(50%) Galacy Strand SLCCD8 Solt 1 ATGC-TTCTTGACKCOCKARTTATTGARCAGAGE GALGAGAGE ACCORD STATTCOCKACT S Solt 2007 937 SLCCD8 Solt 1 ATGC-TTCTTGCTCTTCACACCCARATTATTGARCAGAGE GALGAGAGE ACCORD STATTCOCKACT S Solt 2007 937 Galacy Solt 1 ATGC-TTCTTGCTCTTCACACCCARATTATTGARCAGE GALGAGE ACCORD STATTCOCKACT S Solt 2007 937 SLCCD8 Solt 1 ATGC-TTCTTGCTCTTCACACCCARATTATTGARCAGE GALGAGE ACCORD STATTCOCKACT S Solt 2007 937 SLCCD8 Solt 1 ATGC-TTCTGACACCARATTATTGARCAGE TCTCACAGE TTCTCACAGCCTCTGAGE TS SOLT 2007 937 Solt 1 ATGC-TGALGAGE TTGALGAGE TTGALGAGE TTGALGAGE TTGALGAGE TTGALGAGE TTGALGAGE TTGALGAGE TTGALGAGE TGALGAGE ACCORD SOLT 2007 937 Ourry 95 GALGACCTGATGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGCTCTCACAGCCTCTCT 155 Ourry 95 GALGACCTGATGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGA	
D -1181 914/1828(50%) 339/1828(18%) Plus/Plus CucCD1A Ouery 1 ATG6GAAAGAAAAGCTGSAGA-AACGAOGTGSAGA-AACGAOGTGSAGA-AACGAOGTGSAGA-AACGAOGTGSAGA-AACGAOGTGSAGA-AACGAOGTGSAGA-AACGAOGTGSAGA-AACGAOGTGSAGA-AACGAOGTGAGAOGTAGCTOGAAGAG TATTACTGAGAAATTATGCTATUTG-CATG-GATCTOOGCTGTATTCAGACOAAAG SLCCD8 Sbj ct 1 ATG6	
SLCCD8 Sbjet 1 Атерестистетенскиесымынтатитетискиесы Sbjet 89 Tetracusumutatutetictic Tetracusumutatutetictic Sbjet 89 Tetracusumutatutetictic Sbjet 89 Tetracusumutatutetictic Sbjet 80 Tetracusumutatutetictic Sbjet 80 Tetracusumutatutetictic Sbjet 80 Calcusumutatutetictic Sbjet 80 Calcusumutatutetictic Calcusumutatutetictic Sbjet 80 Calcusumutatutetictic Sbjet 80 Calcusumutatutetictic Calcusumutatutetictictic Calcusumutatutetictictic Calcusumutatutetictictictictic Calcusumutatutetictictictictictictictictictictictictict	
Query 6 GRAATAGTG0305TGGAT002AAACOSHGLATGGAGTAACTTOGHAAGT 95 Query 92 GAAAATGGTGAAAGAAAACAAG-CTGATATTOGATGGTGAAAAGAGAAAACAAG-CTGATATTOGATGGTGAAAAGAAGAGAAAACAAG-CTGATATTOGATGGATGGAATGGAAGTGAAGAAGAAGAAGAAAAACAAG-CTGATATTOGATGGAATGGAAGAAGAAGAAAAACAAG-CTGATATTOGATGGAATGGAAGTGAAGAAGAAGAAGAAAAACAAG-CTGATATTOGATGGAATGGAAGTGAAGAAGAAGAAGAAAAACAAG-CTGATATTOGAAGGAATGGAAGTGAAGAAAGA	891
Sbjet 60 БТТГБАТСАТӨЗСААЛСАТБАЛЕТСКАТСТГЕБАТСААЛ6—ТБАЛААЛСАЛОЗААЛАЛ 117 Sbjet 943 ААТТТБЕТБАЛАGSZEGAGCOCACT6-TATAAGTTFGAGGATGGAGCACTCF-TATAAGTTFGAGATGGAGCACTCF-TATAAGTTFGAGATGGAGCACTCF-TATAAGTTFGAGATGGAGCACTCF-TATAAGTTFGAGATGGAGCACTCF-TATAAGTTFGAGATGGAGCACTCF-TATAAGTTFGAGATGGAGCACTCF-TATAAGTTFGAGATGGAGCACTCF-TATAAGTTFGAGGATGGAGCACTCF-TATAAGTTFGAGATGGAGCACTCF-TATAAGTTFGAGATGGAGCACTCF-TATAAGTTFGAGATGGAGCACTCF-TATAAGTTFGAGATGGAGCACTCF-TATAAGTTFGAGATGGAGCACTCF-TATAAGTTFGAGATGGAGCACTCF-TATAAGTTFGAGATGGAGCACTCF-TATAAGTTFGAGATGGAGCACTCF-TATAAGTTFGAGATGGAGCACTCF-TATAAGTTFGAGATGGAGCACTGATGGAGCACTGATGGAGGAGCACTGATGGAGAGCACTGATGGAGCACTGATGGAGAGCACTGATGGAGAGCACTGATGGAGAGCACTGATGGAGAGCACTGATGGAGAGCACTGATGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGA	942
Query 96 GATAGACITITGTGAGAAACTGATAGTGAAGTGGATGTAGGATTCTCACAGOCICTICA 155 Query 940 AAAGCICGTTTTGGTATACTTGCCAAGAATGAGGCCCAATGAATG	939
QUELY SHO HIGH COMMICATION FOR THE HEAD TO THEAD TO THE HEAD TO TH	999
SDJCT 118 AACAAGRAAAAATTGSACTTGAAATTGSACTTGAAATTGST-TACAAAGGTTGCTAGCCAA 165 SDJCT 1000 AAAGCATHTG-TACAAGTGTATGTGTAAAGCCACTGGCAAGTG	995 1051
Query 156 TTATCTCTCCGGGTAACTTOGCTOCAGTTOCTGAOGAGACTCCTCCTACTAAAGAOC 211	1053
Query 995 GTTGAGCTOCTAATGCTTATCCTOAT-AATGCCATCCTGATGGAGAGAGAA Sbjct 166 TTGCCTGTAATAGTCCACCACAGATCAAGAGTGATTAGTAAGGAGAAAAAGC 220 Sbjct 1052TAGAAGTGCCATTAGTAGGAGTAGATAGGAGAAGAAGC	1107
Query 212 TCXAOSTATOSSSCATC-TCODCGATTGCTTGAATGSSGAGTTTGCAGGSTG 285 Query 1054 GAAGTGSTTCTGATCACTTGCCGAGCAAGCCAGGCCAATGSSGACTAGACATGGTCAATGSSGACT	1113
Sbj.ct 1108 GAAGATGGAAGAGTTACC9CTGTGATT-GCAGATTGCTGTGAGCA-TAGC9CA	1158
Query 266 GTCCTAATCOCHAGTTGCCCGATGGCAGAGAGAGAGGCGTTGAT-GGAGA-TG 319 Sbjct 277 G9C3AGTACCA	1173
Diery 320 GOTGATT-CA-TGGTCTG939ATCAAAGAGGAAAGGGCTACATATGTCT0005 37 Sbjct 1159 GACAOCACCTCGACAAAGCT002bCTTGAGAA-TCTT0GTT0CTTCAAOGGCAAG	1215
SULT 33 GUCATASSIGNCTACAATTTIOSTACCTTTTOSTAGCTTASTCACTTASTC05 39 Query 1174 ACTGSCTASTTCACAAAGAGATTATOSSATOSST-GTTGACTAG	1224
Query 372 TITIGTGAGGACTTCAGGTCTTAAACAGGAAGAATTTTCGGAGGTGCTAAATTTATGAA 431 Sbict 1216 GATGTCTTACCTGATGCAGGGTGGAAGATT-CAGAATACCATTAGATGGAAGTCCAT	1273
Sbjet 390 ТСПСАТТНОЗАААТ-БЭАОЗА-ТНАЛСАТБЭЭТСАТАБАСААНОЗА 437 QUEYY 1225 АБЭБТБААТСАСТБЭСА	1268 1333
	1324
Shirt 1334 – Gtátgáátórtattagótágásatáródorttatger tigtgelettag	1392
Query 484 CTTAGA-ACQAAGTACTGGATGTTTCTATAG-GAAATGGGACAGGAAA 536 SQFCC 1534OIATGAACCAGCAGAAATACAGGATATGGTTATGCTTATGCTGGGGAAA SDjct 492 TTCAGAAGTACCTAAAGTACTGGAGATTTCT-ATGCTGCATAGGGGACATGGGAAATGC 550 Query 1325 ATGCTG-AACCAGCGAGGAAA-ACAAAGCTTGGAGGT-AAGT	1378
Query 537 TACAGCTCTTGTATATCACCATGGGAAACTTCTAGCACTCTCAGAGGCGGAC 588 Sbjct 1393 AGGCCTTG AATTTCCCCAACACCCTCACCAAGATTGATTTTGATATGAAAGAAGGCAAAG	1452
SDjct 551CT009516CAT00CTAAC034TAAT6CTAAAUCT63A6T0CTFAAACT6399AT 606 Query 1379 GCATCTI-T6ATC1699002T69AAGATT166TTCA6A69C76TTTT16TT0CTA6AGA Query 589 AAAC-00TA192	1437
Sbjet 607 GSACODSTAGTCTGUTTAACTGAGACSATAAAAGSTTOCATTGTAATTGATCODSALCAC 666 Query 1438 CCTGSAACCCTCTCTGAAGAAGATGATGGTTACTTAATATTCTTCTCTGATGAGAA Query 626 -TGCAAACACTTGGTATGCTGACTATGACAAG-AGATTACAACATTGCTTTACTG 679 Sbjet 1507 GSTGCAACAGAGSAAGATGATGGTGTGTAATCTCAATGATCAGTGACA	1506
	1496
	1496 1556
	1496 1556 1556
Sbjet 725 črčátočástošítaöskičástóásticataácstfsattocsántitaátás 778 query 1557 astigtogantitocalaassatticcataossatticcataossatticcataossatticcataossatticcataossatticcataossatticcataossatticcataossatticcataossatticcataossatticcataossatticcataossattic	1496 1556 1556 1610
	1496 1556 1556 1610
30/00 113 NO COUCATINT CONCONCININT CONCONCININT CONCONNICTINITY COS QUELY TOTA ACAACTIGAAGAAGAAAATIGIGA TOAA	1496 1556 1556 1610
Sbjet 779 ACODB9GATATAOBETGETGAGAATGAG9DXAG9DXAG9DXAGBAATGATAT 833 Query 1617 ACAACTTGAAGAGCAAGCAAAATTGTGA 1644 Query 781 ATGCATGATOCTGTACCAATAACOETATCAGACODCATCATGA-TGCACGACTTTGC 836 Sbjet 1664CAAAGATATAG 1674 Sbjet 834 AGB9AGAGTGAGT-TGTAG-AG9AG9ACCAG-CACCAG9ATG93TCATTCATTCC 887	1496 1556 1556 1610

Fig 14. Homology of CuCCD1A, SLCCD7, SLCCD8

Homology was searched through NCBI. (A)The lower part marked in red is the sequence position for SLCCD7 F98-R200, and the lower part marked in red is the sequence position for SLCCD8-2 F1165-R1400.





Fig 15. Electrophoresis of SLCCD7 and SLCCD8-2.

(A) Gene expression analysis of SLCCD7 F98-R200. Left is MT, middle is 3-5-12, 6-13-29, 20-9-20 3 sense lines, right is 9-6-8, 13-2-17, 5A-6-7 3 Anti -sense line. (B) Gene expression analysis of SLCCD8-2 F1165-R1400. Left is MT, middle is 3-5-12, 6-13-29, 20-9-20 3 sense lines, right is 9-6-8, 13-2-17, 5A-6-7 3 Anti -sense line.



Homology of CuCCD1A, SLCCD(9'10') Like, SLCCD(9'10')-1 Like, SLCCD(9'10')-1 Like2 was searched. CuCCD1A and SLCCD (9'10') Like confirmed 49% homology (Fig. 13A). In a 1.5mL tube, 3 µL of cDNA, 0.5 µL of SLCCD(9'10') Like F731, 0.5L of SLCCD(9'10') Like R972, D.W. After mixing 16L and performing RT-PCR, 5 µL of the PCR product was loaded on a 2% gel and electrophoresed (Fig. 14A). SLCCD(9'10')-1 Like Homology showed 51% Homology(Fig 13B), 3 µL of cDNA in 1.5mL tube, 0.5 µL of SLCCD(9'10')-1 Like F913, SLCCD(9'10') -1 Like R1083 0.5 µL, DW After mixing 16 µL and performing RT-PCR, 5 µL of the PCR product was loaded on a 2% gel and electrophoresed(Fig 14B). SLCCD(9'10')-1 Like2 Homology showed 52% Homology(Fig. 13C), 3 µL of cDNA in 1.5mL tube, SLCCD(9'10')-1 Like2 F544 0.5 µL, SLCCD(9'10') -1 Like2 R817 0.5 µL, DW After mixing 16 µL and performing RT-PCR, 5 µL of the PCR product was loaded on a 2% gel and electrophoresed(Fig. 14C).



A							
~	NW Score -1257	Identities 1020/2078(49%)	Gaps 517/2078(24%)		l Quer Plus Sbjc		GAGATTACAA-CATTOCTTTACTGCCCATOCAAAGGTTGATCCATACACTGGCGAGATGT GAGATTGGA <mark>CTOGATCATTCACAGCCCAT</mark> CAAAGAAAATTOGAGATAC9363TGAGCTTG
CuCCD1A SLCCD(9'10') like	Query 1 Sbjct 1		AAGAAAAGCTGGAGAAOGACG III AAGTTAATTTTTGTTGTTTGAAAAA-ACC		³⁷ Quer ³⁹ Sbjc	y 716 t 1004	TTACCTTTGGCTATGCACACACCACCACATATATCACATACAGAGTTATTTCAAAG TAATAATGGSAACCTATCCACTAAAGCCTT-ATTT-TGAGCTAG-GAGTCATTTCAGCTG
	Query 38 Sbjct 60	CACGAAATGGAATAGTG-GC-	GGTGGATOCCAAACC TCTGTTTCATCTACTTTCAAGCCATTTTT	-GAAGAA 77	Quer	y 773	ATGGTTTCATGCATGATOCTGTACCAATAACOGTATCAGACOOCATCATGATGC
	Query 78	TOGAGTAACTTCG	GTGATAGACTITGTGGAGAAACT	G-ATAG 12	19 Sbjc 21 Quer		AtosaaaacaaatoAttoAtaAgg-tosAtotoaAattaaAt-Adgstgtattotttoo -acgactiigotaiiactoagaaaitaigotaiiiicatogatotioogetgtaiii
	Sbjct 12 Query 12		GTTATTAAGGAAATTTCTTTCAAATTATT CTTCAC-AGCCTCTTCATTATCTCTCOG-		79 Sbjc 72 Quer		catgaaattiggagttacaaaaaggtataatgtgattttggattttocactaactatg
	Sbjct 18	0 TITIGTAGATITCACCITIGAATIT	GIGGACCAACCITIGC-TCCCATCICAGA	AGTAATT Z	38 Sbjc	,	AGACCAAAGGAAATGGTGAA-AGAAAAACAAGCTGATATTCACATTTGATGATACA GACCTAAAAAGACTTATTATTGGAGGAGAATTAATAAAATACAATGAAGATGGA
	Query 17. Sbjct 23	3 TOGCTOCAGTTOCTGAOGAGACTOC 9 TTGCAOCTGTTGAAGAGATTGG	TOCTACTA-AAGACCTOCAOGTCATO AGAAGCTGTAAGAGTCACTACAATTCAAG	JOGOCAT 22 JOGAAAA 20	28 Quer 195 Sbio	y 937 t 1228	AAGAAAGCTOSTTITGSTATACTTOCCCGATATGCAAAGAATGAGGCTCAAATGAAA TATCCAAGAATGGAATAGCACSTTATGGTGATGCTAATTCCATTAAA
	Query 22	9 CIOCCGATIGCIIGAATG996A		23	270 Quer		IB0111040C11CCTAAT10C111A1C11CCAI4410CCAA10C11000400400400
	Sbjct 29 Query 27		GTTTACATAAGAAATGGAGCAAATCCTCT -TTGCCCC-TGTGGCCGGATACCACTGGT		853 Sbjc 813 Quer		TGGTTTGAAGTTGAACCATGTTATGTATTTCACCTAATAAATTGTTTTGAAGATTATG ATGAAGTQSTTCTGATCACTTGCCGCCTAGACAAGC
	Sbjct 35 Query 314				109 Sbjc		ATGAGGTGGTGGTGAGGGCATGTAGAGCTCGTCGATGGATTATACCAAGACCAAATGAGT
	Query 314 Sbjct 411		САТСАААБАОЭЗАААG9СТАСА СТТТАСААААБАААААБАААААБЭСААСА		866 Quer 169 Sbjc		C956TCTAGACAT66TCAAT69595CT-GTCAAAGAAAA9CTAGAAAATTTC CAGAGTTGAGTTCA6ATACSTTGAAC5AGACAAGTTTCAGTAAAGATAATATTGAATC
	Query 36 Sbict 47	7 TCCCGTTTTGTGAG	AGAAACAGACACATTTAACATAGAAAAAG	AGGA 40	101 Quer 329 Sbic		-TCAAATGAACTGTATGAGA-TG-AGATTCAACTTGAAAACTGG GTCAAAAGATTTATTTTCCTTTTTTAATGTTTGTGAATGGAGATTAAACATGAAAACAAG
	Query 400	2 AGAATTTTTC	GGAGGTGCT	4	120 Quer		CCTAGCTTC&C&AA&G&G&T-T&TCGGC&TCCGCTGTTG&TTTTOCT&
	Sbjct 53 Query 42	-	ĠĠĂAĠġġġattctctîġĉîġttttcatgġ ĄŢtgġġġġcct		389 Sbjc 146 Quer	t 1515 v 1226	TAAAG-TGAAGATGAAAAATCTAACTACGAACGATCATCGGTTATTGGAACAATACTAGG GGGTGAATGAGTGCTACACTGGCAGGAAGCAAAGATATGTGTATGGAACAATACTAGA
	Sbjct 59 Query 44		ATACTATACCTCTTTCATCCAATTTTTTG	a Attitic	649 Sbjc	t 1574	999TGAATGAGTGCTACACTGGCAGGAAGCAAAGATATGTGTATGGAACAATACTAGA TGATCAATGAACACTTCATTGGTCTCAAAAACAAATTTGGTTATC-AACAACTTATTAAT
	Query 44 Sbjct 65		TCATGST-TAACATGCAAAT-GC- ATTTCAACTAATATTATATTCATATOSCS	GCAAGAC 7	184 Quer 109 Sbjc	y 1284 t 1633	T-AGCATIGCAA-AAGTCA-CAGGGATCATCAAATLIGATCIGCATGC TTAGAAACTTTCTCTAACTCAGCAAAGAATGTATCAGGAGGATTTCCTAAATTIGGAGGG
	Query 48 Sbjct 711	• IIIII IIIII IIII	AGTACTGGATGTTTCA-TATGGAAAT AGTAGGGTACGAGGTTTCA-TATGGAAAT	5	25 Quer	y 1329	TGAACCAGACGAAGAGAAAACAAAGCTTGAAGTTGGAGGAAATGTGAGAG CTAGCCAAACTTAATTTTGAAGAAATTTCAAAGGATGACAAAAAGGAGATTTGATAAG
	Query 52	6GGGACAGCTAATACA	GCTCTTGTATATCACCA	TGG 56	nte Sbjo 360 Quer	t 1693 y 1379	GCATCTITGAICTGGGCCCTGGAAGATTTGGTTCAGAG9CTGTTTTTGTTC
	Sbjct 771 Query 561		наТСТТААGATTTGGTGTATTTCACAAAT ТСАGAG		329 Sbjo 398 Quer	t 1753 v 1430	gttgagtatcacattttcccaaaaaacacattttgtagtggagcaacatttgttc
	Sbjct 83	0 GCAATACAAATGTGTTTGAGCACTC	AAAGAAATACTATTCAATTGCTGA-AAAT	rcatata e	188 Sbjc	t 1808	CTAGAGAGOCTGGAACCTCTTCTGAAGAAGATGATGGTTACTTAATATTCTTCTCTCATG CAAAACTTGATGSTGTTGATGAAGATGATGGATGGATTGTGGCATTCACACACA
	Query 59 Sbjct 88	3 COGTCAA-AGTTCTGGAAGATGGAG 9 CC-TCAAGAGATAAATATAC-G	ATCTGCAAACACTT-GGTATGCTTGACTA AACTTTAGAAACTTTGGGAAATTGGACTA	TGACAA 68 TTAATG 9	356 Quer 343 Sbjc		ATGAGAAAACCOGGAAAGTCATCAGTGAACGTGATTGATGCAAAAAACAATGTCAGCTG ATGAAAATACAAATCAATCTCAAGTTTATATACTCGATGCAAAAAACTTTGAAAATG
В		Identities		trand			
	-996	925/1821(51%)	332/1821(18%) P	lus/Plus	Query		
	Query 1 Sbjct 1		GAOGTOGTAGCAOGAAATGGAATAG 99AAGTTATTTTCAAATTATTGGACAAT				SAAA-TGGTGAAAGAAAACAAGCTGATATTCACATTTGATGATACAAAGAAA-SCTOGT CAGACTTATTATTGGAGAGCAATTAATAAAATATGATAA-AGATGGACATGCAAGA
		GCGGTGGATCCCAAACCGAAGAATG	GAGTAACTTOGAAAGTGATAGACT GATCAAOCTTTATOODCATCTCAGAGTAA	105	400.7	949 T	TTGGTATACTTCCCCGATATGCAAAGAATGAGGCTCAAATGAAATGGTTTGAGCTT TTGGAATAATGCCTCATTATGGTGATGCTAATTCCATTAAATGGTTTGAAATT
	Sbjct 57 Query 106	GTGGAGAAACTGATAGTGAAGTGGATGTA	OGATTCT-TCACAGCCTCTTCATTATCTC			903 A 1006 -	OCTAATTGCTTTATCTTCCATAATGCCAATGCTTG
	Sbjct 113 Query 165		-GAAGCTGTAAGAGTCACTACAATTC	00 224			AACCATGTTG-TGTATTTCACATAATAAATTGTTTTGAAGATAATGATGAGAAAAGGTT
	Sbjct 163	GISAAAATTC-CAAATGATTTTCCAGAGG	BGTGTTTACATAAGAAATGGATCAAAT	TG 217			GAGGAGGAGGATGAAGTGGTTCTGA_TCACTTGOCOCTAGAC TAGTAAGGGCATGTAGAGCTCGTAAATOGATTATACCAAGACI <mark>AGATATTATAGTAGAC</mark>
	Query 225 Sbjct 218	CCATCTCCCCCGATTGCTTGAATGGGGGGGT CTCTCTTTGGAGGATTAAAA	TTGTCAGGGTTGGTCCTAATCCCAAGTTTG	GC 284		1084 A	AGCC-GGATCTAGACATGGTCAATGGGGCTGTCAAAGAAAAGCT
(CCCTGTGGCC29GATACCACTGGTTTGATG AAATCAAGCCATATATG9GTTGAAG				1076 G	AGCTTOST TGAGTTTAGACGCGTTGAACGAGATGAGTTTCAGTAAAGATAACATTAAA AGAAAATTTCTCAAATGAACTGTATGAGATGAG
	Sbjct 259 Query 345	AAA†CAAĠĊĊATÆÆĠĠĠ††ĠĂAĞ AGACO3GAAAGGCTACATATG	GTGÁAGGAÁTGCTTCÁTGCTTTATACTTT/ TCTCCQGTTT-TGTGAGG				CSTCAGAAGATTATTTTCTTTTCTAAAGTTTGTGAATGGAGATTAAACATGAAAACA
	Sbjct 315		ATATATTATACAAGAACAAATATGT-AGA	AA 373	400.7		SCCTAGCTTCACAAAAGAGATTATCGSC-ATCCSCTGTTGATTTTCCT STGAAGTGAAGATGAAAAATGTAACTACGAACGATCATCGATTTATAATGGAATTCCCA
	Query 384 Sbjct 374	TTCACGTCTTAAACA-GGAAGAATTTTCC CAGACACATTCAACATGGAAAAGAATCTA		400		1225 A	GƏGTGAATGAGTGÇTAÇACTOQCAGGAAGCAAAGATATGTGTATGGAACAATACTAGAT
	Query 440 Sbjct 431	ACCTTAAGGGGCTATTTGGATTAC	TCATGGTTAACATGCAAATGCTTAGAGCAA				téa telá télá a calettela téléstettá la a la chala titlégetá tetá caletta titlék
		GTTGAAAGTACTGGATGTTTCATATGGAA	ATGGGACAGCTAATACAGCTCTTGTATAT	CA 554		1285 - 1316 T	АОСАТТОСААААОТСАСАОЗОАТСАТСАААТТТОАТСТОСАТОСТОААОСА САОААОСТТСТСТААТТАТТТТООАОЗАТТОСТАААТТООАОЗОСТАОССАААСТТ
	Sbjct 476 Query 555		ATATCTAAGC-AATACAAATGTGTTTGAG			1336 G	ACGAAGAGAAAACAAAGCTTGAAGTTGGAGGAAATGTGAGAGGCATCTT-T ATTTTGAAGAAATTTCAAAGGATTTTAAAGAAGAAGAAGAATTTGATTAAGGTTGAATAT
\$	Sbjct 528		OGGACAAACCSTATGCCSTCAA-AGTTCTO CTGA-AAATCATATGCC-TCAAGAGATA			1387 G	AICIGGGCCCIGGAAGAIIIGGIICAGAGGCIGIIIIIGIICCIAGAGAGCCIGGA
	Query 614 Sbjct 577	AAGATGGAGAT-CTGCAAACACTTGGTAT AACATACAAACACTTGAAACT-TTGGGAA	GCTTGACTATGACAAGAGATTACAA-CAT ATTGGACTGTTAATGGGGGCTTGGAGTCAA	IC 635			ататтттоссалалаатасаттттстадтвелестасатттеттосалалсттел
	Query 672 Sbict 636		TACACTGBCGAGATGTTTACCTTTGGCTAT GATACGGGTGAACTTGTTATAATGGGA-AT	IG 730		1444 A 1492 T	ССТСТСТСКАА ВАА ВАТВАТВАТТАСТТААТАТТСТТСТСТСАТВАТВАВААА ОЗВА ЭВТВТТ
	Query 731	CACACAC-ACCACCATATATC-ACATACA	GAGTTATTTCABAGGATO	ĢŢ 777		1504 A	AGTCATCAGTGAACGTGATTGATGCAAAAAACAATGTCAGCTGATCCTGTTGCAGTTGTC
	Sbjct 684 Query 778		GAATTATTTCAGCTGATGGAAAAACAAATG ISTATCAGACCCCATCATGATGCACGACTT		,		AATCACAAGTTTATATATTGATGCAAAAAAATTTGAAAGTGAGGCTGTTGCCATAATC AATTACCACAAAGGGTTCCATACGGATTTCATGCCTTCTTTGTGTCAGAGGAACAACTT
	Sbjct 743	TCACAAGGTGSATGTCAAACTTAATAGGT			Sbjct	1610 A	AATTACCACAAAGGSTTCCATACGGATTTCATGCCTTCTTTGTGTCAGAGGAACAACTT TATTACCTAGTAGAGTGCCATATGGATTTCATGGGGCATTTATGTCCTC
1	Query 836		ATGGATCTTCCGCTGTATTTCAGACCA	AA 890	Ouery 1		
(CTATTACTGAGAATTATGCTATTTTC GAGTTACAAAGAQGTACAATGTGATTTT-		845	Shict	1624 G 1660 -	AAGAGCAAAGCAAAATTGTGA 1644
(Sbjct 793	GAGTTACAAAGAGGTACAATGTGATTT-				1660 -	ААБАВСААВСАААЛТТБТБА 1644 САЛТТАА 1666
С	Sbjct 793 NW Scort -996	GAGTTACAAAGAGGTACAATGTGATTTT-	Gaps 379/1895(20%)	Strand Plus/P	d Plus		ОАТТАА 1666
	Sbjct 793	GAGTTACAAAGAGGTACAATGTGATTTT-	Gaps	Strand Plus/P	d Plus		ОАТТАА 1666
	Sbjct 793 NW Scorr -996 Query 1 Sbjct 1 Query 4	GAGTTACAAAGAGGTACAATGTGATTTT- " Identities 991/1895(52%) ATGGC	Gaps 379/1895(20%) -AAAGAAAAGCTGGAGAAOGACGTC AAAATTAATTGCTTTTTCAAAGSCTTC 31G9036196ATQCCAAAOS	Strand Plus/F C-GTAGCA C CAGTTOCA E DGAAGAAT	d Plus 39 Que 60 Sbj- 78 Que	ry 803 ct 915 ry 863	
	Sbjct 793 NW Scorr -996 Query 1 Sbjct 1	Gudt Acuades of Acuators Attended 991/1895(52%) 1 ATGS	Gaps 379/1895(20%) никоник-стбаасикозностс амистаматтесттитомизостто от сталитеститомизостто сомптосстояти былоситто сомптосстояти обраноситто сомпсосто с база	Strand Plus/F C-GTAGCA (CAGTTOCA (DSAGGAT DSAGGGAT ATAGTGAA	d Plus 39 Que 60 Sbj- 78 Que	ry 803 ct 915 ry 863 ct 965	CHATTIAN 1866 CONTAINED AND AND AND AND AND AND AND AND AND AN
	Sbjct 793 NW Scorr -996 Query 1 Sbjct 1 Query 4 Sbjct 6 Query 7 Sbjct 1	ант на на на на на на на на на на на на на	Сарк 379/1895(20%) никотика остояност полтоника остояност полтоника остояности солтостости остояности солтостости остояности солтостояни остояности солтосности остояности солтосно солтосно солто солтосно со	Strand Plus/F C-GTAGCA CAGTTODA DSAAGAAT DSAGGGAT ATAGTGAA CTATCAAA	d Plus 39 Que 60 Sbj- 78 Que 120 Sbj- 125 Que 180 Sbj-	ry 803 ct 915 ry 863 ct 965 ry 920 ct 102	Снаттыа 1866 совтаточеморостаточно совтоточемото по по совтато на поста станато ото — ото — ото на совточно по по совточно по совто по по 1994 годи совточно по совточно по совточно по совточно по совто 1994 годи совточно по совто
	Sbjct 793 NW Scorr -996 Query 1 Sbjct 1 Query 4 Sbjct 6 Query 7	sectflexuelasticuutorettti- * jennise A100 0947. D0 0944	Сере 379/1895(20%) нирона - 07(696 - норост лакатантесттитомерост голитистсени быех солитистсени быех	Strend Plus/F C-GTAGCA CAGTICCA DSAGAGAT SAGGGAT ATAGTGAA CTATCAAA ATTATCTC -TICCATC	d plus 39 Que 60 Sbji 78 Que 120 Sbji 125 Que 180 Sbji 162 Que	ry 803 ct 915 ry 863 ct 965 ry 920 ct 102	CONTATION 1866 CONTATION CO
	Sbjct 793 NW Scorr -996 Query 1 Sbjct 1 Query 4 Sbjct 6 Query 7 Sbjct 1 Query 1 Sbjct 1	sectflexuelashcurtorettti- * jernore *	Сере 379/1895(20%) нирони - 07(696 - нориот лакатитесттитомерости гоаттостсетот Какато сантостсетот Какато сантост Санто Какато сантост Санто Какато сантост Сантост Сантоско Какато сантост Сантоско Какато сантоско br>сантоско сантоско сантоско сантоско сантоско сантоско сантоско сантоско сантоско санто	Strend Plus/F C-GTAGCA CAGTICCA DSAGAGAT SAGGGAT ATAGTGAA CTATCAAA ATTATCTC -TICCATC	d Plus 39 Que 60 Sbj- 78 Que 120 Sbj- 125 Que 180 Sbj- 162 Que 239 Sbj-	ry 803 ct 915 ry 863 ct 965 ry 920 ct 102 ry 979 ct 107 ry 103	CONTATION 1866 CONTATION CO
	Sbjet 733 NW Seen -946	sectflexuelasticuutorstitti- * 39/1/1955(52%) A109	Снет 379/1998(20%) 4400446 - сстарана 1000400 - сстарана 10004000000000000000000000000000000000	Strand Plus/F C-GTAGCA CAGTTOCA DGAAGAAT CAGTGCA CTATCAAA ATTATCTC -TTCCATC -TTCCATC -TCCATC -TCCATC -TCCACA -GTCCCAC	d Plus 39 Que 60 Sbji 78 Que 120 Sbji 125 Que 180 Sbji 162 Que 239 Sbji 216 Que 239 Sbji 216 Que 239 Sbji 213 Que	ry 803 ct 915 ry 863 ct 965 ry 920 ct 102 ry 979 ct 107 ry 103 ct 112 ry 103	Синттый 1886 Сортанскородство по короно транева практок по по по по по по по по по по по по по
	Sbjet 733 NW Scon- -996 Ouery 1 Sbjet 1 Ouery 4 Sbjet 2 Sbjet 1 Ouery 4 Sbjet 1 Ouery 4 Sbjet 1 Ouery 1 Sbjet 1 Ouery 1 Sbjet 2 Sbjet	Construction of the second secon	Снет 379/1893(20%) нариан срада с нарадо нариан срада с нарадо с нариан с нари с нариан с на	Strand Plus/F C-GTAGCA CAGTTOCA DDAAGAAT SAGGGAT CTATCAAA ATTATCTC -TTOCATC -TTOCATC -TTOCATC STOCTAAT STOCAAT	d plus 39 Que 60 Sbji 78 Que 120 Sbji 125 Que 128 Sbji 126 Que 239 Sbji 216 Que 239 Sbji 216 Que 239 Sbji 216 Que 239 Sbji 216 Que 239 Sbji 216 Que 239 Sbji 216 Que 239 Sbji 217 Que 239 Sbji 218 Sbji 2	ry 803 ct 915 ry 863 ct 965 ry 920 ct 102 ry 979 ct 107 ry 103 ct 112 ry 109 ct 118	
	Sbjet 793 NW Scene -996 Query 1 Sbjet 1 Query 4 Sbjet 2 Query 7 Sbjet 1 Query 7 Sbjet 1 Query 4 Sbjet 2 Query 2 Sbjet 2 Sbjet 2 Sbjet 2 Sbjet 3 Sbjet 2 Sbjet 2 Sbjet 3 Sbjet 2 Sbjet 3 Sbjet 3 Sbjet 4 Sbjet	sectflexuldischlouiding identities 9/1/1995(25%) 1000	Снет злутлячасоны нарман состанит-ностоят состанито- с	Strand Plus/P C-GTAGCA : CAGTTOCA I DEAGBAAT DEAGBAAT DEAGBAAT CTATCAAA ATTATCTC -TTOCATC : ACTOCAC : STOCTAAA : STOCTAAAT : STOCTAAAT : STOCTAAAT : STOCTAAAT : STOCTAAAT : STOCTAAAT : STOCTAAAT : STOCTAAAT : STOCTAAAT : STOCTAAAT : STOCTAAAT : STOCTAAAT : STOCTAAAT : STOCTAAAT : STOCTAAAT : STOCTAAAT :	d Plus 39 Que 60 Sbjr 78 Que 120 Sbjr 125 Que 180 Sbjr 162 Que 239 Sbjr 162 Que 239 Sbjr 216 Que 294 Sbjr 273 Que 354 Sbjr 306 Que 414 Sbjr	ry 803 ct 915 ry 863 ct 965 ry 920 ct 102 ry 979 ct 107 ry 103 ct 112 ry 109 ct 118 ry 112 ct 124	
	Sbjet 733 New Scen- New Scen- Sbjet 1 Ouery 1 Sbjet 8 Ouery 1 Sbjet 1 Ouery 1 Sbjet 2 Ouery 2 Sbjet 2 Ouery 2 Sbjet 2 Ouery 2	actTH2cuklosthcuk16rs(htt)- * 5600000 * 991/1995(52%) * 991/1995(52%) * 991/1995(52%) * 992/1995(52%) * 9040	<u>379</u> 1995(20%) алу 1995(20%) алу 1995(20%) сонтостсят темератися тосто сонтостсят темератися тосто сонтостсят темератися темератися тосят сонтостся темератися темератися темератися br>темератися темерат	Strand Pluster C-GTAGGA CAGTICCA CAGTICCA CAGAGAAT CAGGGAAT CAGGGGAAT CACCAA- CTACCA- CTACA-	d Phus 39 Que 60 Sbj. 78 Que 120 Sbj. 125 Que 126 Que 1273 Que 294 Sbj. 273 Que 354 Sbj. 356 Que 414 Sbj. 362 Que 414 Sbj. 362 Que	ry 803 ct 915 ry 863 ct 965 ry 920 ct 102 ry 979 ct 107 ry 103 ct 112 ry 103 ct 112 ry 103 ct 112 ry 112 ry 112 ry 112 ry 115 ry 112 ry 115 ry 113 ry	CONTATIONAL 1866 CONTATIONAL 1866
	Sbjet 733 NUY Score Overy 1 Sbjet 1 Sbjet 2 Overy 4 Sbjet 2 Overy 7 Sbjet 1 Overy 2 Sbjet 2 Overy 2 Sbjet 2 Sbjet 2 Sbjet 3 Overy 3 Sbjet 3 Overy 4 Sbjet 2 Sbjet 2 Sbjet 2 Sbjet 3 Overy 4 Sbjet 2 Sbjet 2 Sbjet 3 Sbjet 3 Sbjet 3 Sbjet 3 Sbjet 3 Sbjet 3 Sbjet 3 Sbjet 3 Sbjet 3 Sbjet 4 Sbjet 4	actTH2cuklosthcuk16rs(htt)- * 5600000 * 991/1995(52%) * 991/1995(52%) * 991/1995(52%) * 992/1995(52%) * 9040	<u>379</u> 1995(20%) алу 1995(20%) алу 1995(20%) сонтостсят темератися тосто сонтостсят темератися тосто сонтостсят темератися темератися тосят сонтостся темератися темератися темератися br>темератися темерат	Strand Pluster C-GTAGGA CAGTICCA CAGTICCA CAGAGAAT CAGGGAAT CAGGGGAAT CACCAA- CTACCA- CTACA-	d Phus 39 Que 60 Sbj. 78 Que 120 Sbj. 125 Que 126 Que 1273 Que 294 Sbj. 273 Que 354 Sbj. 356 Que 414 Sbj. 362 Que 414 Sbj. 362 Que	ry 803 ct 915 ry 863 ct 965 ry 920 ct 102 ry 979 ct 107 ry 103 ct 112 ry 103 ct 112 ry 103 ct 112 ry 112 ry 112 ry 112 ry 115 ry 112 ry 115 ry 113 ry	CONTATIONAL 1866 CONTATIONAL 1866
	Solid 733	actTH2cukledit/cukltrichttH- identities 9.1/1995/252%) 1000	ото пределять п	Strand Plus/P 2-GTAGA : 2AGTICA I 2BAGGAAT 3BAGGAAT 3BAGGAAT 3BAGGAAT -TACAAA ATTACTCAA -TACAAA ATTACAA -TACAAA -TACAAA -TACAAA -TACAAA -TACAAA -TACAAA -TACAAA -TACAAA -TACAAA -TACAAA -TACAAA -TACAAA	d Pluss 39 Que 60 Sbj. 120 Sbj. 120 Sbj. 180 Sbj. 180 Sbj. 180 Sbj. 180 Sbj. 180 Sbj. 224 Sbj. 354 Sbj. 364 Sbj. 362 Sbj. 363 Sbj. 364 Sbj. 528 Sbj.	ry 803 tt 915 ry 863 st 965 ry 920 tt 102 ry 979 979 979 103 ct 112 ry 103 ct 112 ry 103 ct 112 ry 112	
	Solid 733 NUY Score 996 0.ery 1 Solid 7 Solid 7 Solid 1 0.ery 4 Solid 7 Solid 1 0.ery 7 Solid 2 0.ery 7 Solid 1 0.ery 7 Solid 1 0.ery 7 Solid 1 0.ery 7 Solid 1 0.ery 7 Solid 1 0.ery 7 Solid 1 0.ery 7 Solid 1 Solid 2 Solid 2 Solid 2 Solid 2 Solid 2 Solid 2 Solid 2 Solid 2 Solid 2 Solid 3 Solid 4 0.ery 7 Solid 4 0.ery 7 Solid 3 Solid 4 0.ery 7 Solid 4 Solid 5 Solid 4 Solid 4 Solid 5 Solid 4 Solid 5 Solid 5 Solid 4 Solid 5 Solid 5 Solid 5 Solid 5 Solid 4 Solid 5 Solid 5 Solid 5 Solid 4 Solid 5 Solid	sectflaculdischlouiding identities 9 991/18952290) 1000	Сортанования 379/1998(20%) 440,444,62(36,44) 440,444,62(36,44) 440,444,64) 440,444,444,444 441,444,444,444,444,444,444,444,444,444	Strand Pluspe C-GTAGCA : 2AGTTOCA I 2BAAGAAT 2BAAGAAT 2BAAGAAT 2BAAGAAT 2TATCAAA ATTATCAC THICAAAT 2TACTCAAAT 2GCCAAT 2GCCAAT 2GCCAAT 2GGCAA 2GGCAAT 2GCCAAT 2GGCAA 2GGCAAT 2GGCAGCAAT 2GGCAGCAAT 2GGCAGCAAT 2GGCAGCAAT 2GGCAG	d plus 39 Que 60 Sol- 120 Sol- 121 Sol- 122 Que 123 Sol- 124 Sol- 125 Que 239 Sol- 354 Sol- 355 Que 354 Sol- 355 Que 356 Que 357 Que 358 Sol- 359 Que 350 Que 351 Que 352 Que 353 Sol- 354 Sol- 355 Sol-	ry 803 ct 915 ct 965 ct 965 ct 965 ct 107 ry 103 ct 112 ry 103 ct 112 ry 103 ct 112 ry 103 ct 112 ry 115 ct 132 ry 125 ct 132 ry 125 ct 142	CONTRICT ON CONTRICT OF CONTRICT ON CONTRINCONTRICT ON CONTRICT ON CONTRICT ON CONTRICT ON CONTRICT ON CONTRI
	Solici 733 Jurr Scene Query 1 Solici 1 Query 4 Solici 1 Query 2 Solici 1 Query 2 Solici 1 Query 2 Solici 2 Query 2 Solici 2 Query 2 Solici 2 Query 3 Solici 2 Query 3 Solici 2 Query 4 Solici 2 Query 4 Solici 2 Query 4 Solici 2 Query 5 Solici 2 Query 5 Solici 2 Query 5 Solici 2 Query 4 Solici 2 Solici 2	Gentiles Bentiles * Sections	Зато резентато на селото со стато и со стат	Втелен Валания в Валания	d Bhas 9 0.02 60 Sbj. 60 Sbj. 78 0.02 120 Sbj. 121 0.02 120 Sbj. 121 0.02 122 0.02 123 Sbj. 124 Sbj. 125 0.02 354 Sbj. 354 Sbj. 354 Sbj. 354 Sbj. 354 Sbj. 354 Sbj. 355 Sbj. 356 0.02 1418 0.02 1428 Sbj. 1431 0.02 1532 0.02 1541 Sbj. 1552 0.02 1565 Sbj.	ry 803 ry 803 ry 915 ry 920 ry 920 ry 920 ry 920 ry 102 ry 102 ry 112 ry 112 ry 112 ry 112 ry 112 ry 112 ry 112 ry 125 ry 125 ry 126 ry 122 ry 126 ry 122 ry 125 ry 126 ry 126 ry 127 ry 126 ry 126 ry 127 ry 126 ry 127 ry 126 ry 127 ry 126 ry 126 ry 127 ry 126 ry 127 ry 126 ry 127 ry 126 ry 127 ry 126 ry 127 ry 126 ry 127 ry 127 ry 126 ry 127 ry 127 ry 126 ry 127 ry	CONTAINA 1866 CONTAINAL 1866 <
	Solict 733 New Scene -996 Ouery 1 Solict 1 Ouery 4 Solict 1 Ouery 7 Solict 1 Ouery 7 Solict 1 Ouery 7 Solict 1 Ouery 7 Solict 2 Ouery 2 Solict 2 Ouery 2 Solict 2 Ouery 3 Solict 2 Ouery 4 Solict 4 Ouery 4 Solict 4 So	Gentiles Bentiles * Sections	Зато резентато на селото со стато и со стат	Втелен Валания в Валания	d Bhas 9 0.02 60 Sbj. 60 Sbj. 78 0.02 120 Sbj. 121 0.02 120 Sbj. 121 0.02 122 0.02 123 Sbj. 124 Sbj. 125 0.02 354 Sbj. 354 Sbj. 354 Sbj. 354 Sbj. 354 Sbj. 354 Sbj. 355 Sbj. 356 0.02 1418 0.02 1428 Sbj. 1431 0.02 1532 0.02 1541 Sbj. 1552 0.02 1565 Sbj.	ry 803 ry 803 ry 915 ry 920 ry 920 ry 920 ry 920 ry 102 ry 102 ry 112 ry 112 ry 112 ry 112 ry 112 ry 112 ry 112 ry 125 ry 125 ry 126 ry 122 ry 126 ry 122 ry 125 ry 126 ry 126 ry 127 ry 126 ry 126 ry 127 ry 126 ry 127 ry 126 ry 127 ry 126 ry 126 ry 127 ry 126 ry 127 ry 126 ry 127 ry 126 ry 127 ry 126 ry 127 ry 126 ry 127 ry 127 ry 126 ry 127 ry 127 ry 126 ry 127 ry	CONTAINA 1866 CONTAINAL 1866 <
	Solici 733 Jurr Scene Query 1 Solici 1 Solici 1 Solici 1 Solici 1 Solici 1 Solici 1 Solici 1 Solici 1 Solici 1 Solici 2 Solici	actTH2cukloshcuktortH1 * Sectors *	<u>379</u> /1995(20%) 440040 - 261(20%) - 440(20%) 440040 - 261(20%) - 440(20%) 156 - 261(20%) - 450(20%) 166 - 261(20%) - 450(20%) 161 - 170-261(20%) - 450(20%) 170-260(20%) - 450(20%) - 450(20%) - 450(20%) 170-260(20%) - 450(20	Втенно Расле Состана Валания Состана Валания Валан	d b 38 0.02 60 Sbj. 78 0.02 120 Sbj. 121 Sbj. 120 Sbj. 121 Sbj. 122 0.02 123 Sbj. 124 Sbj. 125 0.02 126 0.02 1273 0.02 126 0.02 1273 0.02 126 0.02 1273 0.02 1283 Sbj. 1294 Sbj. 1295 0.02 1206 0.02 121 0.02 122 0.02 123 Sbj. 124 Sbj. 1252 0.02 126 Sbj. 127 Sbj. 128 Sbj. 129 Sbj. 129 Sbj. 129 Sbj. <td>ry 803 tt 915 ry 983 st 965 ry 920 tt 102 ry 939 ry 103 tt 112 ry 103 ry d></td>	ry 803 tt 915 ry 983 st 965 ry 920 tt 102 ry 939 ry 103 tt 112 ry 103 ry	
	Solicit 733 New Scene 	actTickukseriscuktronttti- istensisse activities activities <tr< td=""><td>3000 странатор с слоза с сло</td><td>Strand Strand PluyF PluyF PluyF PluyF PluyF Staticol Staticol Staticol</td><td>Plass 39 0.02 39 0.02 60 Sbj-1 125 0.02 1125 0.02 1125 0.02 125 0.02 125 0.02 125 0.02 125 0.02 126 0.02 1273 0.02 024 Sbj-1 125 0.02 126 0.02 1273 0.02 128 Sbj-1 129 0.02 1214 Sbj-1 1215 0.02 1216 0.02 1217 0.02 1218 0.02 1219 0.02 1210 0.02 1211 0.02 1211 0.02 1211 0.02 1211 0.02 1211 0.02 1211 0.02 1211 0.02</td><td>ry 803 ry 963 ry 965 ry 920 ry 920 ry 920 ry 102 ry 103 ry 103 ry 103 ry 112 ry /td><td></td></tr<>	3000 странатор с слоза с сло	Strand Strand PluyF PluyF PluyF PluyF PluyF Staticol Staticol Staticol	Plass 39 0.02 39 0.02 60 Sbj-1 125 0.02 1125 0.02 1125 0.02 125 0.02 125 0.02 125 0.02 125 0.02 126 0.02 1273 0.02 024 Sbj-1 125 0.02 126 0.02 1273 0.02 128 Sbj-1 129 0.02 1214 Sbj-1 1215 0.02 1216 0.02 1217 0.02 1218 0.02 1219 0.02 1210 0.02 1211 0.02 1211 0.02 1211 0.02 1211 0.02 1211 0.02 1211 0.02 1211 0.02	ry 803 ry 963 ry 965 ry 920 ry 920 ry 920 ry 102 ry 103 ry 103 ry 103 ry 112 ry	
С	Solict 733 New Scene Ouery 1 Solict 1 Ouery 1 Solict 1 Ouery 7 Solict 1 Ouery 7 Solict 1 Ouery 7 Solict 2 Ouery 2 Solict 2 Solict 2 Ouery 2 Solict	actTickukserikowithichtti- identities 901/1995229b) 1000		Strand PlayF Play	d d 39 0.02 39 0.02 50 30 100 30 1125 0.02 1125 0.	ry 803 ry 803 ry 863 ry 955 ry 920 ry 920 ry 920 ry 102 ry 102 ry 103 ry 102 ry 103 ry 112 ry 112 ry 112 ry 112 ry 112 ry 125 ry	CONTRICT AND AND AND AND AND AND AND AND AND AND
	Solici 733 New Scene Ouery 1 Solici 1 Solici 1 Solici 1 Solici 1 Solici 1 Solici 1 Solici 1 Solici 1 Solici 2 Solici	actTH2cukloshcuktortH1 * Sectors *	3000 странатор с слоза с сло	Втинование в страна с с с с с с с с с с с с с с с с с с	d p 39 0.22 60 Sbj. 78 0.25 125 0.22 125 0.24 239 Sbj. 125 0.24 239 Sbj. 336 0.24 336 0.23 336 0.23 414 Sbj. 551 Sbj. 552 0.25 553 Sbj. 554 Sbj. 555 0.25 564 0.24 565 0.26 566 0.26 567 0.26 568 0.26 569 0.26 569 0.26 569 0.26 569 0.26 569 0.26 569 0.26 569 0.26 569 0.26 569 0.26 569 0.26 <t< td=""><td>ry 803 ry 803 ry 950 ry 960 ry 920 ry 979 ry 102 ry 979 ry 103 ry /td><td></td></t<>	ry 803 ry 803 ry 950 ry 960 ry 920 ry 979 ry 102 ry 979 ry 103 ry	



Fig 16. Homology of CuCCD1A, SLCCD(9' 10')like, SLCCD(9' 10')-1 like, SLCCD(9' 10')-1 like2 Homology was searched through NCBI. (A)The lower part marked in red is the sequence position for SLCCD(9'10') like F731-R972, (B) The lower part marked in red is the sequence position for SLCCD(9'10')-1 like F913-R1083, (C) The lower part marked in red is the sequence position for SLCCD(9'10')-1 like2 F544-R817.





Fig 17. Electrophoresis of SLCCD(9'10') Like, SLCCD(9'10')-1 Like and , SLCCD(9'10')-1 Like2.

(A) Gene expression analysis of SLCCD(9'10') Like F731-R972. Left is MT, middle is 3-5-



12, 6-13-29, 20-9-20 3 sense lines, right is 9-6-8, 13-2-17, 5A-6-7 3 Anti -sense line.
(B) Gene expression analysis of SLCCD(9'10')-1 Like F913-R1083. Left is MT, middle is 3-5-12, 6-13-29, 20-9-20 3 sense lines, right is 9-6-8, 13-2-17, 5A-6-7 3 Anti -sense line.
(C) Gene expression analysis of SLCCD(9'10')-1 Like2 F544-R817. Left is MT, middle is 3-5-12, 6-13-29, 20-9-20 3 sense lines, right is 9-6-8, 13-2-17, 5A-6-7 3 Anti -sense line.

As a result of confirming the homology of SLLCYB1 and SLLCYB2, 85% of homology was shown (Fig. 15A). 3 µL of cDNA, 0.5 µL of SLLCYB1 F297, 0.5 µL of SLLCYB1 R519, D.W. 16 µL / cDNA 3 µL, SLLCYB1 F651 0.5 µL, SLLCYB2 R919 0.5 µL, D.W. After mixing 16 µL and performing RT-PCR, 5 µL of the PCR product was loaded on 2% gel and electrophoresed (Fig. 16A,B). As a result of confirming the homology of SLCHYB1 and SLCHYB2, the homology was 75% (Fig. 15B). In a 1.5 mL tube, 3 µL of cDNA, 0.5 µL of SLCHYB1 F81, 0.5 µL of SLLCYB1 R374, D.W. 16 µL / cDNA 3 µL, SLCHYB2 F477 0.5 µL, SLLCYB2 R697 0.5 µL, D.W. After mixing 16 µL and performing RT-PCR, 5 µL of the PCR product was loaded on a 2% gel and electrophoresed (Fig. 16C,D). SLZEP primer was synthesized as follows (Fig. 15C). 3 µL of cDNA, 0.5 µL of SLLCYB1 F297, 0.5 µL of SLLCYB1 R519, D.W. After mixing 16 µL and performing RT-PCR, 5 µL of the PCR product was loaded on a 2% gel and electrophoresed (Fig. 16E).



- 40 -

SLVDE primer was synthesized as follows (Fig. 15D). 3 μ L of cDNA, 0.5 μ L of SLLCYB1 F297, 0.5 μ L of SLLCYB1 R519, D.W. After mixing 16 μ L and performing RT-PCR, 5 μ L of the PCR product was loaded on 2% gel and electrophoresed (Fig. 16F).



Fig 18. Gene expression analysis from LCYB1 to VDE in carotenoid biosynthesis.

Homology was searched through NCBI. (A) Homology was searched through NCBI. The upper part marked in red is the sequence position for SLLCYB1 F297-R519, and the lower part marked in red is the sequence position for SLLCYB2 F651-R919, (B) Homology was searched through NCBI. The

- 41 -



upper part marked in red is the sequence position for SLCHYB1 F81-R374, and the lower part marked in red is the sequence position for SLCHYB2 F477-R697, (C)The yellow part marked is the sequence position for SLZEP F121-R402, (D)The yellow part marked is the sequence position for SLVDE F435-R730.



Fig 19. Electrophoresis results from SLLCYB1 to SLVDE.

(A) Gene expression analysis of SLLCYB1 F297-R519. Left is MT, middle is 3-5-12, 6-13-29, 20-9-20 3 sense lines, right is 9-6-8, 13-2-17, 5A-6-7 3 Anti -sense line. (B) Gene expression analysis of SLLCYB2 F651-R519. Left is MT, middle is 3-5-12, 6-13-29, 20-9-20 3 sense lines, right is 9-6-8, 13-2-17, 5A-6-7 3 Anti -sense line. (C) Gene expression analysis of SLCHYB1 F81-R374. Left is MT, middle is 3-5-12, 6-13-29, 20-9-20 3 sense lines, right is 9-6-8, 13-2-17, 5A-6-7 3 Anti -sense line. (D) Gene expression analysis of SLCHYB2 F477-R697. Left is MT, middle is 3-5-12, 6-13-29, 20-9-20 3 sense lines, right is 9-6-8, 13-2-17, 5A-6-7 3 Anti -sense line. (D) Gene expression analysis of SLCHYB2 F477-R697. Left is MT, middle is 3-5-12, 6-13-29, 20-9-20 3 sense lines, right is 9-6-8, 13-2-17, 5A-6-7 3 Anti -sense line. (E) Gene expression analysis of SLZEP

- 42 -



F121-R402. Left is MT, middle is 3-5-12, 6-13-29, 20-9-20 3 sense lines, right is 9-6-8, 13-2-17, 5A-6-7 3 Anti -sense line. (F) Gene expression analysis of SLVDE F435-R730. Left is MT, middle is 3-5-12, 6-13-29, 20-9-20 3 sense lines, right is 9-6-8, 13-2-17, 5A-6-7 3 Anti -sense line.

3-5. Anti oxidant activity analysis

3-5-1. Total carotenoid content

The results of measuring the total carotenoid content by flower, leaf and fruit development are as follows (Fig. 5). MT leaf showed values of 4.18 ± 0.17 and flowers, 1.4 ± 0.07 , Sense leaves 3.18 ± 0.2 , flowers 1.26 ± 0.06 , Anti sense, leaves 3.52 ± 0.22 , flowers 1.04 ± 0.08 value of (Fig. 5a).

MT-Immature green showed a value of 0.19 ± 0.009 , and mature green showed a value of 0.07 ± 0.004 . Turning showed a value of 0.1 ± 0.006 , and Orange showed a value of 0.11 ± 0.004 . Orange red showed a value of 0.22 ± 0.11 , and red showed a value of 0.22 ± 0.29 . Sense immature green showed a value of 0.13 ± 0.008 , and mature green showed a value of 0.07 ± 0.005 . Turning showed a value of 0.11 ± 0.006 , and Orange showed a value of 0.09 ± 0.006 . Orange red showed a value of 0.17 ± 0.01 , and Red showed a value of 0.26 ± 0.017 . Anti sense immature green showed a value of 0.14 ± 0.009 , and mature green showed a value of 0.07 ± 0.004 . Turning showed a value of 0.07 ± 0.005 , and orange showed a value of 0.01 ± 0.004 . Turning showed a value of 0.07 ± 0.005 , and orange showed a value of 0.01 ± 0.004 . Turning showed a value of 0.07 ± 0.005 , and orange showed a value of 0.11 ± 0.004 . Turning showed a value of 0.07 ± 0.005 , and orange showed a value of 0.11 ± 0.004 . Turning showed a value of 0.02 ± 0.004 , and red showed a value of 0.01 ± 0.004 .



- 43 -



Fig 20. Total carotenoid content of control MT, Sense line, Anti sense line.

(a) Total carotenoid content in flowers and leaves, (b) Total carotenoid content by fruit development in sense, anti-sense, and control

3-5-2 Total flavonoid content.

The flavonoid content of three control and sense lines and the flavonoid content value of three anti sense lines were confirmed (Fig. 6). As a result of obtaining the average value of the total flavonoid content of the three Sense lines and the total flavonoid content of the three Anti Sense lines, Control leaves showed values of 23.15 ± 0.73 and flowers of 41.36 ± 0.55 , while leaves of sense showed values of 22.31 ± 1.08 and flowers of 44.5 ± 14.56 . In the case of anti sense, the leaf showed a value of 20.72 ± 2.73 and the flower showed a value of 44.97 ± 4.49 .

As a result of checking the total flavonoid content in the fruit, control immature green showed a value of 17.40 ± 1.21 , and mature green showed a value of 13.57 ± 0.73 . In addition, Turning showed a value of 11.01 ± 0.73 , and Orange showed a content of 14.21 ± 1.00 . Orange red showed a value of 12.61 ± 1.00 , and red showed



a value of 9.73 ± 1.54 . Sense Immature green showed a value of 13.43 ± 1.09 , and mature green showed a value of 11.79 ± 1.33 . Turning showed a value of 8.51 ± 1.14 and Orange showed a value of 9.79 ± 0.92 . Orange Red showed a value of $10.1\pm$ 0.84, and Red showed a value of 9.52 ± 1.13 . Anti Sense Immature green showed a value of 9.68 ± 1.78 , and mature green showed a value of 6.71 ± 2.69 . Turning showed a value of 8.47 ± 2.6 , and Orange showed a value of 10.28 ± 1.04 . Orange red showed a value of 9.53 ± 2 , and red showed a value of 8.7 ± 1.28 .





Fig 21. Total flavonoid content of control MT, Sense line, Anti sense line.

(A) Total flavonoid content of MT, (B) Total flavonoid content of Sense line, (C) Totlal flavonoid content

of Anti-Sense

3-5-3 Total phenolic content

The total polyphenol content of the three control and sense lines and the total



polyphenol content of the three anti sense lines were checked (Fig. 7). As a result of obtaining the average of the total polyphenol content of the three sense lines and the total polyphenol content of the three anti sense lines, the control leaf showed a value of 9.32 ± 0.04 , and the flower showed a value of 15.43 ± 0.008 . The value of Sense leaves was 7.67 ± 0.63 and that of flowers was 15.00 ± 2.95 . Anti Sense leaves showed a value of 9.16 ± 0.91 , and flowers showed a value of 19.76 ± 1.47 .

As a result of measuring the total polyphenol content in the fruit, control immature green showed a value of 6.71 ± 0.002 , and mature green showed a value of 5.13 ± 0.003 . Also, Turning showed a value of 5.94 ± 0.0006 , and Orange showed a value of 7.07 ± 0.004 . Orange Red showed a value of 8.05 ± 0.002 , and Red showed a value of 6.92 ± 0.004 . Sense immature green showed a value of 6.83 ± 0.93 , and mature green showed a value of 6.40 ± 0.41 . Turning showed a value of 5.99 ± 0.57 , and orange showed a value of 7.63 ± 0.35 . In the case of orange red, a value of 11.21 ± 1.54 was shown, and in the case of red, a value of 11.74 ± 1.75 was shown.





Fig 22. Total Phenolic content of control MT, Sense line, Anti sense line.

(A) Total Phenolic content of MT, (B) Total Phenolic content of Sense line, (C) Total Phenolic content

of Anti-Sense



3-5-4. DPPH radical scavenging activity

The DPPH radical scavenging ability of three control and sense lines and the DPPH radical scavenging ability of three anti sense lines were confirmed (Fig. 8). As a result of obtaining the average value of the DPPH radical scavenging activity of the dog sense lines and the DPPH radical scavenging activity of the three anti sense lines, the control leaves showed a scavenging activity of 35.9 ± 0.33 , and the flowers showed a scavenging activity of 70.75 ± 0.16 . Sense leaves exhibited a scavenging activity of 31.75 ± 7.32 , and flowers exhibited a scavenging activity of 67.77 ± 7.68 . Anti sense leaves exhibited a scavenging activity of 26.81 ± 3.43 and flowers of 75.56 ± 2.25 .

As a result of measuring DPPH radical scavenging activity in fruits, control immature green showed a scavenging activity of 25.29 ± 0.17 and mature green showed a scavenging activity of 13.19 ± 0.08 . Turning showed a scavenging ability of 19.84 ± 0.2 and 23.76 ± 0.31 for orange, 25.59 ± 0.18 for orange and 24.01 ± 1.45 for red. Sense immature green showed a scavenging ability of 23.16 ± 10.87 and mature green showed a scavenging ability of 19.97 ± 7.28 . Turning showed a scavenging ability of 21 ± 4.75 , orange showed a value of 29.06 ± 6.50 , orange red showed a scavenging ability of 32.3 ± 6.79 and red showed a scavenging ability of 30.91 ± 5.08 . Anti-sense immature green showed a scavenging ability of 21.36 ± 3.77 , and mature green showed a scavenging ability of 21.66 ± 3.67 . Turning showed a scavenging ability of 27.44 ± 3.75 , orange showed a scavenging ability of 24.54 ± 3.14 , orange red showed a scavenging ability of 32.02 ± 3.35 , and red showed a scavenging ability of 38.44 ± 3.57 .

- 49 -





Fig 23. DPPH radical scavenging activity of control MT, Sense line, Anti sense line.

(A) DPPH radical scavenging activity of MT, (B) DPPH radical scavenging activity of Sense line, (C)

DPPH radical scavenging activity of Anti-Sense



3-5. HPLC(High Performance Liquid Chromatography)

As a result of HPLC on leaves in the MT, Sense, and Anti-sense lines (Fig. 22), the peaks were high at 3.5 min and 15 min in the case of MT. S3-5-12 had a high peak at about 12.5 minutes, and in the case of S 6-13-29, the peak was high at 14 minutes. In addition, in S 20-9-20, the peaks were high at 10.5 and 13.5 minutes, and in the case of AS 9-6-8, high peaks were shown at about 14 minutes. AS 13-2-17 showed a high peak at 14 min, and AS 5A-6-7 showed a high peak between about 10 min, 10.5 min, and 13 min to 13.5 min.

Time	Α	В
	MeOH:Water (95:5)	MTBE
0.01	80	20
2.00	70	30
13.00	50	50
18.00	45	55
23.00	0	100
28.00	80	20

Table 9. HPLC condition.





Fig 24. HPLC on leaves among MT, Sense, and Anti sense lines.

(A)MT Leaf, (B)S 3-5-12 Leaf, (C)S 6-13-29 Leaf, (D)S 20-9-20 Leaf, (E)AS 9-6-8 Leaf, (F)AS 13-2-17 Leaf, (G)AS 5A-6-7 Leaf

As a result of HPLC for flowers in the MT, Sense, and Anti-sense lines (Fig. 23), the peaks were high at 3 min and 12.5 min in the case of MT. S3-5-12 had a high peak at about 12.5 min, and in the case of S 6-13-29, the peak was high at 9 min. In addition, in S 20-9-20, the peaks were high at 15 min and min, and in the case of AS 9-6-8, a high peak was shown at about 12.5 min. AS 13-2-17 showed a high peak at 13.5 min, and AS 5A-6-7 showed a high peak at about 3.5 min.





Fig 25. HPLC on Flower among MT, Sense, and Anti sense lines.

(A)MT Flower, (B)S 3-5-12 Flower, (C)S 6-13-29 Flower, (D)S 20-9-20 Flower, (E)AS 9-6-8 Flower, (F)AS 13-2-17 Flower, (G)AS 5A-6-7 Flower

As a result of HPLC for immature green in MT, Sense, and Anti-sense lines (Fig. 24), MT peaks were high at 11 and 11.5 minutes. S3-5-12 had a high peak at about 13 to 13.5 minutes, and in the case of S 6-13-29, the peak was high at 13 to 14 minutes. In addition, in S 20-9-20, the peak was high at 12.5 min and min, and in the case of AS 9-6-8, a high peak was shown at about 14.5 min. AS 13-2-17 showed a high peak at 12.5 min, and AS 5A-6-7 showed a high peak at about 11 min.





Fig 26. HPLC on Immature green among MT, Sense, and Anti sense lines.

(A)MT Immature green, (B)S 3-5-12 Immature green, (C)S 6-13-29 Immature green, (D)S 20-9-20 Immature green, (E)AS 9-6-8 Immature green, (F)AS 13-2-17 Immature green, (G)AS 5A-6-7 Immature green

As a result of HPLC for mature green in MT, Sense, and Anti-sense lines (Fig. 25), the peak at 11.5 min for MT was high. S3-5-12 had a high peak at about 3.5 min, and in the case of S 6-13-29, the peak was high at 3.5 min and 11~11.5 min. In addition, in S 20-9-20, the peaks were high at 3.5 and 12 minutes, and in the case of AS 9-6-8, high peaks were shown at about 12 to 12.5 minutes. AS 13-2-17 showed high peaks at 7 and 9 min, and AS 5A-6-7 showed low peaks.





Fig 27. HPLC on Mature green among MT, Sense, and Anti sense lines.

(A)MT mature green, (B)S 3-5-12 mature green, (C)S 6-13-29 mature green, (D)S 20-9-20 mature green, (E)AS 9-6-8 mature green, (F)AS 13-2-17 mature green, (G)AS 5A-6-7 mature green

As a result of HPLC for turning in MT, Sense, and Anti-sense lines (Fig. 26), in the case of MT, the peak was high at 11.5 to 12. S3-5-12 had a high peak at about 11.5 to 12.5 minutes, and in the case of S6-13-29, the peak was high at 13 minutes. In addition, in S 20-9-20, the peak was high at 12.5~13 min and min, and in the case of AS 9-6-8, a high peak was shown at about 13 min. AS 13-2-17 had a high peak at 12.5-13 min, and AS 5A-6-7 had a low peak.





Fig 28. HPLC on Turning among MT, Sense, and Anti sense lines.

(A)MT Turning, (B)S 3-5-12 Turning, (C)S 6-13-29 Turning, (D)S 20-9-20 Turning, (E)AS 9-6-8 Turning, (F)AS 13-2-17 Turning, (G)AS 5A-6-7 Turning

MT, Sense, Anti-sense라인 에서의 Orange에대한 HPLC 결과로(Fig. 27), MT의 경우 3.5분과 The peak was high between 11 and 11.5 minutes. S3-5-12 had a high peak at about 13 minutes, and in the case of S 6-13-29, the peak was high at 10-12 minutes. In addition, in S 20-9-20, the peaks were high at 9 and 13 minutes, and in the case of AS 9-6-8, high peaks were shown at about 12.5 minutes. AS 13-2-17 showed a high peak at 11-12 min, and AS 5A-6-7 showed a high peak at about 17 min.





Fig 29. HPLC on Orange among MT, Sense, and Anti sense lines.

(A)MT Orange, (B)S 3-5-12 Orange, (C)S 6-13-29 Orange, (D)S 20-9-20 Orange, (E)AS 9-6-8 Orange, (F)AS 13-2-17 Orange, (G)AS 5A-6-7 Orange

As a result of HPLC for Orange-red in MT, Sense, and Anti-sense lines (Fig. 28), the peak at 16.5 min for MT was high. S3-5-12 showed a low peak, and in the case of S 6-13-29, the peak was high at 17.5 min. In addition, in S 20-9-20, the peak was high at 17.5 min and min, and in the case of AS 9-6-8, the peak was high at about 17 min. AS 13-2-17 showed a high peak at 17.5 min, and AS 5A-6-7 showed a high peak at about 17.5 min.





Fig 30. HPLC on Orange-Red among MT, Sense, and Anti sense lines.

(A)MT Orange-red, (B)S 3-5-12 Orange-red, (C)S 6-13-29 Orange-red, (D)S 20-9-20 Orange-red, (E)AS 9-6-8 Orange-red, (F)AS 13-2-17 Orange-red, (G)AS 5A-6-7 Orange-red

As a result of HPLC for Red in the MT, Sense, and Anti-sense lines (Fig. 29), the peaks were high at 3 min and 16.5 min for MT. S3-5-12 had a high peak at about 17 minutes, and in the case of S 6-13-29, the peak was high at 17 minutes. In addition, in S 20-9-20, the peak was high at 17.5 min and min, and in the case of AS 9-6-8, a high peak was shown at about 17.5 min. AS 13-2-17 showed a high peak at 17.5 min, and AS 5A-6-7 showed a high peak at about 17.5 min.





Fig 31. HPLC on Red among MT, Sense, and Anti sense lines.

(A)MT Red, (B)S 3-5-12 Red, (C)S 6-13-29 Red, (D)S 20-9-20 Red, (E)AS 9-6-8 Red, (F)AS 13-2-17 Red, (G)AS 5A-6-7 Red



4. Summary and Conclusions

Homo line was found from transgenic tomatoes introduced with CuCCD1A carotenoid gene from citrus fruits, and gene expression analysis, activity analysis, and HPLC analysis were performed. As a result, the transformed microtomato was compared with the general control group in the gene expression analysis, but in the experimental group and the control group did not feel any difference.

Also, in the activity assays Total Carotenoid Content, Total Phenolic Content and Total Flavonoid Content, and DPPH radical scavenging, no significant difference was found between the control group and the experimental group.

Therefore, it is necessary to additionally conduct experiments on gene expression analysis and activity analysis, and it is also necessary to perform additional analysis of changes in the composition of substances by performing HPLC measurement several times.

In the case of gene activity analysis, when the genes of the overexpressed Sense line and the suppressed Anti Sense line function normally, the difference in DNA band and the difference between the overexpressed Sense line and the suppressed Anti sense line in the case of activity analysis should be shown. Through this experiment, the change in carotenoid biosynthesis can be observed when the CCD1A gene isolated from citrus is transformed into tomato, and it has great academic and commercial value through activity analysis and material analysis.



감사의 글

대학원 석사 과정으로 있는 동안 저 뿐 만이 아닌 실험실 소속 대학원 생들과 학부생들을 지도해 주신 지도교수님께 감사의 인사를 드리고 싶습니다. 또한 실험적인 부분이나 궁금한 점이 있을 때 저희에게 가 르쳐 주신 실험실 박사님께도 감사의 인사를 드립니다.

또한 대학원과 학교에 발전을 위해 힘을 써 주시는 여러 조교선생님 들에게도 감사의 인사를 드리는 바입니다.



Referense

Zou, Z., Xi, W., Hu, Y., Nie, C., & Zhou, Z. (2016). Antioxidant activity of Citrus fruits. *Food chemistry*, *196*, 885-896.

Aoshima, H., et al. (2004). "Aging of whiskey increases 1, 1-diphenyl-2-picrylhydrazyl radical scavenging activity." Journal of agricultural and food chemistry **52**(16): 5240-5244.

Chang, C.-C., et al. (2002). "Estimation of total flavonoid content in propolis by two complementary colometric methods." Journal of food and drug analysis **10**(3): 3.

Dóka, O., et al. (2013). "Simple and rapid quantification of total carotenoids in lyophilized apricots (Prunus armeniaca L.) by means of reflectance colorimetry and photoacoustic spectroscopy." <u>Food Technology and Biotechnology</u> **51**(4): 453-459.

Fraser, P. D., et al. (2009). "Genetic engineering of carotenoid formation in tomato fruit and the potential application of systems and synthetic biology approaches." <u>Archives of</u> Biochemistry and Biophysics **483**(2): 196-204.

Hai, N. T. L., et al. (2012). "Involvement of carotenoid cleavage dioxygenase 4 gene in tepal color change in Lilium brownii var. colchesteri." <u>Journal of the Japanese Society for</u> <u>Horticultural Science</u> **81**(4): 366-373.

Halliwell, B. (1991). "Reactive oxygen species in living systems: source, biochemistry, and role in human disease." <u>The American journal of medicine</u> **91**(3): S14-S22.

Hermanns, A. S., et al. (2020). "Carotenoid Pigment Accumulation in Horticultural Plants." <u>Horticultural Plant Journal</u>.

Lamuela-Raventós, R. M. (2018). "Folin–Ciocalteu method for the measurement of total phenolic content and antioxidant capacity." <u>Measurement of Antioxidant Activity & Capacity</u> <u>Recent Trends and Applications</u>: 107-117.



Liou, G.-Y. and P. Storz (2010). "Reactive oxygen species in cancer." <u>Free radical research</u> **44**(5): 479-496.

Liu, J., et al. (2013). "Carotenoid cleavage dioxygenase 7 modulates plant growth, reproduction, senescence, and determinate nodulation in the model legume Lotus japonicus." Journal of Experimental Botany **64**(7): 1967-1981.

Liu, Y., et al. (2012). "History, Global Distribution, and Nutritional Importance of Citrus Fruits." Comprehensive Reviews in Food Science and Food Safety **11**(6): 530-545.

Mittal, M., et al. (2014). "Reactive oxygen species in inflammation and tissue injury." Antioxidants & redox signaling **20**(7): 1126-1167.

Sabzevari, A. G. and R. Hosseini (2014). "A quick, efficient, and cost-effective method for isolating high-quality total rna from tomato fruits, suitable for molecular biology studies." Preparative Biochemistry and Biotechnology **44**(4): 418-431.

Stadtman, E. R. (2004). "Role of oxidant species in aging." <u>Current medicinal chemistry</u> **11**(9): 1105-1112.

Yuan, H., et al. (2015). "Carotenoid metabolism and regulation in horticultural crops." Horticulture research 2(1): 1-11.

Zacarías-García, J., et al. (2021). "Antioxidant capacity in fruit of Citrus cultivars with marked differences in pulp coloration: Contribution of carotenoids and vitamin C." <u>Food Science</u> and Technology International **27**(3): 210-222.

