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ORIGINAL PAPER

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Isolation of a partial sequence of a putative nucleotide sugar epimerase, which may involve in stamen development in cucumber (*Cucumis sativus* L.)

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Abstract Sex determination is the most widely studied subject in cucumber. The sex of cucumber plants can be monoecious, hermaphrodite, gynoecious, androecious, or andromonoecious. Besides environmental factors, three major genes, *F*/*f*, *M*/*m*, and *A*/*a* mainly govern the sex types in cucumber. Regardless of their sex all floral buds are bisexual at the early bud stage. A stage specific arrest of either stamen or carpel leads to unisexual flower development. The possible downstream product of the interaction of the sex determining genes that may directly allow the growth or selectively arrest stamen or pistil is not yet identified. Therefore, in the current study, we performed suppression subtractive hybridization using floral buds from nearly isogenic gynoecious and hermaphrodite cucumber plants and identified for the first time a cDNA homologous to nucleotide sugar epimerase. The expression level of the isolated putative nucleotide sugar epimerase is weak in female floral buds but strong in bisexual and male flowers. The weak level of the putative nucleotide sugar epimerase may be an indication for its improper functioning, which may influence stamen development in cucumber plants.

Keywords Cucumber · *Cucumis sativus* · Flower development · Putative nucleotide sugar epimerase · Sex expression · Stamen development

Introduction

Floral induction and differentiation is perhaps the most significant developmental transition in the life cycle of higher plants. It is a step that directly affects the agricultural yield by determining the time of flowering, the number of flowers and fruits, as well as the diversion of resources from vegetative growth. Cucumber plants are originally monoecious but can be androecious, gynoecious, hermaphrodite, or andromonoecious. The embryonic flower bud has both stamen and ovary primordia, and all are thus morphologically bisexual at a very early bud stage (Atsmon and Galun 1960; Goffinet 1990; Perl-Treves 1999). Unisexual male flowers develop from the bisexual buds as a result of inhibition of carpel primordia as the stamens develop whereas unisexual female flowers form when stamen primordia are arrested as the carpels develop. There has been no evidence for programmed cellular death and there has been speculation that the organ primordia may simply cease to grow upon sexual differentiation of the bud (reviewed in Perl-Treves 1999).

The cucumber sex expression is mainly determined by three major genes, *F*/*f*, *M*/*m*, and *A*/*a* (Galun 1961; Shifriss 1961; Kubicki 1969a–d; Pierce and Wehner 1990; Malepszy and Niemirowicz-Szczytt 1991). The *F*/*f* gene regulates the degree of female flower expression, whereas the *M*/*m* gene controls bisexual flower expression (Galun 1961; Kubicki 1969d; Pierce and Wehner 1990). The recessive *m*-allele allows the formation of bisexual flowers; the dominant *M*-allele controls the selective arrest of stamens. Gene *A*/*a* is suppressed by the dominant *F*-allele, however the recessive *a*-allele intensifies the male tendency in *ff* genotypes (reviewed in Perl-Treves 1999). Thus the genotypes (*M*-*F*—) are gynoecious, (*M*-*ffA*-) are monoecious, (*mmF*—) are hermaphrodite, (*mmffA*-) are andromonoecious and (–*ffaa*) are androecious (reviewed in Tatlioglu 1993; Perl-Treves 1999). Although sex in cucumber plants is genetically controlled mainly by the above-mentioned three genes, it can also be modified by plant hormones and environmental conditions (Galun 1961). Ethephon (2-chloroethylphosphonic acid), auxins, short days, and low temperature promote femaleness, whereas $AgNO_3$, aminoethoxyvinyl-glycine (AVG), gibberellins, and other

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substances counteracting the ethylene effect promote maleness (reviewed in Malepszy and Niemirowicz 1991; Perl-Treves 1999). Amongst these sex modifying plant hormones, ethylene acts more directly on sex expression and is thought to be the most important sex hormone in cucumber (Yin and Quinn 1995).

Series of studies have been carried out to investigate the relationship between ethylene and the *F*/*f* gene in cucumber. Gynoecious cucumber plants produce more ethylene than monoecious plants (Rudich et al. 1972). The ethylene synthesis related gene, *CsACSl*, is found closely linked to the *F* locus (Trebitsh et al 1997). Recently, Mibus and Tatlioglu (2004) isolated the sequence of the promoter region of the *CsACS1G* postulated by Trebitsh et al. (1997) and confirmed as a dominant *F*-allele. It has also been shown that a second ethylene synthesis related gene, *CsACS2*, and ethylene receptor genes, *CsETR2, CsERS* are expressed more intensively in gynoecious than in monoecious plants (Kamachi et al. 2000; Yamasaki et al. 2000).

Considering ethylene as a sex hormone, a fixed female receptor on the ethylene scale, *F*/*f* gene to control the level of ethylene and *M*/*m* gene to control the sensitivity level of male receptors, Yin and Quinn (1995) explained the interaction between these genes to form the different sex types in cucumber. However, the possible downstream product of the interaction of these genes and ethylene that may directly allow the growth or selectively arrest stamen or pistil is not yet understood. In this investigation, we used cDNA subtraction on two nearly isogenic gynoecious and hermaphrodite cucumber lines in an attempt to find differentially expressed cDNA. To our knowledge here we report for the first time the difference in the transcript levels of putative nucleotide sugar epimerase gene between the two nearly isogenic cucumber lines within three genetic backgrounds and discuss the possible role of the nucleotide sugar epimerase gene in relation to stamen development in cucumber plants.

Materials and methods

Plant materials

Nearly isogenic gynoecious (*FFMMaa*) and hermaphrodite (*FFmmaa*) cucumber lines were drawn from a backcross program with three genetically different gynoecious lines as recurrent parents (Tatlioglu 1983). A minimum of ten backcrosses was made between hermaphrodite and gynoecious lines. Thus, gynoecious and hermaphrodite lines with about 99.8% identical genes were used for the studies described herein with three genetic backgrounds WrD, ECD, and ED. Additionally, monoecious (*ffMMA*-) and androecious (*ffMMaa*) cucumber lines from the three genetic backgrounds were used. Seeds were germinated on a 35 quick-pot plate at a temperature of 28°C. The seedlings were transferred to clay pot filled with Terreau Professional Gepac Einheitserde Typ T. Plants were grown at day/night temperatures of 24/18°C and 16 h of assimilation light with day light supplemented by fluorescent lamps (Son-T Agro 400, Philips Licht GmbH, Hamburg, Germany).

Chemical application

Gynoecious cucumber plants were treated with 300 ppm $AgNO_3$ at about 4-leaf stage to get male flowers. At the same time androecious cucumber plants were treated with 20 ppm ethephon (2-chloroethylphosphonic acid) to get female flower. The $AgNO_3$-induced male floral buds from gynoecious and ethephon-induced female floral buds from androecious cucumber plants were then used for RNA isolation. In addition the normally developed female floral buds from gynoecious and male floral buds from androecious cucumber plants, which were not affected by the chemical application, were also used for RNA isolation.

RNA and messenger RNA isolation

Samples were taken from floral buds of gynoecious, hermaphrodite, monoecious, and androecious cucumber plants. In addition, leaf tissues from gynoecious and hermaphrodite; $AgNO_3$-induced male floral buds from gynoecious and ethephon-induced female floral buds from androecious cucumber plants were used. Flower buds at about 1–2 mm stage and newly emerging leaves were harvested and immediately frozen in liquid nitrogen. The materials were then milled by using an oscillating mill (Retsch) under liquid nitrogen. RNA isolation was performed using 30 mg of the ground sample according to the RNA isolation kit (Machery and Nagel, Düren, Germany). The concentration of the RNA was estimated by using spectrophotometer (Pharmacia, MI, USA). About 250 μg of the isolated total RNA was used for the mRNA isolation by using mRNA isolation kit (Qiagen, Hilden, Germany). The mRNA isolated was also estimated by using spectrophotometer (Pharmacia, MI, USA).

Suppression subtractive hybridization analysis

Suppression subtractive hybridization (SSH) analysis was performed as described by Diatchenko et al. (1996) using the Clontech PCR-Select cDNA Subtraction Kit (BD Biosciences, USA). Complementary DNAs (cDNAs) from flower buds of nearly isogenic gynoecious (*FFMMaa*) and hermaphrodite (*FFmmaa*) cucumber plants were used as driver and tester and vice versa. The subtracted cDNAs were cloned by using TOPO TA Cloning kit (Invitrogen, UK). After cloning, the subtracted cDNA library was screened by dot blot analysis. Inserts were amplified by using standard T3/T7 primers. The PCR products were denatured in 0.2 M NaOH at

37°C for 15 min and manually dotted onto Hybond-N+ membrane (Amersham, Freiburg, Germany). The driver and tester cDNA probes were labeled by PCR using the DIG system (Roche, Penzberg, Germany) and the nested PCR primers (BD Biosciences, USA). The membranes were prehybridized in a hybridization glass tubes (Bachofer, Germany) containing 15 ml of DigEasyHyb (Roche, Penzberg, Germany) at 42°C for 30 min. The main hybridization took place overnight under the same conditions with 100 ng of denatured probe in 7 ml of DigEasyHyb. Posthybridization washes were performed twice for 5 min each in 2×SSC/0.1% SDS at room temperature, twice for 15 min each in 0.5×SSC/0.1% SDS at 65°C. Detection of the Dig-labeled probes was performed according to the instructions of the supplier, using CDP-Star (Amersham, Freiburg, Germany) as substrate. Then the membrane was exposed to X-ray film (Amersham, Freiburg, Germany) for about 1 h.

Complementary DNA sequencing and analysis

Plasmids for cDNA clones found to be up regulated were recovered using QiaPrep-Mini kit (Qiagen, Hilden, Germany) and sequencing was done by MWG-Biotech. The obtained cDNA sequences were analyzed by using the CLUSTAL W programme, European Bioinformatics Institute (EMBL; Thompson et al. 1994) and the BLUSTN programme, National Center for Biotechnology Information (NCBI; Altschul et al. 1997).

Semi-quantitative reverse transcriptase (RT)-PCR

Northern blot analysis revealed no hybridization signal. Therefore, we used semi-quantitative RT-PCR (Marone et al. 2001) for the subsequent expression study. Oligonucleotide primers (sense, 5′-ATTCATTCTCGGTCCATA-3′; antisense, 5′-GCCTCGCCGATCTTCTAC-3′) from subtracted cDNA sequence highly homologous to putative nucleotide sugar epimerase was designed and synthesized by MWG-Biotech. Semi-quantitative RT-PCR was performed by using 160 pg of total RNA. The reaction components were 400 µM of each dNTP, 0.6 µM of each sense and antisense primers, in 1×QIAGEN OneStep RT-PCR buffer and QIAGEN OneStep RT-PCR enzyme mix as recommended by the manufacturer, filled to a final volume of 50 µl with RNase-free water (OneStep RT-PCR Kit, Qiagen, Hilden, Germany). The following RT-PCR condition was used: 50°C for 30 min—reverse transcription, 95°C for 15 min—for activation of the DNA polymerase, and deactivation of the RT followed by 30 cycles of 94°C for 30 s, 58°C for 1 min and 72°C for 2 min, and a final extension of 72°C for 10 min. A primer pair, sense, 5′-GGCAGTGGTGGTGAACAT-′3; antisense, 5′-CTGGTATCGTGCTGGATT-′3, obtained from cucumber mRNA for actin (accession number AB010922) was used as internal control in the semi-quantitative RT-PCR. The obtained amplicons were fractionated on flatbed electrophoresis using 1.5% agarose gels in 1×TAE buffer and the sizes estimated by comparison to a 100-bp ladder (Amersham Pharmacia Biotech Inc., Chalfont, UK).

DNA extraction, digestion and Southern blot hybridization

Total DNA was isolated according to the method of Bentzen et al. (1990) modified by Engelke and Tatlioglu (2000). Twenty micrograms DNA from the nearly isogenic gynoecious and hermaphrodite cucumber lines were digested using 30 U of the randomly selected restriction enzymes *BamH*I, *Bgl*II, *Dra*I, *Eco*RI, *Eco*RV, *Hind*III, *Msp*I, *Pst*I, *Xba*I, *Bcn*I, *Hin6*I, *Hinf*I, *Mva*I, *Pae*I, *Sac*I, *Sal*I, *Rsa*I, *Xho*I, and *Bfa*I (MBI Fermentas Inc., Germany) according to the manufacturer instructions. The restriction fragments were separated by flatbed electrophoresis, using 0.8% agarose gels in 1×TAE buffer for 16 h (2 V/cm length of the gel). The DNA was cracked by soaking the gel in 0.25 M HCl for 10 min and denatured for two times 15 min in denaturation solution (0.5 M NaOH, 1.5 M NaCl). Following denaturation, the DNA was transferred to Hybond-N+ nylon membranes (Amersham, Freiburg, Germany) in 20×SSC, using a vacuum-system (Pharmacia: 60 mbar, 45 min). After cross-linking by irradiation with UV light, the membranes were rinsed with water and stored until hybridization. The cDNA probe highly homologous to putative nucleotide sugar epimerase was labeled by PCR using the DIG system (Roche, Penzberg, Germany) for non-radioactive hybridization. Plasmid-DNA containing the cDNA clone was amplified by using standard T3/T7 primers. Hybridization and detection procedures were performed as described above for the dot blot. The obtained fragments were estimated by comparison with the DNA Molecular Weight MarkerIII (Dig-labeled, 0.56–21.2 kb; Roche, 1 µg).

Results

Differentially expressed cDNA

A total of 178 cDNA clones were obtained by using SSH procedure. From the 178 putatively up-regulated cDNA clones, 21 were selected by dot blot analysis (Fig. 1). From the 21 cDNA clones, 11 were differentially expressed in the floral buds of hermaphrodite and the remaining ten in gynoecious plants (Table 1). Sequence analysis and database search for cDNA clone-38 (differentially expressed in hermaphrodite floral bud), indicated more than 90% homology on amino acid level to nucleotide sugar epimerase from *Arabidopsis thaliana* and other plant species (*Prunus armeniaca, Cicer arieti-*

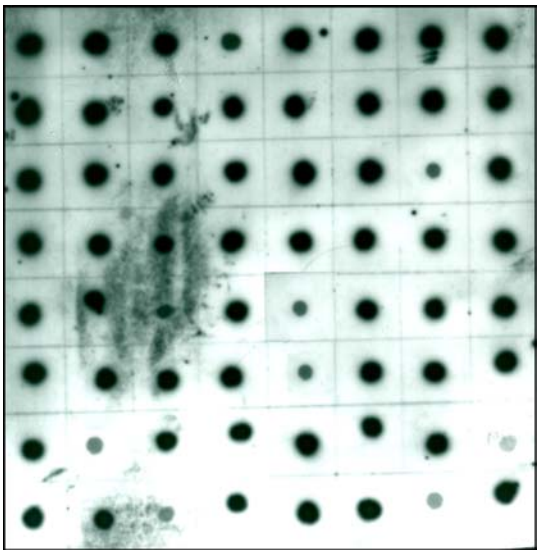

Fig. 1 Dot blot analysis of subtracted cDNA clones from floral buds of nearly isogenic hermaphrodite cucumber lines after hybridization with driver cDNA from floral buds of nearly isogenic gynoecious cucumber lines. Clones showing strong hybridization signals were classified as false-positives

num, Pisum sativum, Phragmites australis). This 416 bp cDNA from clone-38 and homologous to nucleotide sugar epimerase is hereafter named as cucumber putative nucleotide sugar epimerase.

Semi-quantitative RT-PCR

When 10 μg total RNA was used to study the level of gene expression by using Northern blot hybridization technique, no hybridization signal was detected in all the three genetic backgrounds, indicating that the transcript level of the putative nucleotide sugar epimerase is too weak. The expression level of the selected gene, putative nucleotide sugar epimerase was then analyzed by using semi-quantitative RT-PCR. Strongly expressed putative nucleotide sugar epimerase was detected in the floral buds of hermaphrodite cucumber; whereas, weak transcript level was detected in gynoecious lines, in all the three genetic backgrounds (Fig. 2a). However, identical transcript level of putative nucleotide sugar epimerase was detected in leaves of both gynoecious and hermaphrodite cucumbers (Fig. 2b).

Expression analysis of the putative nucleotide sugar epimerase on female and male floral buds obtained from a single monoecious (*ffMMA*-) cucumber plant showed weak transcript level in female floral bud and strong expression in male floral bud. This was confirmed in all the three genetic backgrounds (Fig. 3a).

Ethephon-induced female flower buds showed weak expression level of the putative nucleotide sugar epimerase, whereas, male flower buds showed strong expression level (Fig. 3b). After ethephon application some male flowers were still available and used as a control. A strong expression level of putative nucleotide sugar epimerase was still detected in the male flowers developed together with ethephon-induced female flowers (Fig. 3b). The $AgNO_3$-induced male flower buds showed strong expression level of putative nucleotide sugar epimerase (Fig. 3c). After $AgNO_3$ application some female flowers were still available and used as a control. A weak expression level of putative nucleotide sugar epimerase was detected in the female flowers developed together with the male flowers formed after $AgNO_3$ application (Fig. 3c).

Table 1 List of up-regulated cDNA clones selected by dot blot analysis and their size, and homology to the sequences available at the GenBank

Clones	Up-regulated in	Size (bp)	Homology
cl-3	Hermaphrodite	370	*A. thaliana* sugar transporter family protein (NM_116261)
cl-4	Hermaphrodite	390	*Trypanoplasma borreli* Tt-JH Mitochondrion apocytochrome b (U11684)
cl-6	Hermaphrodite	276	No clear homology
cl-7	Hermaphrodite	353	*Betula pendula* glutathione reductase (CAB66332)
cl-11	Hermaphrodite	290	*Brassica rapa* putative ubiquitin extension protein (BAA11389)
cl-12	Hermaphrodite	106	No homology
cl-21	Hermaphrodite	208	*Cucumis sativus* lipoxygenase mRNA (CSU36339)
cl-33	Hermaphrodite	404	*A. thaliana* putative calcium-dependent protein kinase (AY050981)
cl-38	Hermaphrodite	416	*A. thaliana* NAD-dependent epimerase/dehydratase family protein (NM_128436)
cl-49	Hermaphrodite	192	*A. thaliana* transducin family protein (NM_115199)
cl-52	Hermaphrodite	589	*C. sativus* lipoxygenase mRNA (AAA79186)
cl-57	Gynoecious	310	*Rattus norvegicus* similar to hexose-6-phosphate dehydrogenase precursor mRNA (XM_233688)
cl-66	Gynoecious	127	*A. thaliana* putative mitochondrial translation elongation factor G (AAD32833)
cl-78	Gynoecious	564	*Oryza sativa* (japonica cultivar-group), mRNA (XM_550078)
cl-83	Gynoecious	564	*O. sativa* (japonica cultivar-group), mRNA (XM_550078)
cl-90	Gynoecious	313	*R. norvegicus* similar to hexose-6-phosphate dehydrogenase precursor mRNA (XM_233688)
cl-98	Gynoecious	306	*R. norvegicus* similar to hexose-6-phosphate dehydrogenase precursor mRNA (XM_233688)
cl-110	Gynoecious	255	*A. thaliana* cDNA from flowers and buds (BX819336)
cl-115	Gynoecious	422	*Nicotiana tabacum* ethylene-induced protein kinase (T04125)
cl-118	Gynoecious	314	*R. norvegicus* similar to hexose-6-phosphate dehydrogenase precursor mRNA (XM_233688)
cl-128	Gynoecious	456	*Schizosaccharomyces pombe* ras-associated protein (T37921)

cDNA clones: cl-57, cl-90, cl-98, and cl-118 have identical sequences;
cl-78 and cl-83 have identical sequences

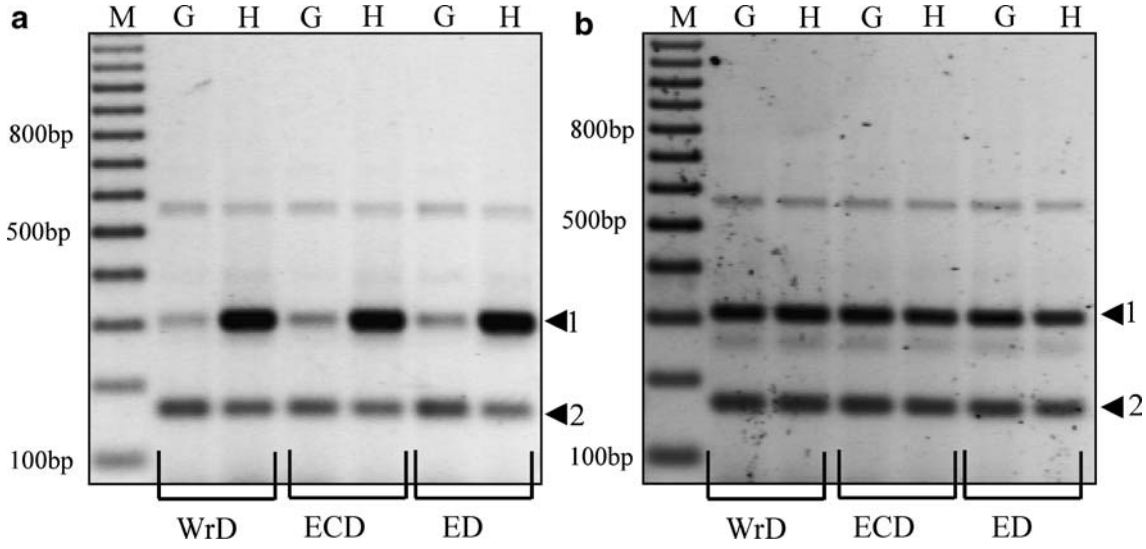

Fig. 2 Semi-quantitative RT-PCR analysis of putative nucleotide sugar epimerase gene on the floral buds and leaves of gynoecious and hermaphrodite cucumber lines from three genetic backgrounds, WrD, ECD and ED. **a** Total RNA obtained from floral buds (1–2 mm) of nearly isogenic gynoecious and hermaphrodite cucumber lines. **b** Total RNA obtained from leaves of nearly isogenic gynoecious and hermaphrodite cucumber lines. *G* gynoecious, *H* hermaphrodite, *M* 100 bp ladder. The *arrowhead 1* indicates putative nucleotide sugar epimerase (315 bp), the *arrowhead 2* indicates actin (167 bp) as internal control

Southern blot hybridization

To look for a possible polymorphism on genomic DNA level between the nearly isogenic gynoecious and hermaphrodite cucumber plants, we performed Southern blot hybridization by using 19 randomly selected restriction enzymes and the 416 bp cDNA for putative nucleotide sugar epimerase. However, no polymorphism was detected between the nearly isogenic gynoecious and hermaphrodite cucumber lines (Fig. 4). The restriction enzymes, *Bgl*II, *Mva*I, and *Xho*I, each resulted in three different restriction fragments (size between ca. 1.6 and 10 kb), which may indicate the existence of more than one copy of the putative nucleotide sugar epimerase.

Discussion

Nucleotide sugar epimerase families are involved in catalytic activity of nucleotide-sugar metabolism. For example the enzyme uridine 5′-diphosphate (UDP)-glucose 4-epimerase (UDP-glucose 4-epimerase) is used in the interconversion of UDP-glucose and UDP-galactose. UDP-galactose is needed for the synthesis of arabinogalactan-proteins (AGPs) and cell wall polysaccharides (reviewed by Reiter and Vanzin 2001). The AGPs are ubiquitous in plants and involved in controlling cell expansion (Willats and Knox 1996). The loss of UDP-glucose 4-epimerase function affects the morphology of root epidermal cells by interfering with the synthesis of AGPs (reviewed by Reiter and Vanzin 2001). Mutation in the UDP-glucose 4-epimerase gene of Arabidopsis cause bulging of the root epidermal cells (Schiefelbein and Sommerville 1990). Some of the UDP-glucose 4-epimerase isoform play important roles in specific cell types. For example the *ROOT HAIR DEFICIENT1* (*RHD1*) mutant of *Arabidopsis* carries a defect in the UDP-glucose 4-epimerase gene (reviewed by Reiter and Vanzin 2001). The weak transcript level of putative nucleotide sugar epimerase in female floral buds of cucumber plants might be the indication for improper functioning of the gene in this specific part of the cucumber plant. Such speculated improper functioning of the putative nucleotide sugar epimerase gene might affect cell expansion. The arrest in the development of stamen primordia in female flowers of cucumber plants may be due to the weak transcript level of putative nucleotide sugar epimerase. The anticipated proper functioning (strong expression level) of putative nucleotide sugar epimerase in male and bisexual flowers may enable the preformed stamen primordia continue to develop to sexual maturity. Previous studies have indicated that sex determination in cucumber is limited to specific floral whorl (Kater et al. 2001), yet the specific site is unknown. Recently Hao et al. (2003) detected DNA damage in cells of early primordial anther of female flowers. Such DNA damage could be part of the process in stamen arrest possibly caused by inadequate transcription level (improper functioning) of the putative nucleotide sugar epimerase gene in female flower buds as discussed above.

Interestingly, epimerase belongs to the superfamily of "short-chain" dehydrogenases (Baker and Blasco 1992; Holm et al 1994; Photon et al. 1997). The sex determination gene *TASSELSEED2* (*TS2*) of maize, that cause feminization of the tassel, belongs to the family of short-chain alcohol dehydrogenases (DeLong et al. 1993). Unlike the *TS2* of maize the putative nucleotide sugar epimerase isolated here for the first time from cucumber probably cause stamen arrest and lead to female flower. Although the anticipated role of the putative nucleotide

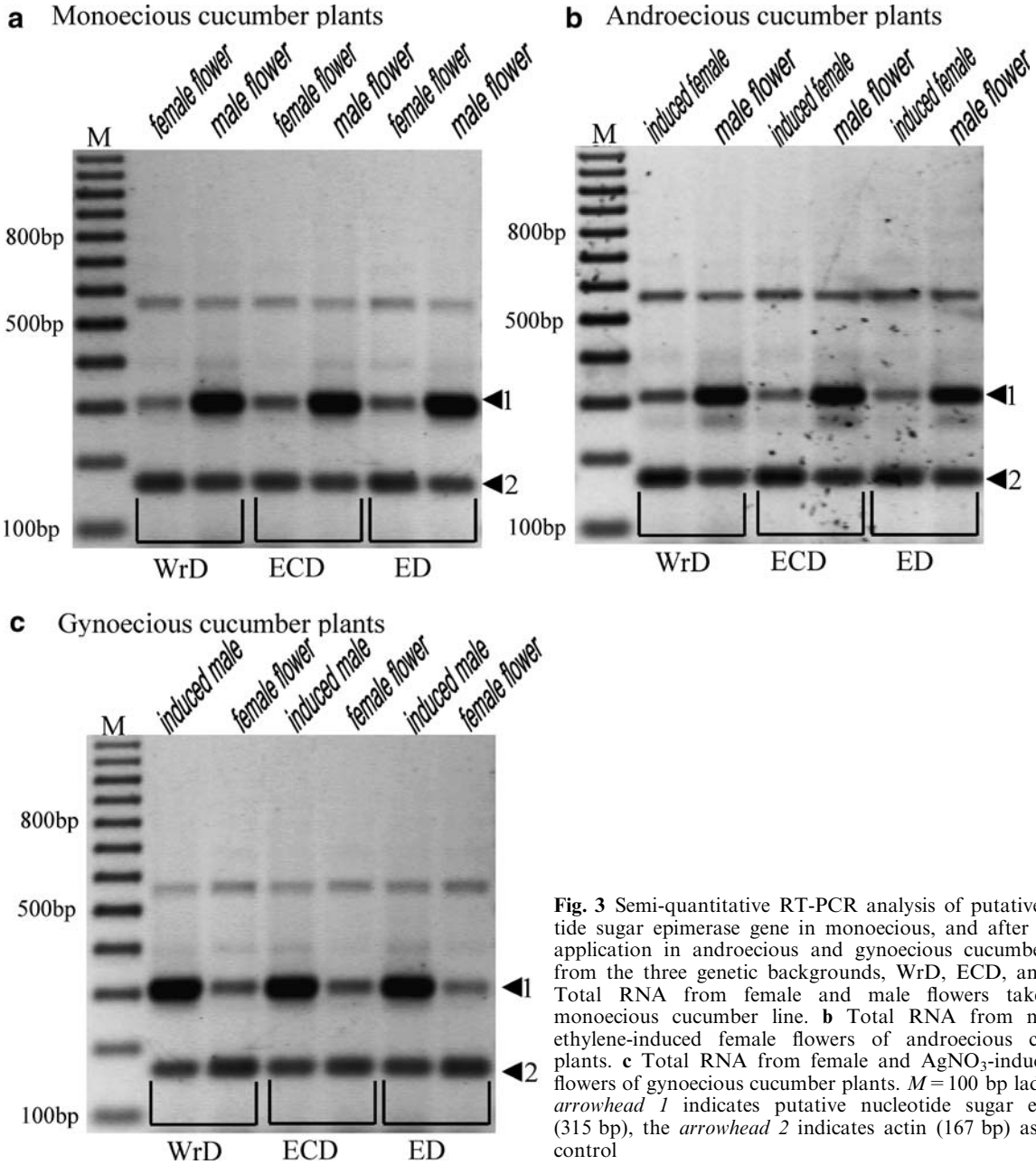

Fig. 3 Semi-quantitative RT-PCR analysis of putative nucleotide sugar epimerase gene in monoecious, and after chemical application in androecious and gynoecious cucumber plants from the three genetic backgrounds, WrD, ECD, and ED. **a** Total RNA from female and male flowers taken from monoecious cucumber line. **b** Total RNA from male and ethylene-induced female flowers of androecious cucumber plants. **c** Total RNA from female and $AgNO_3$-induced male flowers of gynoecious cucumber plants. $M = 100$ bp ladder. The *arrowhead 1* indicates putative nucleotide sugar epimerase (315 bp), the *arrowhead 2* indicates actin (167 bp) as internal control

sugar epimerase in cucumber is opposite to that of *TS2* in maize, it can be understood that gene families of short-chain dehydrogenase may be responsible for the arrest of either stamen (the case in cucumber) or pistil (the case in maize) primordia in plant species.

As suggested by Yin and Quinn (1995) if ethylene is indeed the sex hormone and the *F*-allele controls the level of ethylene production, speculation can be made for the possible role of *M*/*m* gene in controlling the expression level of putative nucleotide sugar epimerase in cucumber plants. In our nearly isogenic gynoecious (*FFMMaa*) and hermaphrodite (*FFmmaa*) cucumber lines, the *F*-allele is dominant in both plants and the expected ethylene level due to the *F*-allele is identical. The dominant *M*-allele may control the level of ethylene response in a specific floral part (somewhat in accordance to the sensitivity level of male receptor as explained by Yin and Quinn (1995). Through its effect on ethylene response the *M*-allele or its downstream products could negatively interfere with the expression level of putative nucleotide sugar epimerase in gynoecious floral bud and may lead to the arrest of stamen development. In monoecious cucumber plants in which first male flowers develop followed by a mixed male and female and at the later stage female flowers, the strength from the influence of the dominant *M*-allele or its downstream products may be plant growth-stage specific. The loss of the dominant *F*-allele could lead to lower level of ethylene, hence the expression level of putative nucleotide sugar epimerase remain normal and

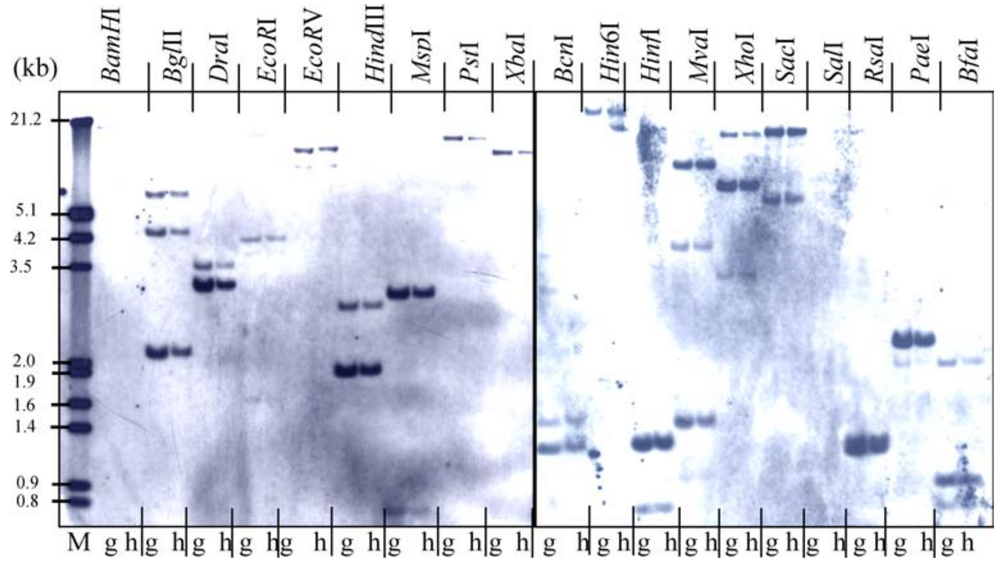

Fig. 4 Southern blot hybridization analysis of cucumber putative nucleotide sugar epimerase gene. Each lane contains ca. 20 μg genomic DNA isolated from nearly isogenic gynoecious and hermaphrodite cucumber lines subjected to digestion with 19 different randomly selected restriction enzymes. *M* DNA molecular weight marker III, *g* gynoecious, *h* hermaphrodite

male flowers continue to form from the beginning. As the monoecious plants continue to grow, the strength of the influence from the *M*-allele or its downstream products may increase and may slowly start to interfere with the expression level of putative nucleotide sugar epimerase. This could lead to the formation of mixed male and female flowers. At the later developmental stage of the plant, the strength of the influence from the *M*-allele or its downstream products may further increase and more intensively suppress the expression level of putative nucleotide sugar epimerase. This may then lead to the arrest of the stamen development and the formation of female flowers at the later developmental stage of the monoecious plants. It is difficult to explain the development of hermaphrodite and androecious cucumber sex-types in relation to the expression level of putative nucleotide sugar epimerase. Although, these two sex-types show different sex expression patterns, the expression level of the putative nucleotide sugar epimerase is strong and similar in the floral buds of these sex-types. For the occurrence of these sex-types, interactions between the putative nucleotide sugar epimerase and the genes *F*/*f* and/or *A*/*a* may be responsible. This speculation is very premature and further experiments are necessary to elucidate the relationship between the sex-determining genes and the expression level of the putative nucleotide sugar epimerase in cucumber plants.

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