THE ISOLATION SINGLE *ET1*, *ET2*, *ET3* MUTANTS AND GENERATION DOUBLE *ET1/ET2* MUTANTS OF THE ET GENE FAMILY IN *Arabidopsis Thaliana*

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SUMMARY

EFFECTORS OF TRANSCRIPTION (ET) are plant-specific regulatory proteins characterized by highly conserved ET repeats. These proteins are involved in zinc and DNA binding. Therefore, It was supposed that ETs might act as epigenetic regulators. Isolation single mutant alleles *et1*, *et2*, *et3* and generation double *et1/et2* mutants have been proposed as a powerful tool for identifying, confirming the function of ET family. Here, we describe the structural characterization of the three member *Arabidopsis thaliana* ET gene family. Then, Identifying a mutation in the genes of interest by PCR screening of pools of insertion lines, using one primer corresponding to interest genes and one primer corresponding to the end of the insertion element (T-DNA). The two double mutants have been generated by crossing the homozygous mutant *et1-1* both with homozygous *et2-1* and *et2-3* mutants. The homozygous double mutants *et1-1 et2-1* and *et1-1 et2-3* have been selected and characterized in the F2 generation. In this study we got two *et1* lines : homozygous *et1-1* with the T-DNA insertion is positioned in the second exon and heterozygous *et1-5* with the T-DNA insertion in *et1-5* is now located far up in the 5`flanking region, 699 bp in front of the translation start; Two *et2* lines : homozygous *et2-1* with T-DNA insertion are positioned in the second exon and homozygous *et2-3* with T-DNA insertion are positioned in first exon; Two *et3* lines : heterozygous *et3-2* and heterozygous *et3-3* with T-DNA insertion are located at promoter 239bp and 216bp in front of the translation start, respectively. And we have been generated two lines double mutants *et1/et2*: homozygous *et1-1/et2-1* and homozygous *et1-1/et2-3*.

Keywords: double mutant, EFFECTORS OF TRANSCRIPTION (ET), heterozygous, homozygous, mutant, T-DNA insertion.

1. INTRODUCTION

EFFECTOR OF TRANSCRIPTION (ET) genes were originally discovered by using South Western screens aiming to the isolating of transcription factors important for embryonic gene regulation (Ellerström et al., 2005; Ivanov et al., 2008).

They represent a strictly plant specific class of gene regulators of barley, broad bean, rape seed and Arabidopsis (Raventós et al., 1998; Ellerström et al., 2005; Ivanov et al., 2008) designated as HORDEUM REPRESSOR OF TRANSCRIPTION (HRT) in monocots and EFFECTOR OF TRANSCRIPTION (ET) in dicots. ET proteins share variable numbers of highly conserved cysteine-histidine containing, zinc- and DNA binding repeats also found in lower plants such as the moss *Physcomitrella patens* demonstrating their evolutionary conservation (Ellerström et al., 2005; Ivanov et al., 2008).

In Arabidopsis the ET family consists of three genes: *AtET1* (AT4G26170); *AtET2* (AT5G56780); *AtET3* (AT5G56770). *AtET1* is located on the fourth chromosome, while the other genes, *AtET2* and *AtET3* are located on the fifth chromosome (Ellerström et al., 2005; Ivanov, 2005; Ivanov et al., 2008). The *AtET1* and *AtET2* genes are intact coding sequence, whereas *AtET3* is a truncated version of *AtET2* due to the lack of the ET repeat domain.

Besides these DNA binding ET-repeats, ET factors share a characteristic DNA single strand cutting domain (GIY-YIG) with structural similarity to that of bacterial UVRC proteins and so called homing nucleases (Derbyshire et al., 1997; Aravind et al., 1999; Verhoeven et al., 2000; Stoddard, 2005). The bacterial UVRC protein is essential for DNA excision repair (Friedberg et al., 1995; Moolenaar et al., 1998). It is targeted to UV-induced DNA lesions like thymidine-dimers

and introduces two single strand cuts 8 bp 5` and 4 bp 3` of the lesion. The two single strand cuts are processed by two structurally and functionally distinct domains. A C-terminal domain consisting of an Endonuclease V (EndoV) and Helix-hairpin-Helix (HhH) domain is required for the 5`cut, whereas the N-terminal GIY-YIG domain inserts the 3`nick (Lin and Sancar, 1992; Derbyshire et al., 1997; Aravind et al., 1999; Kowalski et al., 1999; Verhoeven et al., 2000; Van Roey et al., 2002). The sequence similarity between plant ET factors and UVRC is only restricted to the single strand cutting GIY-YIG domain. This suggests that an ancestral bacterial GIY-YIG domain has been recruited by ET proteins and attached to the DNA-binding ET repeats to create a novel plant specific regulatory protein. In all known ET genes the GIY-YIG domain is encoded by the separate second exon, consistent with a corresponding domain shuffling event during protein evolution. A conceivable hypothesis is that the nicking activity of the plant ET factor GIY-YIG domain may be involved in the catalysis of changes in higher order DNA structures, such as, for example, nucleosome sliding or the relaxation of supercoiled chromatin domains as a prerequisite for regulated gene expression (Choi et al., 2002; Xiao et al., 2003; Haince et al., 2006; Ju et al., 2006). Alternatively, the domain could be involved in active de-methylation processes as described for the plant regulators DEMETER (DME) and REPRESSOR OF SILENCING1 (ROS1) (Choi et al., 2002; Gong et al., 2002; Xiao et al., 2003; Choi et al., 2004; Morales-Ruiz et al., 2006).

Remarkably, the HhH domain as the separate second nicking domain of the UVRC protein, can be considered as the ancestor protein domain for both of these regulatory proteins. DME can introduce single strand nicks in the MEDEA (MEA) promoter as part of a DNA de-methylation pathway involved in the epigenetic imprinting of the MEA gene. ROS1 is described as protein that represses homology-dependent transcriptional silencing by de-methylating the target promoter DNA (Gong et al., 2002). Thus, a possible evolutionary scenario is that plant-specific ET factors have recruited a single GIY-YIG domain from prokaryotic repair-related proteins by a domain shuffling process, joining this domain to the DNA-binding ET repeat. The regulatory mechanism in part analogous to the function of DME and ROS1 might include the insertion of nicks, with an impact on higher order structures of chromatin packed DNA or on the genomic DNA methylation pattern required for differentiation processes for instance during seed development.

A principally similar evolutionary process combining an ancient endonuclease domain with a DNA-binding domain has been described for the transcription factors family AP2/ERF (Magnani et al., 2004).

2. RESEARCH METHODOLOGY

2.1. Plant materials

Arabidopsis thaliana ecotypes Columbia-0 (Col) and *Wassilewskija-2* (Ws) were obtained from Gene Regulation Group (IPK, Gatersleben, Germany) and used throughout this study as wild type control experiments. T-DNA insertion lines have been received from Nottingham Arabidopsis stock center. From the genetic and molecular analysis of several SALK lines the following stable mutant lines have been obtained: *et1-1; et1-5; et2-3; et3-2; et3-3*. The line *et2-1* has been isolated from the Arabidopsis Knock-out Facility (AKF) at the University of Wisconsin Biotechnology center.

2.2. Extraction of genomic DNA

Genomic DNA extraction from plants was performed according to Edwards et al., 1991. Leaf tissue (~100 mg) was ground in liquid nitrogen into fine powder and suspended in 400 µl of extraction buffer. The suspension was centrifuged for 10 min at 13.000 rpm in a microcentrifuge. The supernatant was collected into a new tube and the DNA was precipitated with an equal volume of isopropanol. DNA

was collected by centrifugation for 10 minutes, washed in 70% ethanol, dried and resuspended in 50 µl TE buffer. DNA concentration was determined by Nanodrop® ND-1000 spectrophotometer (NanoDrop Technologies Inc., USA).

2.3. Screening and verifying T-DNA insertion mutants

T-DNA insertion lines in *AtET1* were obtained from the SIGNAL T-DNA collection (http://signal.salk.edu/cgi-bin/tdnaexpress) and designated as *et1-1* (SALK_000422), *et1-5* (SALK_006710), *et3-2* (CS431900) and *et3-3* (CS423803). T-DNA insertion lines *et1-1; et1-5; et2-3; et3-3; et3-2* in Columbia (Col) background were isolated from the Salk Institute collection of T-DNA lines transformed with pROK2. The T-DNA specific primer LBa1 (O'Malley *et al.*, 2007) was used in combination with either forward or reversed gene specific primers.

The *et2-1* mutant was selected by pool screening from the collection of *Arabidopsis* Knock-Out-Facility (AKF), University of Wisconsin (Ivanov, 2005). This collection has been transformed with a derivative of the T-DNA vector pD991 in the *Wassilewskija 2* ecotype (Ws) (Krysan et al., 1999; Sussman et al., 2000) and was designated as *et2-1* (Ivanov et al., 2008). The presence of T-DNA was verified by PCR using T-DNA right border XR2 primer (Zhao *et al.*, 2002; Ivanov *et al.*, 2008) in combination with a gene specific primer.

The primer combinations were as follows: Wild type *ET1*: ET1-1HUF/ET1-1HUR; T-DNA *et1-1*: ET1-1HuF/LBa1; Wild type *ET1*: ET1-5F/ET1-RACE1; T-DNA *et1-5*: ET1-RACE1/LBa1; Wild type *ET2*: ET2_RT_ACF/ET2_RT_ACR; T-DNA *et2-1*: ET2_RT_ACR/XR2; Wild type *ET2*: ET2-3HUF/ET2-3HUR; T-DNA *et2-3*: ET2-3HUR/LBa1; Wild type *ET3*: ET3F/ET3R; T-DNA *et3-2*: ET3R/8409-LB; Wild type *ET3*: ET3F/ET3R; T-DNA *et3-3*: ET3R/8409-LB.

PCR conditions: 95°C in 5 minutes; (95°C: 30 seconds, 60°C: 30 seconds, 72°C: 40 seconds) repeat in 40 cycles, 72°C in 5 minutes.

2.4. Cloning methods and sequencing

Basic molecular methods such as enzymatic digestion, DNA ligation, DNA gel electrophoreses were performed according to standard protocols (Sambrook and Russell, 2001). DNA fragments were isolated and purified from agarose gel by QIAquick gel extraction kit (Qiagen, Hilden, Germany) and GeneJET gel extraction kit (Fermentas, Vilnius, Lithuania). PCR products were sequenced at the Leibniz-Institute of Plant Genetics and Crop Plant Research (IPK, Gatersleben, Germany) or commercially by MWG Biotech Company (Ebersberg, Germany). Plasmid extractions and purifications were done using Qiagen Plasmid kit and Fermentas GeneJET plasmid miniprep kit according to the protocols recommended by the manufactures.

2.5. cDNA synthesis and RT-PCR

First strand cDNA was synthesized by reverse transcription from total RNA using Revert Aid H Minus First strand cDNA synthesis kit (Fermentas, Vilnius, Lithuania). 1 µg of total RNA and 1 µl oligo (dT) primer were added to each tube to obtain a total volume of 11 µl. Priming was carried out at 70ºC for 5 minutes, then 1 µl of ribonuclease inhibitor (20 units/µl), 2 µl of 10 mM dNTP mix and 4 µl of 5X RT buffer were added to each reaction tube. The reaction mixture was incubated at 37ºC for 5 minutes and supplemented 1 µl of Reverse transcriptase (200 units/µl). Thereafter, this reaction mixture was incubated at 42ºC for an hour, and then heated for 10 minutes at 70°C and stored at -20°C for further uses.

2.6. Reverse-transcription PCR

RT-PCR reaction to measure transcript amounts was performed using the primers ET1-1HUF/ET1-1HUR for ET1 transcript, ET2_RT_ACF/ET_RT_ACF for ET2 transcript and AP3F/AP3R for APETALA3

(AP3) transcript.

RT - PCR conditions: AP3: 95°C in 5 minutes, (95°C: 30 seconds, 60°C: 30 seconds, 72°C: 1 minutes), 72°C in 7 minutes

RT-PCR conditions: ET1, ET2: 95°C in 5 minutes, (95°C: 30 seconds, 60°C: 30 seconds, 72°C: 1 minutes), 72°C in 5 minutes.

3. RESULTS

3.1. Characterisation of *et* mutants

3. 1.1 Mutants in ET1 gene

About 50 plants for each original mutant line (*et1-1, et1-5*) were genotyped in the first generation to verify the insertions and determine whether the line was heterozygous or homozygous. Genomic DNA was isolated and used for PCR with gene specific primers in combination with the T-DNA-specific primer LBa1. The sizes of PCR products were determined to be 655 bp and 1047 bp for *et1-1* and *et1-5,* respectively.

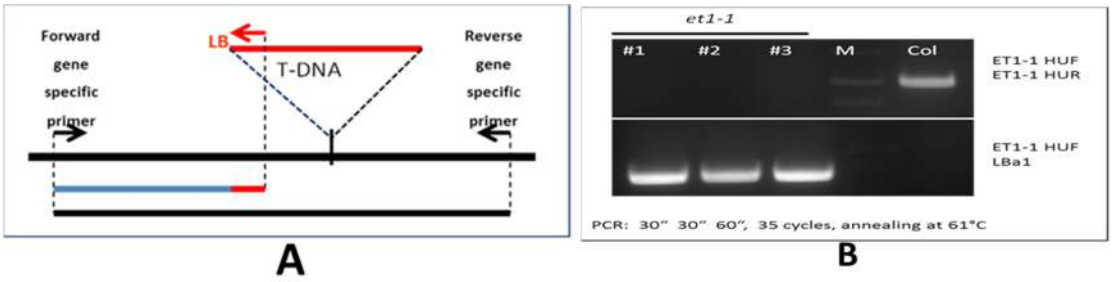

Fig. 1. Detection of the T-DNA insertion in ATET1

(A: Principal strategy for T-DNA detection using T-DNA-specific primer LBa1and two gene specific primers to detect mutant and wild type alleles. B: PCR analysis of three homozygous et1-1 lines (#) and wild type (Col) using ET1-1HUF and ET1-1HUR as gene specific primers and ET1-1HUF and LBa1 to detect the T-DNA insertion)

Connected to the verification of the T-DNA insertions the TAIR proposed gene model for *et1-1* has been investigated by SMART-RACE-technique to amplify and sequence the 5′-terminal part of the transcript. The results suggest that the database predicted gene model of *AtET1* needs to be corrected. The sequence of the RACE amplified fragment does not support the existence of the predicted small upstream exons and introns and the gene start needs to be shifted as shown in Fig.3. Based on this new gene model, the T-DNA insertion in *et1-5* allele is now located far up in the 5`flanking region, 699 bp in front of the translation start and in the *et1-1* allele the T-DNA insertion is positioned in the second exon (Fig. 2).

Up to now it was not yet possible to isolate a homozygous *et1-5* mutant. Although more than 400 plants have been analysed, only heterozygous genotypes could be detected by now. It is rather unexpected since the position of the T-DNA insertion is rather far up in the 5′-flanking region, but it still might destroy the gene promoter activity. Currently, this is investigated further.

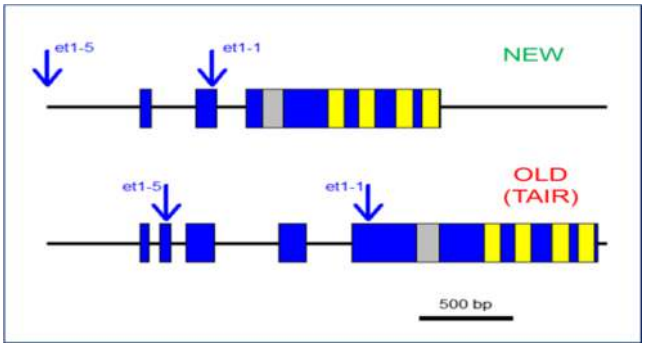

Fig. 2. Comparision of the TAIR data base predicted gene model and the new experimentally confirmed gene model

3.1.2. Mutants in *AtET2*

This mutant was backcrossed repeatedly into ecotype Col at least four times before used for further analyses and renamed *et2-1*. The homozygous mutant status was confirmed by PCR analysis using gene specific primers (ET2_RT_ACF and ET2_RT_ACR) as well as the T-DNA right border primer XR2 in combination with ET2_RT_ACR. The expected fragment length is 851 bp (Fig. 3).

The SALK_151861 line was screened from SIGNAL T-DNA collection and after confirmation and precise localization by sequencing and this line was renamed *et2-3*. The homozygous *et2-3* also was identified by PCR analysis using the same primer of *et2-1* as shown Fig.3. The expected fragment lengths were 1284 bp and 1369 bp for the mutant and wild type allele, respectively.

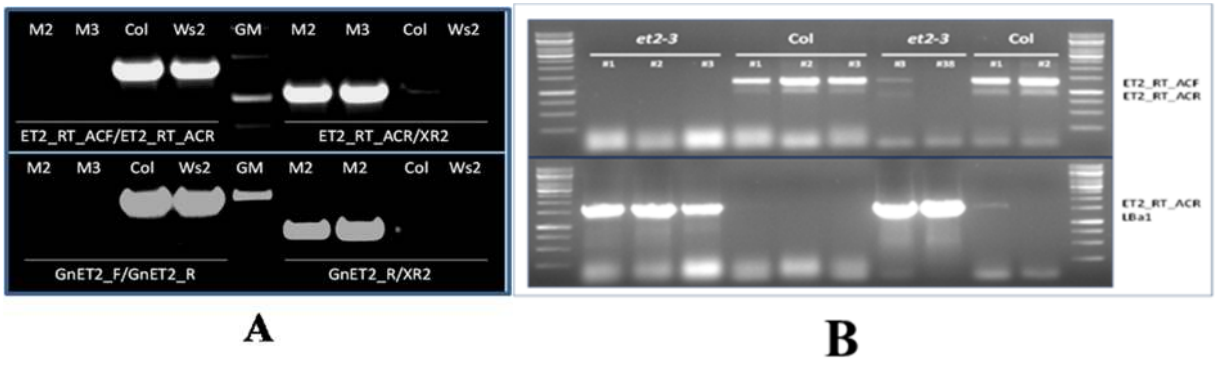

Fig. 3. Detection of T-DNA insertion in AtET2

(A: Genotyping of et2-1 using to different primer sets (RT_ACF/RT_ACR and GnET2_F/GnET2_R). XR2, T-DNA right border primer of pD991; M2, M3, mutant lines; Col, Ws2 ecotypes Columbia and Wassilewskija2; GM, size marker. B: Genotyping of et2-3 using gene specific primers ET2_RT_ACF/ET2_RT_ACR and ET2_RT_ACR/LBa1.)

3.1.3 Mutants in *AtET3*

Two T-DNA insertion lines have been identified and characterized for *AtET3*. Lines CS423803 and CS431900 were obtained from the SIGNAL-collection and after confirmation and precise localization by sequencing were renamed into *et3-3* and *et3-2*, respectively. Both mutants were shown to be homozygous. The insertions in both lines are located close to each other within the 5`-flanking gene region (Fig. 4).

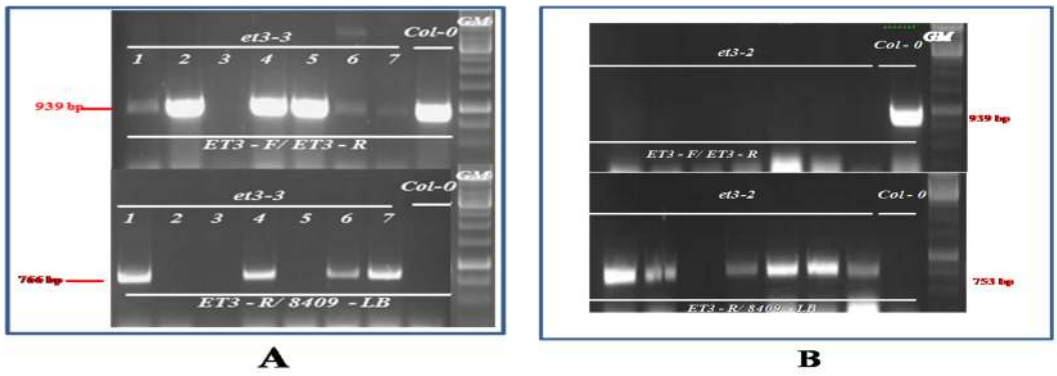

Fig.4. Detection of T-DNA insertion in AtET3

(A: Genotyping of et3-3 using different primer sets (ET3-F/ET3-R and ET3-R/8409-LB). GM: size marker. B: Genotyping of et3-2 using gene specific primers ET3-F/ET3-R and ET3-R/8409-LB.)

In total there are 6 well characterized insertion lines available as summarise in Fig. 5. The single mutant *et1-1* was combined with the single mutants *et2-3* and *et2-1* to generate homozygous double mutants.

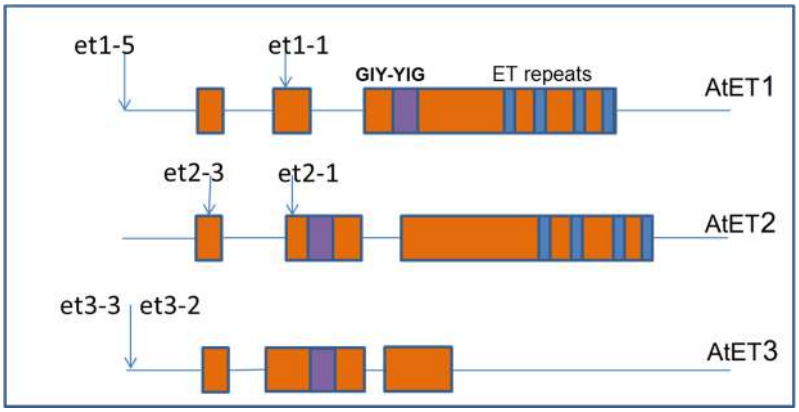

Fig.5. Gene model and positions of T-DNA-insertions of the *ET* gene family

(The positions of T-DNA insertions are indicated by arrows. ET repeats and the GIY-YIG single strand cutting domain are given in green and purple, respectively.)

Tab. 1 Nucleotide positions of 6 T-DNA insertion mutants relative to the ATG start

Gene	Mutant	Position	Insert relative to ATG
ET1	*et1-5*	promoter	-699
	et1-1	Exon2	383
ET2	*et2-3*	Exon1	85
	et2-1	Exon2	518
ET3	*et3-3*	promoter	-239
	et3-2	promoter	-216

3.1.4. Loss of transcripts in single mutants *et1-1, et2-1* and *et2-3*

A final step to verify the homozygous status of single mutants (*et1-1, et2-3, et2-1*) requires to demonstrate the absence of the wild type *AtET1* and *AtET2* mRNA, respectively. Therefore, RT-PCR on total RNA from wild type and homozygous mutant plants was performed and using actin transcript to control RNA quality and quantity. The *AtET1* and *AtET2* products, could be amplified only from the wild type sample, demonstrating the destruction of the intact *AtET1, AtET2* mRNA (Fig.6) in the homozygous mutants. The data demonstrate that the *AtET1* insertion line *et1-1* and the *AtET2* insertion lines *et2-3* and *et2-1* can be used assuitable tools for functional studies of the ET gene family in Arabidopsis.

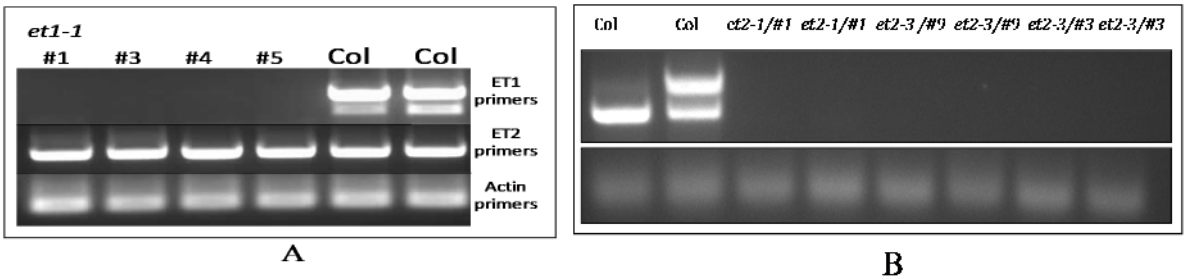

Fig. 6. RT-PCR to demonstrate the knock out character of single mutants *et1-1, et2-1* and *et2-3*

***(A**: et1-1 mutant and wild type probed with ET1 specific primers which span the insertion site show the loss of the ET1 transcript. Priming with ET2 specific primers demonstrates that the ET2 transcript is not present. **B**: ET2 specific primers which span the insertion site were used to show the loss of the ET2 transcript. Actin transcript was used in both cases as loading control.)*

3.2. Generation of double *et1 et2* mutants

AtET1 and *AtET2* are two closely related proteins. They share an overall amino acid identity of 40%, especially in the ET repeats (58%). To analyze possible functional redundancy, as indicated by similar phenotypes (see below), two double mutants have been generated by crossing the homozygous mutant

et1-1 both with homozygous *et2-1* and *et2-3* mutants. Homozygous double mutants *et1-1 et2-1* and *et1-1 et2-3* have been selected and characterized in the F2 generation (Fig. 7).

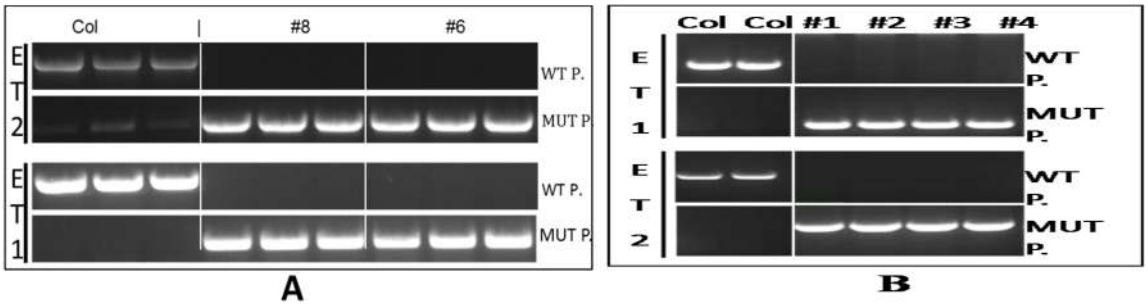

Fig.7. Genotyping of *et1 et2* double mutants.

***(A**: Genotyping of et1-1 et2-1 double mutant demonstrating the homozyogous double mutant status of lines #8 and #6. **B**: Genotyping of et1-1 et2-3 double mutant demonstrating the homozygous mutant status of lines #1, #2, #3, #4.)*

4. DISCUSSION

Genetic studies often rely on the analysis of phenotypic consequences of certain genotypic mutant alleles. Prior to a more detailed discussion of the phenotypic effects of the mutants, it is required to demonstrate the relation between genotypes and phenotypes. Principal possibilities include the analysis of multiple alleles and/or the phenotypic complementation of the genotype. The first option has been applied for *ET1*, *ET2, ET3* by the analysis of the mutant alleles, *et1-1*, *et1-5*, *et2-1*, *et2-3*, *et3-2*, and *et3-3* which obviously exhibit similar phenotypes. However, the mutant lines *et1-5*, *et3-2* and *et3-3* are heterozygous mutants. Therefore, we focus on the homozygous mutant lines *et1-1*, *et2-1*, *et2-3* and homozygous double mutants *et1-1/et2-1* and *et1-1/et2-3*. The second approach has been used for *ET1*. In this case, a 4.5 kb genomic wild type fragment has been amplified, re-sequenced and transformed into the *et1-1* mutant. The phenotypic characterisation of the transformed lines indicates the partial correction of the mutant phenotypes.

The new model derived translation product is also supported by tryptic peptides identified by peptide mass fingerprinting using matrix assisted laser desorption ionization-time-of-flight (MALDI-TOF) mass spectrometry. The AtET1 cDNA has been expressed in *E. coli* and the analysis of the isolated gene product resulted in the peptides given highlighted in red in Fig. 8. Le Hong Diep's thesis, 2011; A. Matros and H. P. Mock, IPK). Therefore, we strongly favour the new gene model.

MFK**RDDYIR**TNHDPFFSKWQGFARSMFLR**KPISETAELRKTFADYSL**
ISRDLGPKPK**ILIGANEKENFR**EGKDLVGRNR**VQGAFQGLYELSHDH**
GRKDDVLVANLGQPESIRSRLRSYSR**SFAHHDLLKQGLSQTILPTTQ**
NKSDNQTEEKKSDSEEEREVSSDAAEK**ESNSLPSILR**LSR**SRPQPVS**
EKHDDIVDESDSASACGVLLEDGTTCTTTPVKGRKRCTEHKGKRLSR
VSPGIHIPCEVPTVRECEETENICGVILPDMIRCRSKPVSRRKRCED
HKGMR**VNAFFFLLNPTER**DKAVNEDKSKPETSTGMNQEGSGLLCEAT
TKNGLPCTRSAPEGSKRCWQHKDKTLNHGSSENVQSATASQVICGFK
LYNGSVCEKSPVKGRKRCEEHKGMRITS

Fig. 8. Tryptic peptides given in red obtained by MALDI-TOF analysis (Le Hong Diep's thesis, 2011)

5. CONCLUSION

We were successfully amplified and sequenced from six the mutant lines *et1-1, et1-5, et2-1, et2-3, et3-2* and *et3-3*. The result indicated that the T-DNA insertion in *et1-1* allele is positioned in the second exon, *et1-5* allele is located in the promoter, *et2-1* allele and *et2-3* are positioned in the second exon and first exon, respectively, *et3-2* allele and *et3-3* allele with T-DNA insertion are located at promoter 239bp and 216bp in front of the translation start. Then, the homozygous single mutants (*et1-1, et2-3, et2-1*) were demonstrated the absence of the wild type *AtET1* mRNA and *AtET2* mRNA by RT-PCR. And two double mutant lines are generated including: homozygous *et1-1/et2-1* and homozygous *et1-1/et2-3* in the F2 generation.

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PHÂN LẬP CÁC ĐỘT BIẾN ĐƠN *et1, et2, et3* VÀ SỰ TẠO THÀNH CÁC ĐỘT BIẾN KÉP *et1/et2* CỦA HỌ GEN ET Ở CÂY *Arabidopsis thaliana*

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TÓM TẮT

ET là protein điều hòa đặc hiệu ở thực vật bởi đoạn lặp ET bảo thủ cao, đoạn lặp ET liên quan tới việc liên kết với kẽm và ADN trong vai trò điều hòa. ET được cho là có thể hoạt động như nhân tố điều hòa ngoài nhân. Việc phân lập các gen đột biến đơn *et1*, *et2*, *et3* và sự tạo thành các đột biến kép *et1/et2* được cho là công cụ hữu ích cho việc nhận biết, chứng thực chức năng của họ gen ET. Chúng tôi mô tả đặc điểm cấu trúc của 3 gen trong họ gen ET. Sau đó, xác định đột biến của các gen quan tâm bằng phương pháp PCR, bằng cách sử dụng một mồi của gen quan tâm và một mồi thuộc đoạn chèn T-DNA. Hai đột biến kép được tạo ra bằng cách lai cây đồng hợp tử đột biến đơn *et1-1* với cây đồng hợp tử đột biến đơn *et2-1* và *et2-3*. Cây đồng hợp tử đột biến kép *et1-1 et2-1* và *et1-1 et2-3* được sàng lọc và xác định ở thế hệ F2. Trong nghiên cứu này chúng tôi đã nhận được hai dòng đột biến đơn *et1*: Cây đồng hợp tử *et1-1* với đoạn T-DNA chèn ở vị trí exon thứ 2 và cây dị hợp tử *et1-5* với đoạn T-DNA chèn ở vị trí khởi đầu (vùng 5') cách xa vị trí khởi đầu dịch mã là 699bp; Hai dòng đột biến đơn *et2*: Cây đồng hợp tử *et2-1* với đoạn T-DNA chèn vởi vùng exon thứ 2 và cây đồng hợp tử *et2-3* với đoạn T-DNA chèn ở vị trí exon thứ nhất; Hai dòng đột biến đơn *et3*: Cây dị hợp tử *et3-2* và *et3-3* với đoạn T-DNA chèn ở vùng khởi động cách vị trí khởi đầu dịch mã là 239bp và 216bp tương ứng. Và chúng tôi đã tạo được hai dòng đột biến kép *et1/et2*: Cây đồng hợp tử đột biến kép *et1-1/et2-1* và cây đồng hợp tử đột biến kép *et1-1/et2-3.*

Từ khóa: dị hợp tử, đoạn chèn T-DNA, đồng hợp tử, đột biến, đột biến kép, *EFFECTORS OF TRANSCRIPTION (ET).*

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