Functional characterization of genes and promoters identified in strawberry fruit development
101195

Introduction

Based on our earlier research and sequencing 130 cDNA fragments (Balogh et al. 2005) we identified genes previously not associated with ripening **(I)** or role of which is not fully understood in non-climacteric ripening **(II** and **III)**. Genes selected for functional analysis and the experimental approaches including plant species and genotypes applied are listed in Table 1.

We functionally characterized the following genes: *SPATULA* (*FvSPT)* encoding bHLH transcription factor, *SPIRAL* (*FvSPR1,2*) operating in directional cell growth, *ACC-synthases* (*FvACS1-5*) and ACC oxidases (*FaACO1-4*) coding for the key enzymes of ethylene biosynthesis, *SAM-synthase* (*FvSAMS1*) producing SAM, the common precursor of ethylene and polyamine pathway, genes of polyamine synthesis/metabolism *(FaSAM-decarboxylase, FaSpermidin-, FaSpermine-synthase, FaSAH-hydrolase*), gene of cynnamil-alcohol dehydrogenase (CAD) and putrescine N-methyltransferase (PMT) producing lignin and nicotine requiring SAM. S-adenosyl-L-methionine decarboxylases (*FaSAMDC1-3*), *arginine decarboxylase (FaADC), spermidine synthase* (*FaSPDS*). For these purposes we isolated genes (promoters and coding sequences), built promoter-reporter gene vectors, transformed plants, analyzed gene expression by qRT-PCR during strawberry ripening and in heterologous transgenic system, by histochemical staning and confocal microscopy based on fusion protein production.

Table 1. Genes selected for functional analysis and the applied methods

# of sub-chapter in the report	Name of the gene	Description	Accession number	Method for functional analysis	Species/genotype	Manuscript
I.	*Fragaria vesca SPATULA (FvSPT)*	bHLH transcription factor	AY679615	Complementation test; qRT-PCR	*Arabidopsis thaliana*	Hidvégi et al.
I.	*Fragaria vesca SPIRAL (FvSPR)*	directional control of cell elongation in *Arabidopsis thaliana*	AY695666	Complementation test; qRT-PCR	*Arabidopsis thaliana*	Hidvégi et al.
II:	*Fragaria x ananassa ACC synthase (FaACS1-4)*	ethylene biosynthesis	*FaACS1*-XM_004298784.2	qRT-PCR	*Fragaria ananassa* cv. 'Asia'	Mendel et al.
			FaACS2-XM_004306687.2			
			FaACS3-XM_011461390.1			
			FaACS4-XM_004288236.2			
II:	*Fragaria x ananssa ACC oxidase (FaACO1-4)*	ethylene biosynthesis	*FaACO1*-XM_004293286.2	qRT-PCR	*Fragaria ananassa* cv. 'Asia'	Mendel et al.
			FaACO2-XM_004304031.2			

			FaACO3-XM_004290864.2			
			FaACO4-XM_004305215.2			
II.	*Fragaria x ananssa SAM synthase (FaSAMS1-3)*	S-adenosyl methionine synthase: synthesis of SAM	*FaSAMS1*-XM_004288294.2	qRT-PCR;	*Fragaria ananassa* cv. 'Asia'	Mendel et al.
			FaSAMS2-XM_004297040.2			
			FaSAMS3-XM_011467929.1			
II.	*Fragaria x ananssa SAM decarboxylase (FaSAMDC1-3)*	Decarboxylation of SAM /polyamine biosynthesis	*FaSAMDC1*-XM_011464655.1	qRT-PCR	*Fragaria ananassa* cv. 'Asia'	Mendel et al.
			FaSAMDC2-XM_004304118.2			
			FaSAMDC3-XM_004296980.2			
II.	*FaADC*	polyamine biosynthesis	XM_004306397.2	qRT-PCR	*Fragaria ananassa* cv. 'Asia'	Mendel et al.
II.	*FaSPDS*	polyamine biosynthesis	XM_004297595.2	qRT-PCR	*Fragaria ananassa* cv. 'Asia'	Mendel et al.
III.	*FvSAMS*	S-adenosyl methionine synthase: synthesis of SAM	XM_004288294.2	overexpression in transgenic system	*Nicotiana benthamiana*	Kovács et al.
				localization of fusion protein SAMS+sGFP reporter		
				qRT-PCR;		
III.	*FvSAMDC*	Decarboxylation of SAM /polyamine biosynthesis	XM_011464655.1	overexpression in transgenic system	*Nicotiana benthamiana*	Kovács et al.
				localization of fusion protein SAMDC+sGFP reporter		
				qRT-PCR;		
III.	*Spermidine synthase*	polyamine biosynthesis	AB304779.1	qRT-PCR;	*Nicotiana benthamiana*	Kovács et al.
III.	*Spermine synthase*	polyamine biosynthesis	AB304780.1	qRT-PCR;	*Nicotiana benthamiana*	Kovács et al.
III.	*ACC synthase*	ethylene biosynthesis	CBMM010006910.1	qRT-PCR;	*Nicotiana benthamiana*	Kovács et al.
III.	*SAHH; SAH hydrolase*	recycling of SAM	LC008356.1	qRT-PCR;	*Nicotiana benthamiana*	Kovács et al.
III.	*Cynnamyl-alcohol dehydrogenase (CAD)*	lignin biosynthesis	EU877915.1	qRT-PCR;	*Nicotiana benthamiana*	Kovács et al.
III.	*Putrescine N-methyltransferase (PMT)*	nicotine biosynthesis	EU165356.1	qRT-PCR;	*Nicotiana benthamiana*	Kovács et al.

I. Functional analysis of strawberry „*SPATULA*" *(FVSPt)* and „*SPIRAL*" *(FVSPR)* genes based on complementation test in *Arabidopsis thaliana*

SPATULA (AY679615) and *SPIRAL* (AY695666) genes were identified among the genes displaying differential expression from green to red ripening stages of strawberry (Balogh et al., 2005, Polgári et al. 2010). *SPATULA* (*SPT*: encoding bHLH transcription factor) was characterized in *Arabidopsis thaliana* L.*,* where its recessive mutation caused degenerative carpel and fruit development (Heisler et al., 2001, Groszmann et al., 2011). The *Atspt2* mutant bears shorter, smaller, wider spatula shaped siliques compared to the wild type. *SPATULA* is expressed in marginal and transmission tract tissues throughout their development confirming its growth regulating role. We have already characterized *FaSPT* gene in strawberry, in which the expression of *FaSPT* increased as ripening proceeded while it decreased on the effect of wounding, auxin and ethylene. RNAi based gene silencing of *SPATULA* retarded fruit development (Tisza et al., 2010).

Members of *SPIRAL* gene family encode small proteins, which regulate the organization of microtubules by affecting the cell growth and elongation (Furutani et al., 2000; Nakajima et al., 2004). SPIRAL genes in *Arabidopsis thaliana* are classified into two main categories *SPR1* and *SPR2* and five subgroups: *SPR1-LIKE1 – SPR1-LIKE5* (Nakajima et al., 2006). *Arabidopsis thaliana* plants harbouring mutant *spiral* gene develop roots of right-handed helical growth.

In this project our aim was to characterize the *Fragaria vesca* L. *SPATULA (FvSPT)* and *SPIRAL (FvSPR)* genes by complementation test using *FvSPT*, *FvSPR1* and *FvSPR2* constructs with pGWB604 vector for transforming Columbia *spt* and *spr2 Arabidopsis* mutants.

Amplification of the *SPATULA* (*FvSPT*) and *SPIRAL* (*FvSPR1, 2*) genes of *Fragaria vesca* L. based on the genomic sequence determined by Shulaev et al. (2011), resulted in a 6600 bp, a 4400 bp and a 3500 bp fragment, respectively, which were built into pGWB401 binary vector. We transformed *spt* and *spr2* mutant *Arabidopsis thaliana* L. plants with these constructions using *Agrobacterium tumefciens* GV3101 strain for the floral dip method (Clough and Bent, 1998).

In PCR-positive T_1 individuals the transcription of the *FvSPT* and *FvSPR* genes were checked in RT-PCR with primers designed to the exon-intron junction of *FvSPT* and *FvSPR* (*GAPDH* housekeeping gene was the reference). The plants were grown in the climatic chamber, their habit, roots, siliques were observed, measured, number of seeds/siliques was determined and compared to wild type and mutant Columbia plants. We repeated the same measurements in the next (T_2) generation applying identical growth conditions as in the case of T_1 plants. Altogether data of 2 x 80 siliques were recorded in T_1 and T_2 progeny.

Our experimental results attested, that *FaSPT* and *FaSPR* genes isolated from the octoploid *Fragaria x ananassa* Duch. by cDNA-AFLP (Balogh et al., 2005) show not only sequential similarity to the *Arabidopsis thaliana AtSPT*, *AtSPR1*, *AtSPR2* and *AtSPR3*, as well as to the *Fragaria vesca* L. diploid

strawberry *FvSPT*, *FvSPR1* and *FvSPR2* but they also have the capability to complement the *A. thaliana* mutant phenotype. Similarly to the result of Heisler et al. (2001) according to which the wild type *AtSPT2* allele complemented the *Atspt2* mutation, *FvSPT* has brought about the same effect, confirming the same functional ability of this strawberry derived gene (Figure 1. left).

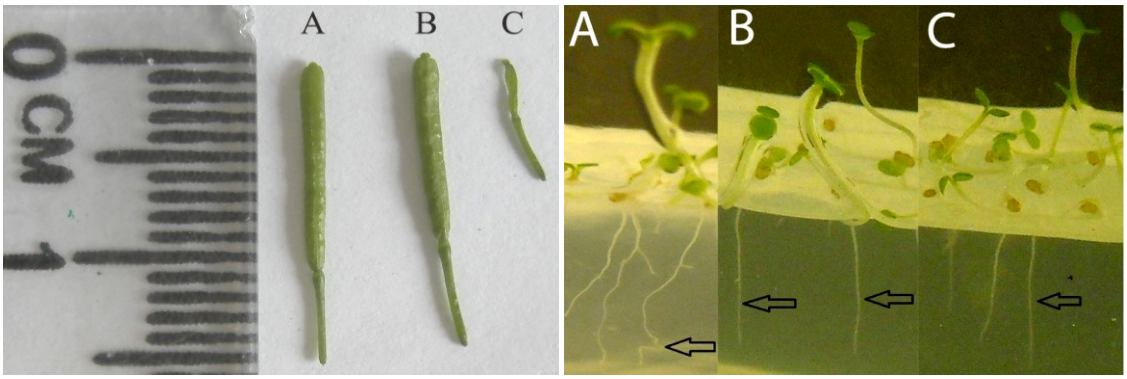


Figure 1. Left: Siliques of Columbia wild type *Arabidopsis* (A); *FvSPT* complemented Columbia *spt* mutant (B) and Columbia *spt* mutant (C); Rightw: Roots of *spiral* mutant Columbia (A), *FvSPR2* complemented Columbia (B) and Columbia wild type plants (arrows point at the different root phenotypes).

In the case of *spr2* mutant plants we also proved that however according to literature data the mutation *SPR1*, *SPR2* and *SPR3* genes cause the same abnormal root malformation symptoms in *A. thaliana* (Furutani et al., 2000) only *FvSPR2* was able to restore the dysfunction of *Atspr2 (*Figure 1, right).

II. Expression pattern of genes related to polyamine and ethylene biosynthesis determined by qRT-PCR

Among the 130 ripening related transcripts mentioned in the introduction there were genes involved in ethylene and polyamine biosynthesis, which are well known participants of plant physiological processes including fruit maturation. The molecular background of ripening is a less clarified mechanism in non-climacteric fruits such as strawberry than in the ethylene inducible climacteric ones. However the ethylene and polyamine biosynthetic genes (1-aminocyclopropane carboxylate synthase and oxidase - *ACS* and *ACO*; spermidine synthase - *SPDS*) transcripts could be identified in achenes and green flesh of strawberry (Balogh et al., 2005). Steps of ethylene biosynthesis in higher plants were described by Yang and Hoffmann (1984). *ACS* and *ACO* genes encode the key enzymes in this pathway. The amount of ethylene is relatively high in the green fruit of non-climacteric strawberry, it decreases at the white ripening stage and then it slightly rises until the end of ripening process (Perkins-Veazie et al., 1996). The moderate growth of ethylene starting from the white ripening stage is accompanied by an increase in the rate of respiration, similarly to the onset of climacteric ripening (Iannetta et al., 2006). In addition to ethylene, polyamines also play a major role in the ripening processes of fruits sharing a common precursor molecule, the S-adenosyl-L-metionin (SAM). Polyamines are polycations of small molecular weight present in all living organisms (Cohen, 1998). Diamine putrescine (Put), triamine spermidine (Spd) and tetraamine spermine (Spm) are the most common polyamine forms in plants (Kaur-Shawhney et al., 2003). Promoting cell division and growth,

influencing cell membrane structure and defense against the oxidative stresses are the main functions of polyamines during fruit development. Earlier studies discussed the antagonistic effects of ethylene and polyamine metabolisms during fruit ripening in avocado and tomato (Winer and Apelbaum, 1986; Saftner and Baldi, 1990). By revealing the expression pattern of genes involved in the ethylene and polyamine metabolic pathways, our primary aim was to better understand the mechanisms underlying non-climacteric fruit ripening, which still have elements to be clarified. Figure 2. shows the ethylene and polyamine metabolism. We measured the relative level of expression of the genes highlighted with red at four different ripening stages (green, white, pink and red). RNA was isolated from *Fragaria x ananassa* Duch cv. Asia and used to synthesize cDNA by reverse transcriptase enzyme, which was applied in qPCR with primers designed for S-adenosyl-L-methionine synthase (*FaSAMS1-3*), S-adenosyl-L-methionine decarboxylase (*FaSAMDC1-3*), arginine decarboxylase (*FaADC*), spermidine synthase (*FaSPDS*), 1-aminocyclopropane-1-carboxylate synthase (*FaACS1-4*) and 1-aminocyclopropane-1-carboxylate oxidase (*FaACO1-4*) gene sequences. We applied the actin housekeeping gene as reference.

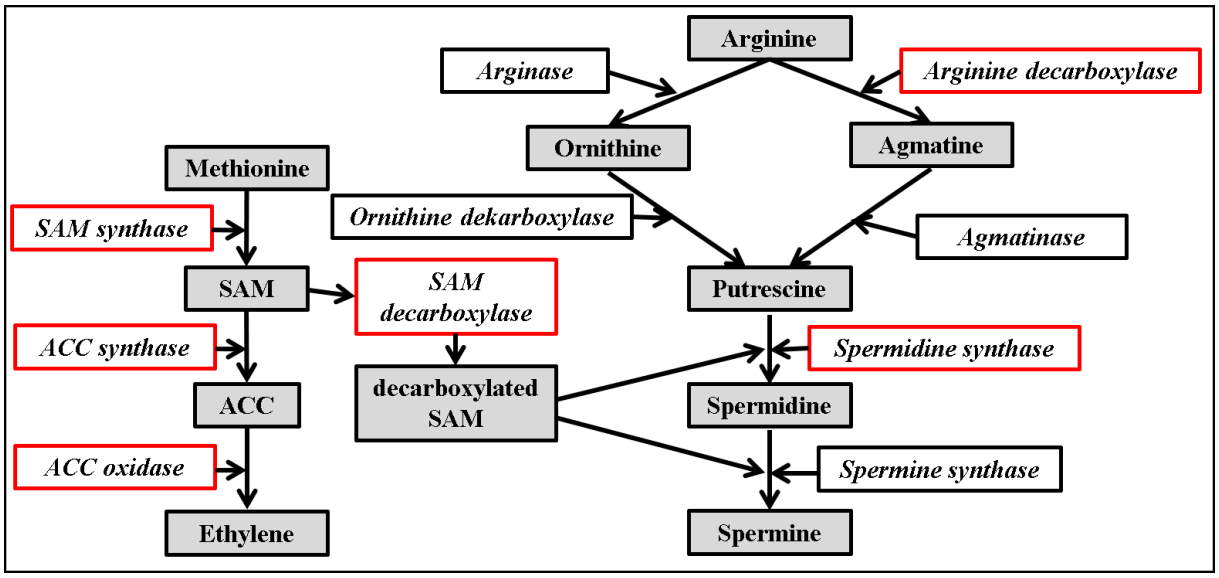
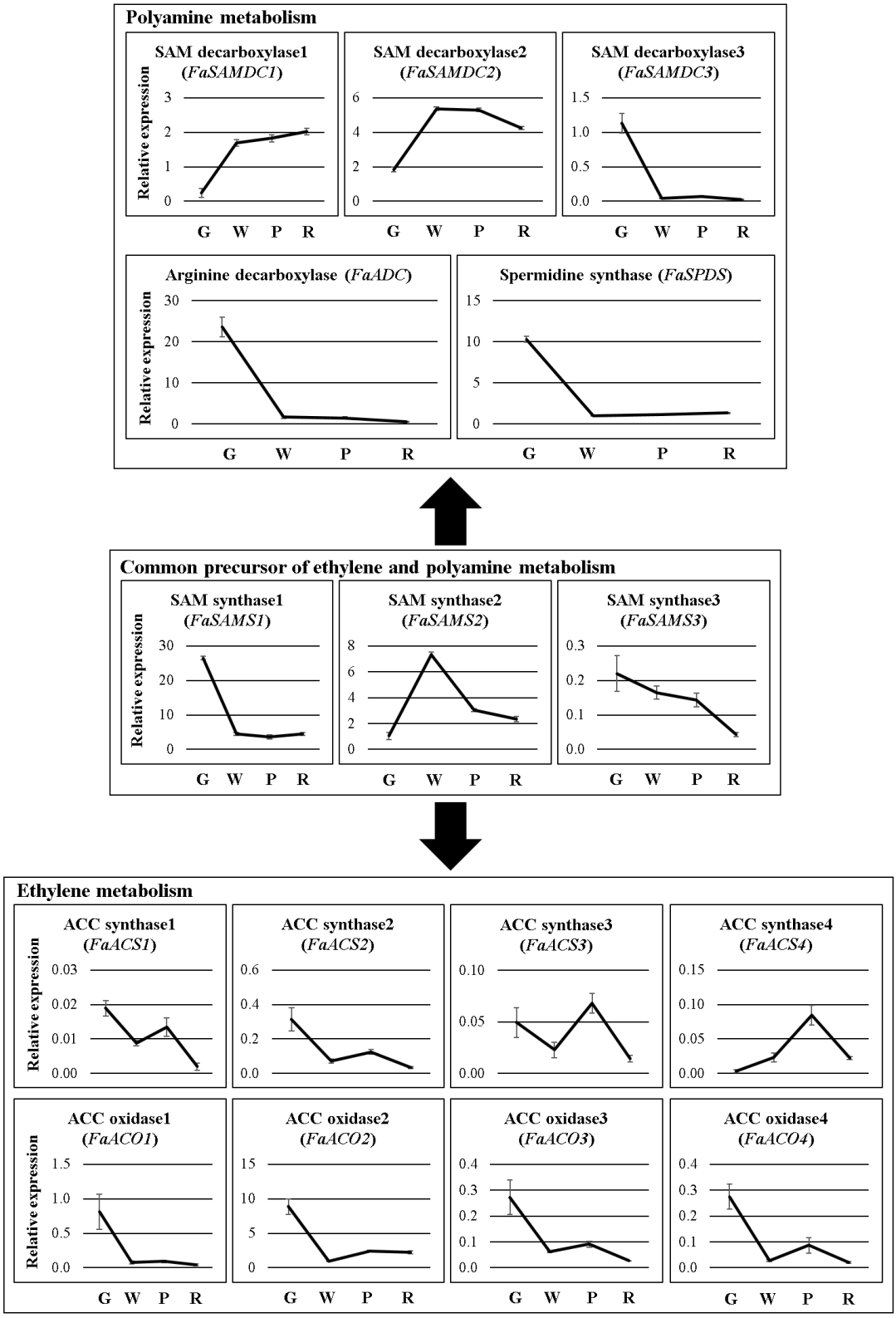


Figure 2. Ethylene and polyamine metabolism. Expression levels of genes highlighted with red were measured in strawberry fruit at four different ripening stages.

Fragaria x ananassa Duch. cv Elsanta *FaACS1* and *FaACO1* genes (AY661301 and Y706156) were isolated and sequenced by Kiss et al. (2007). *FaACS2-4* and *FaACO2-4* were identified by BLAST analysis of *FaACS1* and *FaACO1*. The primers were designed based on the available whole genome sequence of *Fragaria vesca* L. (Shulaev et al., 2011) and tested both on genomic and cDNA of *Fragaria x ananassa* Duch. cv. Asia. Based on the comparison made between the expression patterns of ethylene- and polyamine-related genes and that of the common precursor- and pathway shift-related genes, it appears that with the exception of *FaSAMS2*, *FaSAMDC1* and *FaSAMDC2*, the genes involved in polyamine biosynthesis showed decreasing level of expression as ripening proceeds (Figure 3). However, the increase in the transcription rate of the *FaACS1-4* and *FaACO1-4* genes in the pink fruits indicates that ethylene might act as a signal molecule for the accumulation of secondary metabolites (e.g. anthocyanins) at later stages of ripening, since

the rate of accumulation of these metabolites greatly increases before the fruits are fully ripen. Anthocyanins accumulate in significant amounts from the vérasion/pink ripening stage in grapes and strawberries (Halbwirth et al., 2006; Pilati et al., 2007; Griesser et al., 2008; Carbone et al., 2009). In accordance with these observations, the anthocyanins in our experiments also showed increased accumulation from the pink ripening stage (Figure 4).



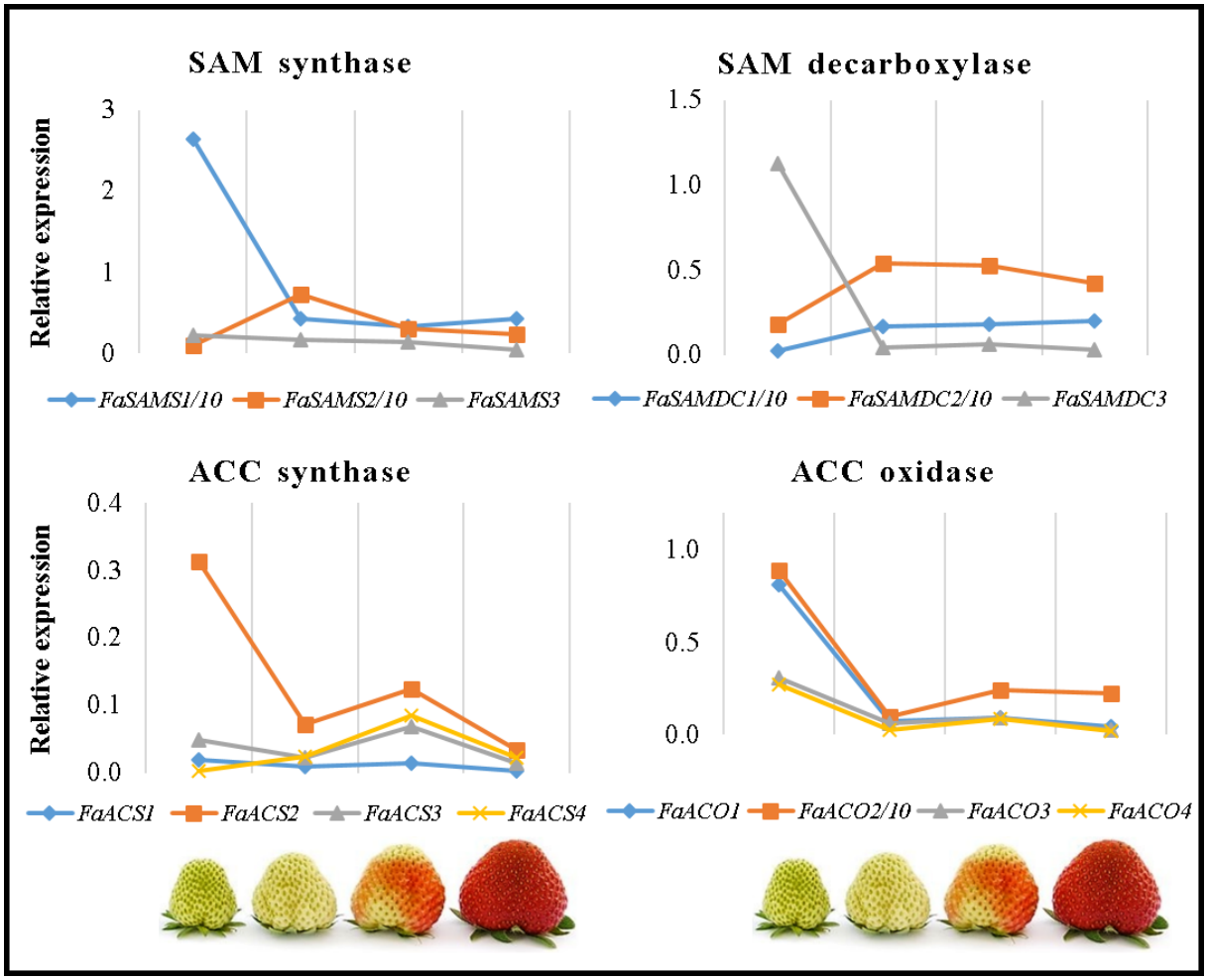


Figure 3. Relative expression levels of genes involved in ethylene and polyamine metabolism in four different ripening stages of *Fragaria x ananassa* Duch. cv. 'Asia'. (G=Green stage; W=White stage; P=Pink stage; R=Red stage). Data are the means of four replicates (±SD). Error bars represent significant differences at $P< 0.001$ (**a**). The relative expression of *FaSAMS1-FaSAMS3*, *FaSAMDC1-FaSAMDC3*, *FaACS1-FaACS4* and *FaACO1-FaACO4* genes merged in one diagram. The expressions *FaSAMS1/10*, *FaSAMS2/10*, *FaSAMDC1/10*,*FaSAMDC2/10* and *FaACO2/10* refer to the relative expression level values divided by ten **(b).**

In an earlier study while investigating the relative expression levels of genes involved in anthocyanin biosynthesis such as chalcone synthase (*CHS*), chalcone isomerase (*CHI*), flavanone 3-hydroxylase (*FHT*), dihydroflavonol 4-reductase (*DFR*), anthocyanidin synthase (*ANS*) and flavonoid 3-glucosyltransferase (*F3GT*) in strawberry the largest amounts of mRNA transcribed from these genes were found in the pink ripening phase (Thill at al., 2012). Therefore, the moderate increase of *FaACS1-FaACS4* and *FaACO1-FaACO4* transcript levels found in the pink fruits may suggest that ethylene is needed both for the completion of the ripening process and for the accumulation of anthocyanins (Figure 4). Furthermore, the ethylene peak coincides with the increased accumulation of pigments during the fruit ripening process in tomato (Van de Poel, 2012). Figure 3b illustrates the relative expression levels of the genes *FaSAMS1-FaSAM3*, *FaSAMDC1-FaSAMDC3 FaACS1-FaACS4* and *FaACO1-FaACO4* merged in one diagram. It can

be seen that the amount of mRNA transcribed from genes *FaACS1-FaACS4* is relatively low compared to that of genes *FaSAMS1-FaSAMS3*, *FaSAMDC1-FaSAMDC3* and *FaACO1-FaCO4*.

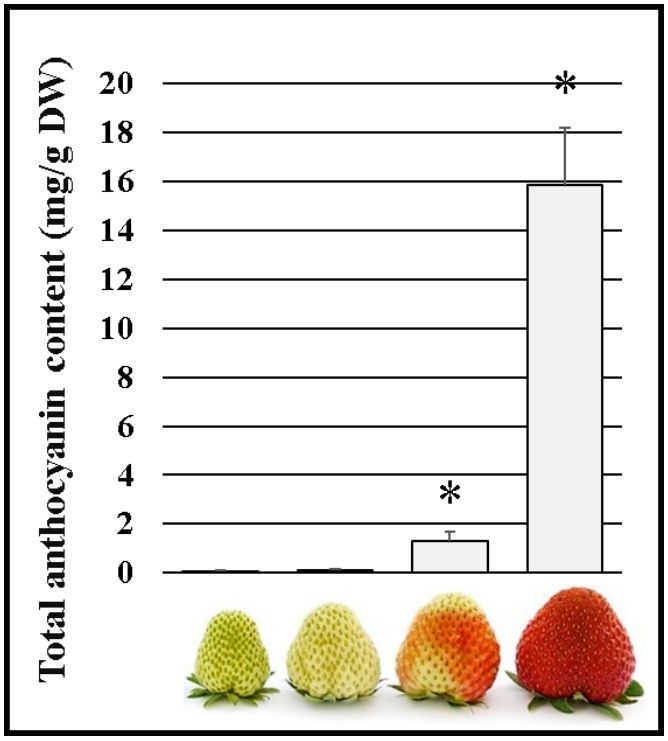


Figure 4. The total amount of anthocyanin in the green, white, pink and red ripening phases in strawberry. Data are the means of three replicates (±SD). The asterisk represent significant differences at P< 0.001.

The relative expression curves of the strawberry ethylene and polyamine biosynthetic genes show partial similarity to the results of several earlier studies (Aharoni and O'Connell 2002; Agudelo-Romero et al., 2013; Sun et al., 2013) and it can be concluded that ethylene plays a fundamental role in the fruit coloration processes of non-climacteric strawberry as well, similarly to the climacteric fruits.

III. Studying the effect of overexpressed *Fragaria vesca* S-adenosyl-L-methionine synthase (*FvSAMS*) and decarboxylase (*FvSAMDC*) under salt stress in transgenic *Nicotiana benthamiana*

We investigated the effect of overexpressing *Fragaria vesca* L. cv. Rügen S-adenosyl-L-methionine synthase (*FvSAMS*) and decarboxylase (*FvSAMDC*) genes on salt stressed *Nicotiana benthamiana* Domin plants. Previous studies have already proven that the overproduction of both proteins enhances the abiotic stress tolerance of plants, but the two enzymes have not yet been studied in one experimental system. In both cases we found that the transgenic plants subjected to long-term salt stress displayed higher levels of tolerance than the WT. In contrast to several earlier studies no antagonistic effect between ethylene and polyamine biosynthesis was observed in our experimental system. Based on the data measured in the FvSAMDC lines there appears to be a positive correlation between the free polyamine (PA) levels and the proline content as well as the amount of ethylene, while there is a negative correlation between the free PA levels and the lignin content when the plants are exposed to salt stress. Overexpression of *FvSAMDC* had

higher impact on the plant physiological parameters, than that of *FvSAMS* with the exception of lignin and proline content in control and salinity conditions. The summary of the measurements is shown in Table 2.

Table 2. The summary of the measurements of physiological parameters, composition and gene expression in FvSAMS and FVSAMDC overexpressing N. bentahmiana plants in control and salt stress conditions

	FvSAMS	FvSAMDC	FvSAMS	FvSAMDC
	Control condition (fold change)		10 mM NaCl stress (fold change)	
Biomass production/WT	~ 1.5 (↑)	~ 1.9 (↑)	~ 2 (↑)	~ 2.6 (↑)
Shoot length /WT	~ 1.4 (↑)	~ 1.5 (↑)	~ 1.6 (↑)	~ 1.9 (↑)
Chlorophyll *a* and *b* content/WT	~ 1.3 (↑)	~ 1.6 (↑)	~ 1.7 (↑)	~ 1.9 (↑)
Electrolyte leakage/WT	~ 1.3 (↓)	~ 1.8 (↓)	~ 1.4 (↓)	~ 1.9 (↓)
H_2O_2 concentration/WT	~ 1.2 (↓)	~ 1.4 (↓)	~ 1.3 (↓)	~ 1.8 (↓)
Proline content/WT	~ 1.1 (↑)	~ 0	~ 1.2 (↑)	~ 4.3 (↑)
Lignin content/WT	~ 2.8 (↑)	~ 1.8 (↑)	~ 1.7 (↑)	~ 1.5 (↓)
Ethylene production/WT	~ 1.1 (↓)	~ 1.1 (↑)	~ 1.1 (↓)	~ 1.5 (↑)
Total free polyamine content				
Put/WT	~ 1.1 (↓)	~ 1.3 (↑)	~ 1.2 (↓)	~ 1.2 (↑)
Spd/WT	~ 1.2 (↑)	~ 1.3 (↑)	~ 1.3 (↑)	~ 1.8 (↑)
Spm/WT	~ 1.2 (↑)	~ 1.3 (↑)	~ 1.3 (↑)	~ 1.8 (↑)
Total free PA/WT	~ 1.1 (↑)	~ 1.3 (↑)	~ 1.2 (↑)	~ 1.7 (↑)
Relative expression measurement				
***SAMS*/WT**	~ 1.8 (↑)	~ 2.0 (↑)	~ 3.9 (↑)	~ 2.4 (↑)
***SAMDC*/WT**	~ 1.2 (↑)	~ 1.2 (↑)	~ 1.2 (↑)	~ 1.6 (↑)
***ACS*/WT**	~ 1.2 (↓)	~ 1.1 (↓)	~ 1.4 (↓)	~ 2.2 (↑)
***SPDS*/WT**	~ 0	~ 1.1 (↑)	~ 1.2 (↑)	~ 1.4 (↑)
***SPMS*/WT**	~ 1.2 (↑)	~ 1.4 (↑)	~ 1.2 (↑)	~ 1.5 (↑)
***SAHH*/WT**	~ 1.5 (↑)	~ 1.8 (↑)	~ 1.3 (↑)	~ 1.3 (↑)
***PMT*/WT**	~ 4.8 (↓)	~ 2.4 (↑)	~ 3.0 (↑)	~ 34.9 (↑)
***CAD*/WT**	~ 1.7 (↑)	~ 1.3 (↑)	~ 1.4 (↑)	~ 5.1 (↑)

The transformation vector constructs contained the CaMV35S promoter, the coding sequence of *FvSAMS* and *FvSAMDC* fused with synthetic green fluorescent protein (sGFP). For the identification of sequences, the NCBI database was used. The main ORF of *FvSAMS* is 1182 bp (XM_004288294.2), while the *FvSAMDC* is 1080 bp long (XM_011464655.1). The leaf tissue of *Fragaria vesca* L. cv. Rügen was used for the isolation of RNA and cDNA was synthesized by reverse transcription. The cDNA sequences of *FvSAMS* and *FvSAMDC* were amplified with the primer pairs designed for the main ORFs and the CaMV35S promoter was amplified with specific primer pairs. NetNES 1.1 (La Cour et al., 2004), TargetP 1.1 (Emanuelsson et al., 2007), cNLS Mapper (Kosugi et al., 2009), GPS-SNO 1.0 (Xue et al., 2010), Nucleolar localization sequence Detector (NoD) (Scott et al., 2011) and PHOSIDA (Gnad et al., 2011) applications were used for bioinformatic analysis of the sequences. The cDNA fragments were ligated into pGWB405 (AB294429.1) and the CaMV35S was ligated into pGWB604 (AB543113.1) binary vector according to manufacturer's protocol (Invitrogen™ Gateway®, ThermoFisher Scientific, Waltham, USA). The pGWB405 contained a constitutive CaMV35S promoter and the *sGFP* reporter gene while the

pGWB604 carried only the *sGFP*. The *FvSPDS::sGFP* construct used for microscopic analysis was built by us using the pGWB405 vector. The insert codes for the spermidine synthase gene isolated from *Fragaria vesca* L. cv. Rügen. *FvSPDS::sGFP* fusion protein showed only cytoplasmic localization and was used as a cytoplasmic control (Figure 5).

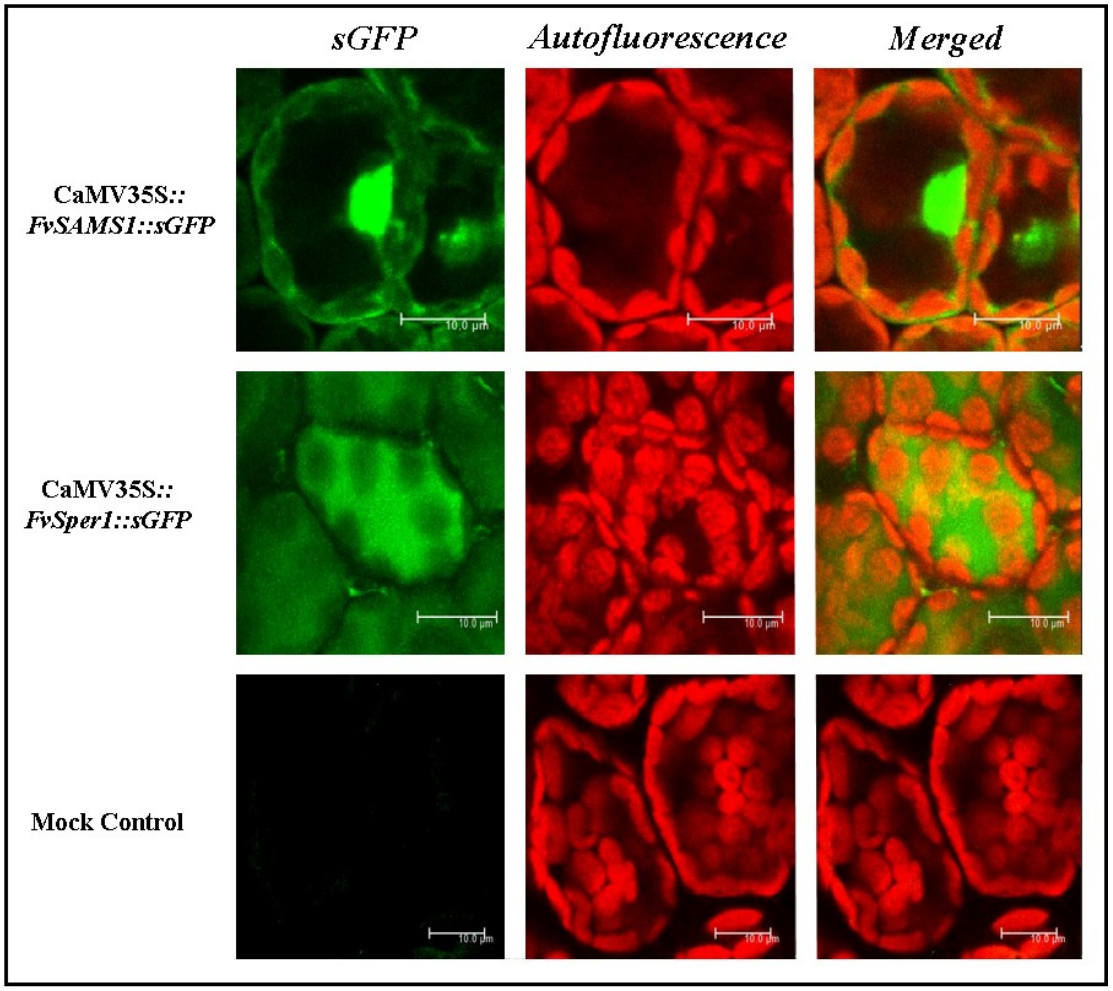


Figure 5. Different cytoplasmic localization of FvSAMS1::sGFP and FvSperS1::sGFP (unpublished data). Bars indicate 10 µm

The *Agrobacterium tumefaciens* GV3101 strain was used for transformation of the *Nicotiana benthamiana* plants based on the method described by Clemente (2006). The *A. tumefaciens* GV3101 strain containing *pGWB405::FvSAMS*, the *pGWB405::FvSAMDC* and the *pGWB604::CaMV35S* vectors was used for the *in vivo* infiltration of the 35-day-old *Nicotiana benthamiana* plants based on the method described by Li (2011). We detected the subcellular localization of both enzymes and examined the possible stress induced changes in their distribution. In the case of *FvSAMS::sGFP* nuclear, nucleolar, cytoplasmic (near to the plasmalemma), plastid membrane, whereas in *FvSAMDC::sGFP* nuclear and homogenous cytoplasmic localization was detected. (Figure 6 and 7)

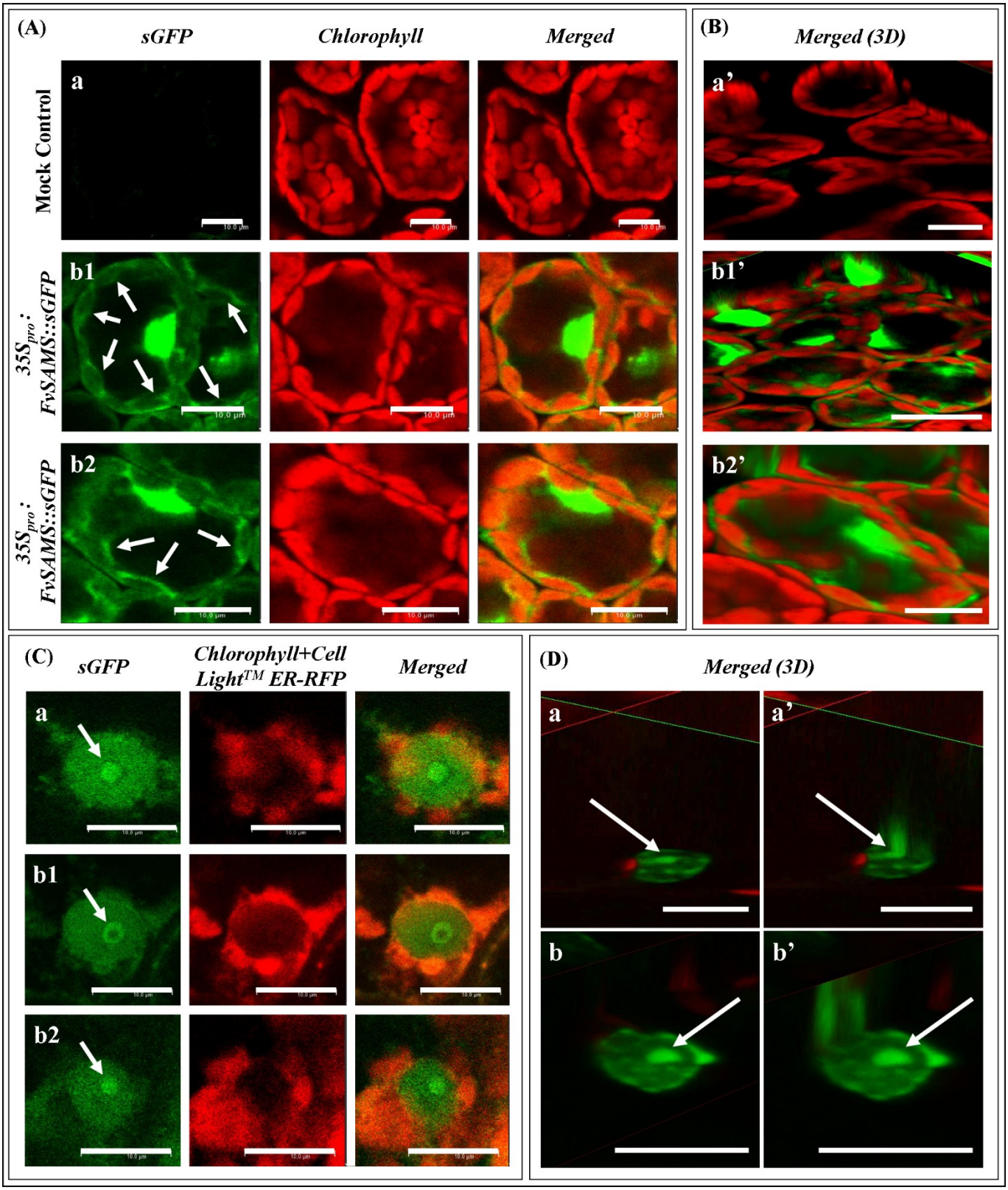


Figure 6. The cytoplasmic and plastid membrane localization of *FvSAMS::sGFP* (**b1, b2**) in palisade parenchyma cells. Bars indicate 10 µm (**A**). The cytoplasmic localization of *FvSAMS::sGFP* (**b1', b2'**) in palisade parenchyma cells in 3D. Bars indicate 20 µm (**B**). *FvSAMS::sGFP* signals in the cell nucleus and nucleolus of epidermal cells with transient expression (**C**) (CellLight™ ER-RFP used only in these samples) and in the palisade parenchyma cells of the stable transformant plants in 3D (**D**).

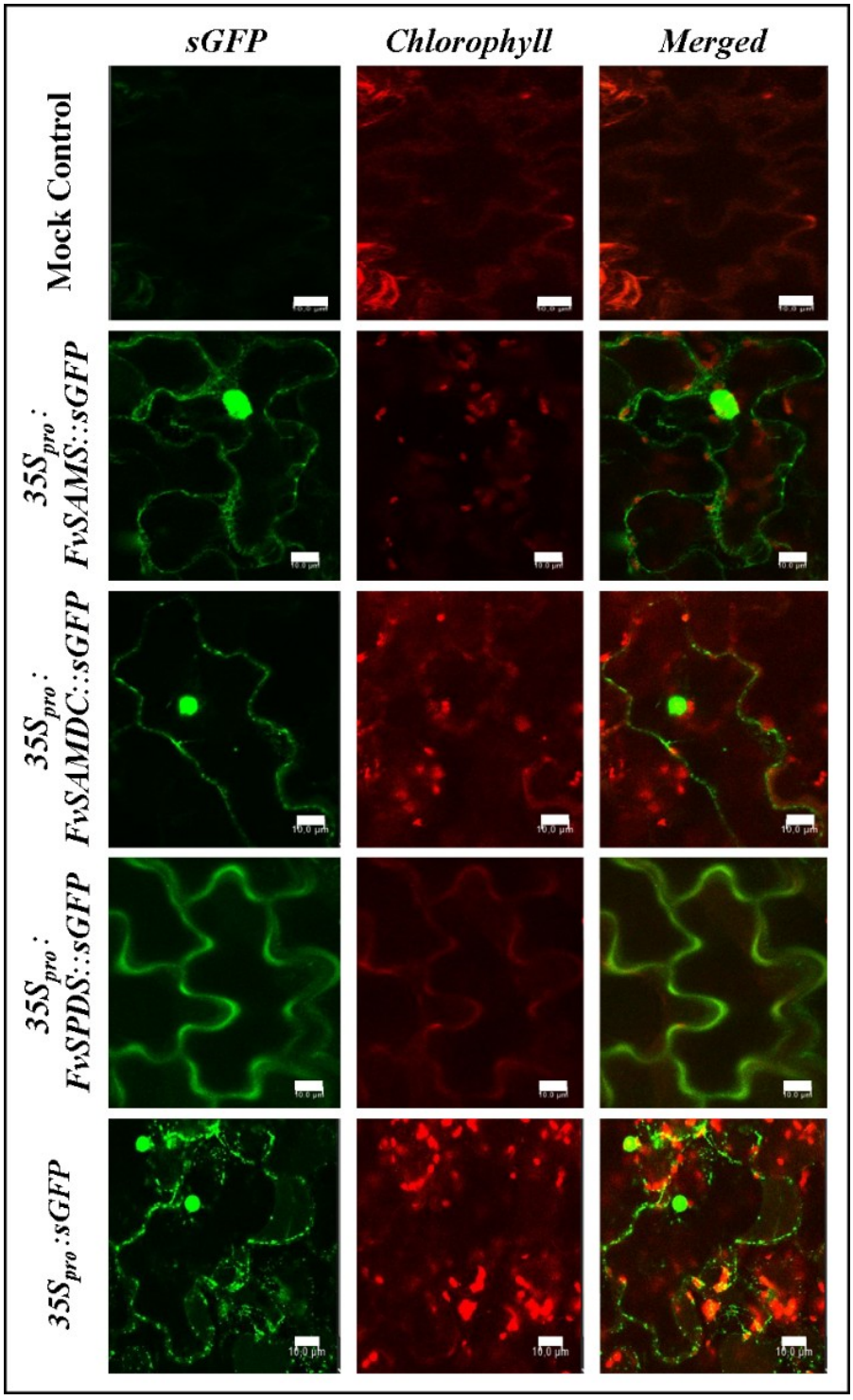


Figure 7. Transient expression pattern of *FvSAMS::sGFP*, *FvSAMDC::sGFP*, *FvSPDS::sGFP* and $35S_{pro}$*:sGFP* in epidermal cells. Bars indicate 10 µm.

Therefore, SAM is assumed to be produced *in situ* for numerous biochemical reactions (e.g. ribosome biosynthesis, methylation of DNA, RNA proteins in nucleus and plastid; lignin biosynthesis). As for FvSAMS it is presumed that the phosphorylation of Thr-112 and Ser-271 might be involved in the inhibition of enzyme activity through Protein kinase C, while its subcellular localization is thought to be determined by the acetylation of Lys-355, Lys-360 and Lys-364 as well as the SUMOylation of Lys-387.

References

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Manuscripts

1. Hidvégi et al.

   Hidvégi N, Gulyás A, Posta K, Kiss E (2018) Functional analysis of strawberry „*SPATULA*" *(FVSPT)* and „*SPIRAL" (FVSPR)* genes based on complementation test in *Arabidopsis* (*in preparation*)

2. Mendel et al.

   Mendel A, Kovács L, Szentgyörgyi A, Fekete S, Posta K, Ercisli S, Kiss E (2018) Expression patterns of ethylene and polyamines biosynthetic genes during fruit ripening in strawberry (*submitted)*

3. Kovács et al.

   Kovács L, Mendel á, Szentgyörgyi A, Fekete S, Söre F, Posta K, Kiss E (2018) Comparative analysis of overexpressed Fragaria vesca S-adenosyl-L-methionine synthase (FvSAMS) and decarboxylase (FvSAMDC) during salt stress in transgenic *Nicotiana benthamiana (submitted)*