Kasetsart J. (Nat. Sci.) 40 : 472 - 485 (2006)

DNA Sequences of Ca^{2+}-ATPase Gene in Rice KDML 105

Sakonwan Prasitwilai[1], Sripan Pradermwong[2], Amara Thongpan[3] and Mingkwan Mingmuang[1]*

ABSTRACT

Total RNA isolated from the leaves of KDML 105 rice was used as template to make complementary DNA (cDNA). Primer combinations of CA1-CA10 which were designed from *Lycopersicon esculentum* and *Arabidopsis thaliana* Ca^{2+}-ATPase gene sequence were used. The nucleotide sequences amplified from 7 primer combinations gave 2,480 bp (fragment A). This fragment was found to be 99% homology to that of the putative calcium ATPase of *Oryza sativa* (Japonica cultivar-group) as shown in the GenBank. Amplification of 3′ end fragment done by Rapid Amplification of cDNA Ends (3′RACE) technique using 3′GSP1, 3′GSP2, and 3′UAP as primers gave a DNA fragment of 933 bp having an overlapping region with DNA fragment A, which resulted in the combined length of 2,944 bp (fragment B). To find the sequence of 5′ end, 5′RACE technique was used having 5′GSP1, 5′GSP2, 5′ AP and 5′UAP as primers. It gave a DNA fragment of 473 bp showing an overlapping region with DNA fragment B, which ultimately resulted in the combined length of 3,331 bp (total CA). The deduced 1,008 amino acid sequence of total CA showed 99% homology to putative calcium ATPase of *O. sativa* (Japonica cultivar-group) cv. Nipponbare. The higher percent homology of this Ca^{2+}-ATPase gene in KDML105 to that of *O. sativa* cv. Nipponbare (99%) than to *O. sativa* cv. IR36 (89%) was not as anticipated since both KDML105 and *O. sativa* cv. IR36 belong to the same Indica type while *O. sativa* cv. Nipponbare is a Japonica. Analysis of the amino acid sequences of Ca^{2+}- ATPase gene indicated that this gene in KDML105 most likely belonged to ER type IIA group.

Key words: KDML 105, cDNA, Ca^{2+}-ATPase gene, DNA sequences

INTRODUCTION

"Khao Dok Mali 105" (KDML 105) is a cultivated Asian rice of *Oryza sativa* L. group. This cultivar grows well in tropical region and is famous for its pleasant jasmine-like aroma with soft texture when cooked. The high demand in the international market results in its high price and preferred cultivar for growth all over the country. However, a vast majority of the rice-growing land in northeastern Thailand is high salinity soil. Salt tolerance KDML 105 is, therefore, an ideal and most prevalent cultivar for rice improvement projects all along. Testing and selecting different rice tolerant cultivars have been carried out (Vaidyanathan *et al*., 1999; Ohta *et al.*, 2002) while

1 Department of General Science, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand.
2 Department of General Science, Faculty of Science and Technology, Nakhon Si Thammarat Rajabhat University, Nakhon Si Thammarat 80000, Thailand.
3 Department of Genetics Science, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand.
* Corresponding author, e-mail: fscimkm@ku.ac.th

Received date : 12/10/05 Accepted date : 06/02/06

genes controlling the homeostasis of ions especially the levels of Ca^{2+}, Na^{+}, K^{+} in plants are also reported (Blumwald *et al.*, 2000; Zhu, 2001). It is known that Ca^{2+} content in cytoplasm is maintained at specific range of only 10^{-7}–10^{-6} M (Rasi-Caldogno *et al*., 1987). Changes in Ca^{2+} cause a wide variety of mechanism changes in the cell while Ca^{2+}-ATPase is needed at this critical period to eliminate the surplus of Ca^{2+} to restore the cellular homeostasis (Briskin, 1990; Bush, 1993). Ca^{2+}-ATPase gene and its expression is, therefore, an indicator of the plant's adjustment to high salt condition. To fully understand this Ca^{2+}-ATPase gene and its role in salt tolerant rice, attempts are made to determine the level of expression in several salt tolerant plants (Wimmers *et al.*, 1992; Kawasaki *et al.*, 2001). This project aimed at finding the DNA sequences of Ca^{2+}-ATPase gene in KDML 105 which had not been reported before. The finding would give a better understanding on the mechanism of salt tolerance in rice enabling to make a feasible plan for development of salt tolerance KDML 105.

MATERIALS AND METHODS

Plant materials

KDML 105 seeds (*Oryza sativa* L.) from Pathumthani Rice Research Center were germinated in a pot containing sandy soil impregnated with Hoagland solution (Hoagland and Arnon, 1950) and kept at room temperature for two weeks. Plantlets were transferred and hydroponically grown for a week in Hoagland solution. The leaves were then collected for RNA extraction.

Isolation of total RNA

Total RNA was extracted from the leaves using guanidinium isothiocyanate according to the method described by Chomczynski and Sacchi (1987) with some modifications. Approximately 1 g of leaves was ground in liquid nitrogen and suspended in a 10 ml buffer solution containing 4M guanidinium isothiocyanate, 25mM sodium citrate, 0.5% sarkosyl, and 0.1M β-mercaptoethanol. One millilitre of 2M sodium acetate (pH 4.0), 10 ml of phenol and 2 ml of chloroform: isoamyl alcohol (24:1 v/v) were added to the sample and vortexed, followed by centrifugation at 3,500 g for 10 min. The supernatant was collected and mixed with an equal volume of cold absolute isopropanol. DNA and RNA were allowed to precipitate at –20 °C for 1 h, followed by centrifugation at 11,000 g for 10 min. The pellet was washed once using 1 ml of cold 70% ethanol and centrifuged at 5,000 g for 5 min. It was then dissolved in 500 μl of 0.1% DEPC-treated water and added with 500 μl of 6M LiCl. The solution was left at 4 °C overnight, followed by centrifugation at 11,000 g for 10 min. The precipitated RNA pellet was washed again in cold 70% ethanol as described above and dissolved in 0.1% DEPC-treated water. The purity and concentration of total RNA was determined using spectrophotometer at 260 and 280 nm. RNA was stored at –80 °C until use.

cDNA synthesis

First strand cDNA was synthesized from total RNA by the process of reverse transcription. Approximately 5 μg of RNA was mixed with 0.5 μg of oligo $(dT)_{12\text{-}18}$ primer and adjusted to the volume of 12 μl with distilled water. The mixture was heated at 70 °C for 10 min and quickly cooled on ice. Superscript II $RNaseH^{-}$ kit solution (Invitrogen) containing 4 μl of 5X first strand buffer, 2 μl of 0.1M dithiothreitol and 1 μl of reverse transcriptase (200U) was added to the sample together with 1 μl of 10mM dNTP. Then the mixture was incubated at 42 °C for 50 min. The reaction was terminated by incubation at 70 °C for 15 min. It was kept at –20 °C until use.

Primers design

Ten specific oligonucleotide primers were designed based on the known Ca^{2+}-ATPase gene sequences of other organisms provided in the

GenBank (http://www.ncbi.nlm.nih.gov) and used to synthesize the sequence of Ca^{2+}-ATPase gene of KDML105 rice (Table 1).

RT-PCR

cDNA was used as the template for PCR amplification with the designed primers of different combinations. The reaction mixture contained 0.2mM dNTP, 0.1 μM of forward and reverse primers and 2.5U of *Taq* DNA polymerase. PCR was carried out at 94°C for 3 min for a single cycle, 94°C for 30 s, 52°C for 1 min and 72°C for 1 min for 30 cycles, followed by a single cycle of 72°C for 5 min. The PCR products were analyzed on 1% agarose gel electrophoresis and stained with ethidium bromide.

Cloning of the amplified products

The PCR products were selected and purified using QIA quick gel extraction kit (QIAGEN), then inserted into pGEM-T Easy Vector System (Promega) and transformed into *Escherichia coli* DH5a bacteria as described by Sambrook *et al.* (1989). Transformed colonies were screened for plasmid using X-gal color system.

DNA sequencing and analysis

Double-strand plasmid DNA was sequenced using the dideoxynucleotide chain termination method done at Bioservice Unit, National Science and Technology Development Agency, Thailand. DNA sequences were analyzed using the following public software package online: http://www.ddbj.nig.ac.jp for homology or similarity search, http://www.ebi.ac.uk for multiple sequence alignment, and http://dot.imgen.bcm.tmc.edu:9331 for possible six-frame reading of amino acid. The sequence was used to design gene specific primers (GSP) to obtain the 3′-end of Ca^{2+}-ATPase gene.

3′-end amplification of cDNAs

The 3′RACE was done by using the method of Frohman *et al.* (1988). The first strand of cDNA was synthesized using 5 μg of total RNA, 3′ adapter primer (3′AP) and Superscript II $RNaseH^-$ reverse transcriptase according to cDNA synthesis method as previously described. Two forward gene specific primers, 3′ GSP1 and 3′ GSP2, were designed from previously identified sequence of Ca^{2+}-ATPase gene. Two rounds of PCR were performed using two different forward primers. The first round PCR was done with 3′ GSP1, gene specific primer and 3′ UAP, an adapter primer, whereas the second PCR was done with 3′ GSP2, gene specific primer and 3′ UAP primer. First PCR reaction was performed in the solution containing 2 μl of cDNA template, PCR buffer, 1.5mM $MgCl_2$, 0.2mM dNTP, 0.1 μM of each primer and 2.5U *Taq* DNA polymerase (Invitrogen) at the final volume of 50 μl. The first PCR products were used as the template for the second PCR amplification. The second round of PCR was carried out for 30 cycles: at 94 °C for 30 s, at 52 °C for 1 min, at 72 °C for 1 min for each cycle, followed by a final extension at 72 °C for 7 min. The PCR products were electrophoresed on a 1% agarose gel. Then it was purified, cloned and sequenced as described above.

5′-end amplification of cDNAs

The 5′RACE was performed according to the method described by Frohman *et al.* (1988). The first strand cDNA was synthesized using 5 μg of total RNA, gene specific primer and Improm-II™ reverse transcriptase (Promega). To eliminate strong RNA secondary structure, partial heat denaturation reverse transcription method of Huttemann (2002) was used. The first stranded cDNA was purified by QIAquick PCR purification kit (QIAGEN). Poly-A tail was added to the 3′ end in the solution containing 25mM dATP, TdT buffer and 1 μl of terminal deoxynucleotidyl transferase (TdT) (Promega). Two reverse gene specific primers, 5′GSP1 and 5′GSP2 were designed from the previously identified sequence of Ca^{2+}-ATPase gene of KDML105. Two rounds

of PCR were performed using different primer pairs. The first round PCR was done using 5′GSP1, a gene specific primer and oligo(dT) primer (5′ AP). Then PCR product was diluted to 1:50 (v/v) with deionized water and subjected to the nested PCR performing with 5′GSP2, a gene specific primer and 5′UAP, an adapter primer. PCR reactions were carried out in the solution containing 1 µl of cDNA template, PCR buffer, 2mM $MgCl_2$, 0.2mM dNTP, 0.4 mM of each primer and 1.25 U of *Taq* DNA polymerase (Invitrogen) at the final volume of 50 µl. To decrease a strong secondary structure in the 5′ end mRNA, DMSO (dimethyl sulfoxide) was added to the reaction mixture at the final concentration of 4% (v/v). To increase annealing specificity, combination of hot start and touch down PCR was used. At first, hot start PCR was carried out by adding *Taq* DNA polymerase after incubation at 94°C for 5 min, followed by a touch down PCR in which annealing temperature was decreased at 1° C per cycle from 59-54°C for the first six cycles. Then PCR amplification for 30 cycles was done by denaturation at 94°C for 30 s, annealing at 53° C for 30 s and extension at 74°C for 2 min, followed by a final extension at 74°C for 7 min. The nested PCR was done under the same condition except in the touch down step where the annealing temperature decreased from 60-55°C in the first six cycles, followed by the annealing temperature at 54°C for the PCR amplification in the next 30 cycles. The PCR product was analyzed on a 2% agarose gel. Then, it was purified, cloned and sequence as previously described.

RESULTS AND DISCUSSION

To find the appropriate primers for the amplification of DNA sequence of the Ca^{2+}-ATPase gene in KDML105, primers were designed from the Ca^{2+}-ATPase sequence of tomato, *L. esculentum*, and *Arabidopsis thaliana* (Table 1). First strand cDNA, resulting from the reverse transcribe of total RNA, was then amplified with 7 selected primer combinations. Figure 1 shows RT-PCR products of Ca^{2+}-ATPase gene on 1% agarose gel ranging from 550 bp using CA1+CA5 primers to as high as 1,800 bp using CA2+CA7 primers (Table 2). These bands correlated well with the expected size of Ca^{2+}-ATPase sequences found in *O. sativa* cv. IR36 (Chen *et al.*, 1997). The products were gel eluted, purified and sequenced. Sequence analysis of the cloned products of Ca^{2+}-ATPase gene revealed several overlapping regions (Figure 2). They were aligned to construct a continuous DNA sequence which gave a combined length of 2,480 bp (fragment A). The deduced amino acid sequences of fragment A was blasted in GenBank database by blastX (translated query vs. protein database). The amino acid sequences

Table 1 Primers used for cloning of Ca^{2+}-ATPase gene sequences.

Primer code	Primer sequence (5′ → 3′)
CA1 (forward)	GACAAGGTCCCGGCAGACATG
CA2 (forward)	GGAACATTGACCACCAACCAGATG
CA3 (reverse)	CGATCGAACTCCAGAGTAGCTAC
CA4 (forward)	GATGGATCTGTTGTGCTCCTGGA
CA5 (reverse)	GCTCCATCACCAGTCATAGCAAC
CA6 (forward)	TGGGTCAACCTCGTGACAGATG
CA7 (reverse)	CGAGGAACGGCACGTAGAGGAT
CA8 (forward)	AGTGGCCAAGTTTGAGCATG
CA9 (reverse)	TTATGTTTCGGCTCAGCCCT
CA10 (reverse)	GCAATCCCAACGTACATCCC

of KDML105 Ca^{2+}-ATPase in this fragment was found to be highly homology with putative calcium ATPase of *O. sativa* (Japonica cultivar-group) cv. Nipponbare, Ca^{2+}-ATPase of *O. sativa* cv. IR36 (Chen *et al*., 1997) and ER-type calcium pump protein from the *A. thaliana* (Liang *et al*., 1997) at 99%, 89% and 80%, respectively. The higher percent homology of this Ca^{2+}-ATPase gene in KDML105 to that of *O. sativa* cv. Nipponbare (99%) than to *O. sativa* cv. IR36 (89%) was contrary to our expectation since both KDML105 and *O. sativa* cv. IR36 belonged to the same Indica type while *O. sativa* cv. Nipponbare was a Japonica.

First strand cDNA at 3′ end was synthesized from total RNA of KDML105 rice using 3′AP (5′-GGCCACGCGTCGACTAG $TACT_{16}$-3′) as primer. The synthesized cDNA was then amplified by PCR method having 3′GSP1 (5′-CACGAAGGAGTGATGACTCG-3′) and 3′UAP (5′-GGCCACGCGTCGACTAGTAC-3′) as primers. PCR product was found to contain a DNA

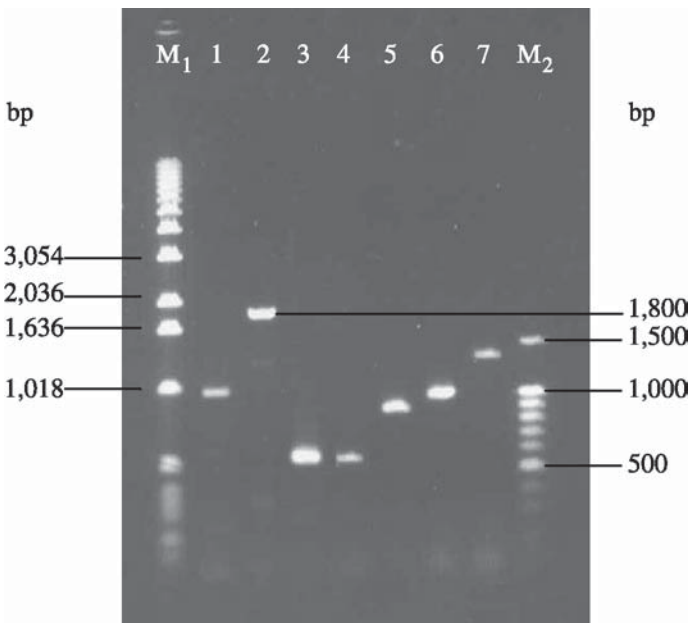

Figure 1 RT-PCR products of total RNA from KDML105 using seven selected combinations of primer: lane1 CA1+CA3, lane2 CA2+CA7, lane3 CA4+CA5, lane4 CA6+CA7, lane5 CA8+CA9, lane6 CA4+CA10 and lane7 CA8+CA10; M_1=DNA ladder 1 kb, M_2= DNA ladder 100 bp.

Table 2 Expected cDNA size and PCR product size of Ca^{2+}-ATPase gene.

Primer combination	Expected size (bp)	PCR product size (bp)
CA1+CA3	1,000	1,000
CA2+CA7	1,800	1,800
CA4+CA5	520	550
CA6+CA7	530	550
CA8+CA9	900	900
CA4+CA10	980	980
CA8+CA10	1,300	1,300

band of 990 bp which was equal in length to the 3′ end of Ca^{2+}-ATPase sequences of *O. sativa* cv. IR36 as expected (Figure 3, lane 1). To confirm the reproducibility of this DNA product, the first PCR product of 990 bp was amplified one more time using 3′GSP2 (5′-ACGTTGGGATTGC CACAGTG-3′) and 3′UAP as nested PCR primers. The DNA band from this second amplification was found to be similar but smaller in size (900 bp, Figure 3, lane 2) than the first one which was validated by the finding of Frohman *et al*. (1988). These two DNA bands were cloned and their base sequences were analyzed. The sequences of 3′ RACE1 and 3′RACE2 amplified DNAs were found to be almost identical and superimposed in every single base position which added to a total length of 933 bp. The deduced amino acid sequences of 3′RACE was also blasted in GenBank database blastX (translated query vs. protein database). The results showed a high amino acid sequence identity with putative calcium ATPase of *O. sativa* cv. Nipponbare, P-type ATPase of the barley, *Hordeum vulgare* and Ca^{2+}-ATPase of *O. sativa* cv. IR36 at 99%, 93% and 88%, respectively. Again, the 3′-end sequence of Ca^{2+}-ATPase gene of KDML105 was found to be higher homology to *O. sativa* cv. Nipponbare (99%) of Japonica type and even to the barley (93%) than to its own Indica type of *O. sativa* cv. IR36 (88%).

Fragment A of the previous amplification was found to have an overlapping segment with 3′RACE amplified DNA which resulted in a continuous alignment of 2,944 bp (fragment B) as shown in Figure 4. The deduced amino acid sequences of fragment B was blasted in GenBank database blastX (translated query vs. protein database). The results showed a high amino acid

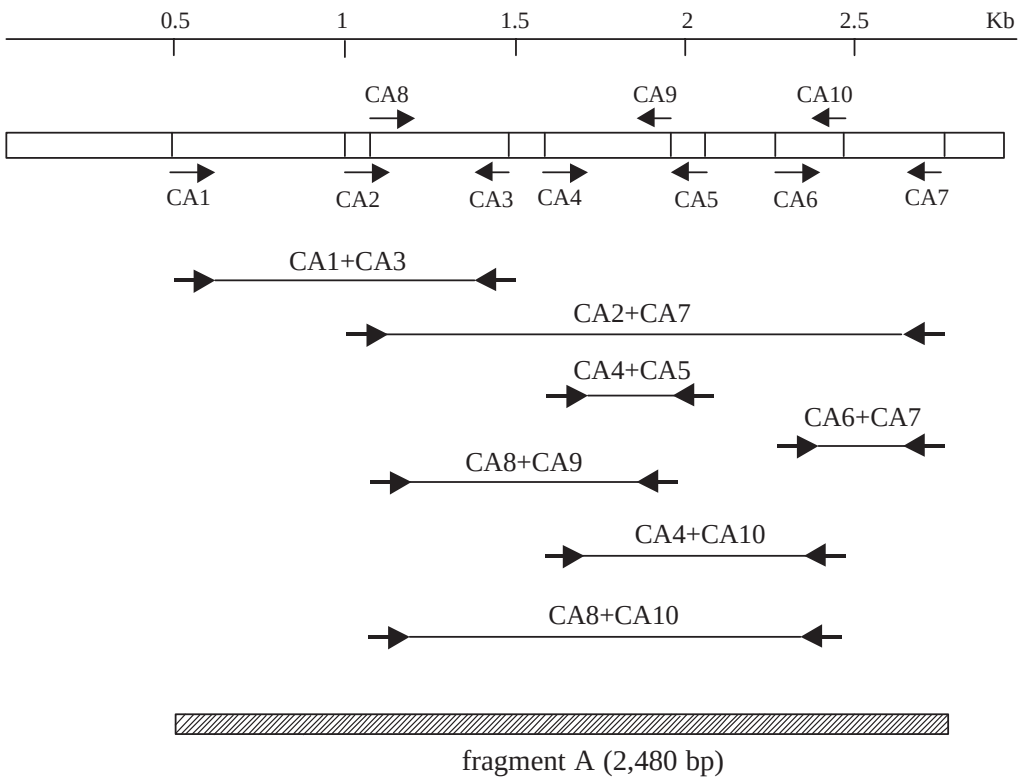

Figure 2 Diagram of PCR products of Ca^{2+}-ATPase gene produced by each primer combination showing the overlapping regions of cDNA. The total alignment resulted in the combined fragmentA of 2,480 bp long.

sequence identity with putative calcium ATPase of *O. sativa* cv. Nipponbare, Ca^{2+}-ATPase of *O. sativa* cv. IR36 and ER-type calcium pump protein from *A. thaliana* at 99%, 88% and 79%, respectively.

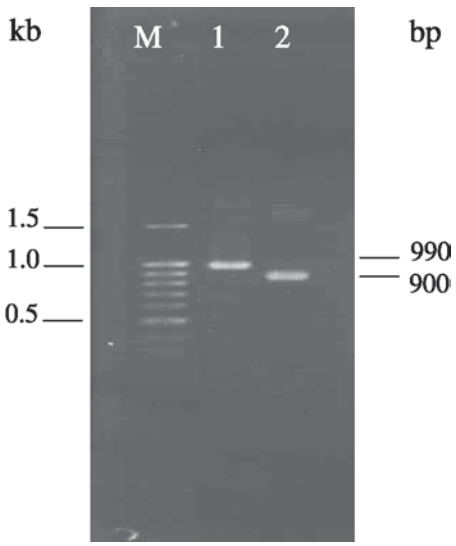

Figure 3 3′RACE RT-PCR products of KDML105 amplified by 3′GSP1+3′ UAP primer (lane1) and 3′GSP2+3′ UAP primer (lane 2); M=DNA ladder 100 bp.

For 5′ end of this Ca^{2+}-ATPase gene, first strand cDNA was synthesized from total RNA using 5′GSP5 (5′-GGAGCACAACAGATCCA TCAAGCA-3′) as gene specific primer. The reverse transcribed cDNA that added poly-A tail at 3′-end (which correspond to the 5′-end of the mRNA) was amplified having 5′GSP1 (5′-GCTGGTCTTGTTAACCGAAG-3′) and 5′AP (5′-CCGGAATTCAAGCTTCTAGAGGATCC T_{16}-3′) as primers. Then the first PCR product was diluted and subjected to the nested PCR using 5′ GSP2 (5′-AGTCTCACCAGTGAGGGAAC-3′) and 5′UAP (5′-CCGGAATTCAAGCT TCTA GAGGATCCT-3′) as primers. Electrophoresis of PCR product showed several bands of cDNA fragment ranging from 350-500 bp (Figure 5). The largest fragment of about 500 bp was selected to be purified and cloned into pGEM-T easy vector. Six clones harboring 5′-end cDNA were sequenced and gave 455-473 bp long (not included the added poly-A tail). Sequence alignment showed that these 5′ cDNA-end shared 99% nucleotide sequence identity and had a total length of 473 bp. The deduced amino acid sequences of 5′RACE was blasted in GenBank database blastX (translated query vs. protein database) and showed

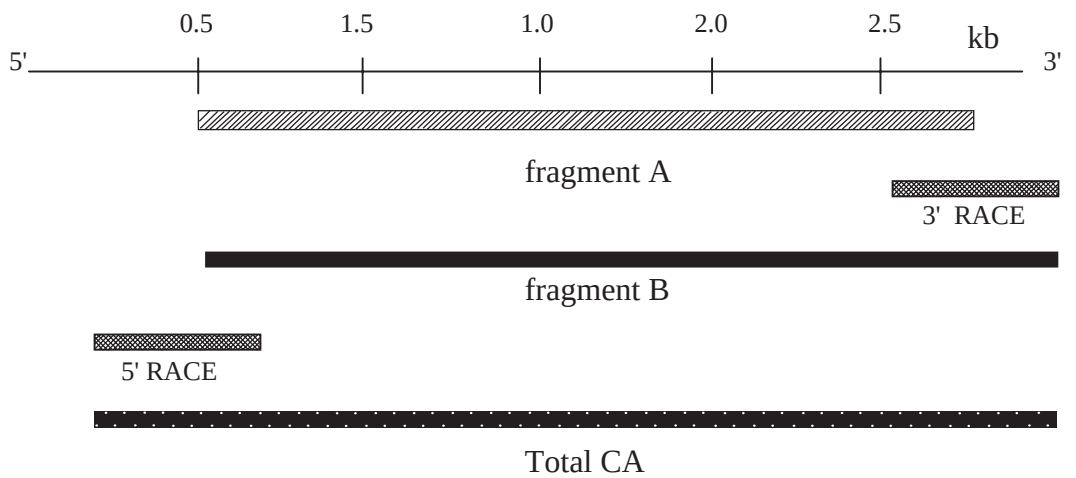

Figure 4 Diagram showing the alignment and overlapping ends of fragment A (2,480 bp) with 3′ RACE (933 bp) to be combined to a continuous fragment B of 2,944 bp and overlapping of fragment B (2,944 bp) with 5′RACE (473 bp) to be combined to a continuous total CA of 3,331 bp.

to have 98%, 80% and 76% homology to putative calcium ATPase of *O. sativa* cv. Nipponbare, Ca^{2+}-ATPase of *O. sativa* cv. IR36 and ER-type calcium pump protein from *A. thaliana*, respectively. Similar to what were seen at 3′-end, the 5′-end sequence of Ca^{2+}-ATPase gene of KDML105 was highly homology to *O. sativa* cv. Nipponbare (98%) of Japonica type. The sequence also showed GC-rich at 5′-end. However, the high GC region could make a rather stable secondary structure formation of RNA, thereby the cDNA synthesis was aborted leading to shorter clones (Moreau *et al*., 1994) which prevented it from being extended to the end of 5′mRNA where start codon and promoter were located.

Fragment B of the previous amplification was found to have an overlapping with 5′RACE amplified DNA which resulted in a continuous alignment of 3,331 bp (total CA) as shown in Figure 4. The deduced 1,008 amino acid sequences (Figure 6) were blasted in GenBank database by

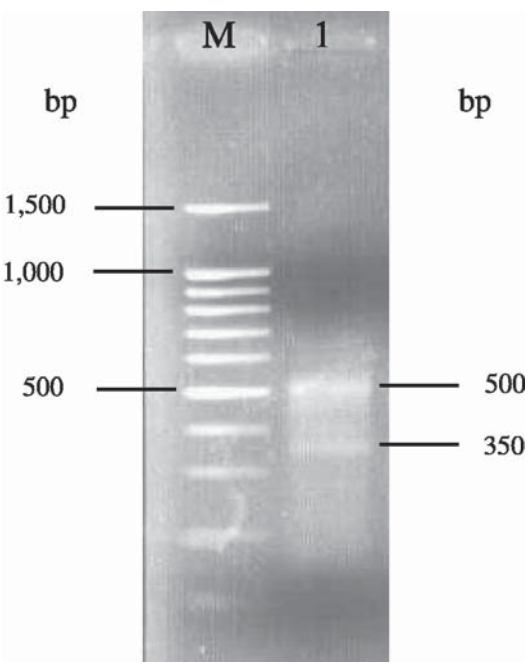

Figure 5 5′RACE nested RT-PCR product of KDML105 amplified by 5′GSP2+5′ UAP primer (lane1); M=DNA ladder 100 bp.

blastp (protein-protein blast) and was found to be 99% homology to putative calcium ATPase of *O. sativa* cv. Nipponbare, 87% to Ca^{2+}-ATPase of *O. sativa* cv. IR36 and 79% to ER-type calcium pump protein from *A. thaliana*.

It was interesting to consistently find the higher percentage of homology in all the fragments of this Ca^{2+}-ATPase gene in KDML105 than that of *O. sativa* cv. Nipponbare even they belonged to different groups of rice type. The differences in amino acid sequences between KDML105 and *O. sativa* cv. IR36 were observed at six locations (Figure 7, Dif 1-6). However, these six specific locations were found to be similar among KDML105, putative calcium ATPase of *O. sativa* cv. Nipponbare, ER-type calcium pump protein from *A. thaliana* and Ca^{2+}-ATPase of *L. esculentum*. Moreover, there were other two respective sites of 431-444 bp and 997-1,008 bp which shared the same amino acid sequences among KDML105 and *O. sativa* cv. Nipponbare. As for the 3′ carboxyl terminal end of Ca^{2+}-ATPase gene, it was found that amino acid sequences of KDML105 were KRKED which was generally found in ER type IIA and was reported to be in the form of (K/X)(K/X)KXX-stop (Jackson *et al.*, 1990).

In addition to the percent homology of Ca^{2+}-ATPase gene among rice cultivars, Geisler *et al*. (1999) also reported on the ten putative transmembrane domains found in *O. sativa* cv. IR36, *L. esculentum*, and *A. thaliana* which corresponded to the locations of M1-M10 in KDML105 (Figure 7). These findings indicated the existence of M1-M10 and the phosphorylation site of DKTGTLT (Figure 7, overlined and P) as well as the conserved amino acids at the transmembrane region of M4, M5, M6 and M8 (Figure 8, boxed and number). These amino acids are known to be used as calcium binding sites as specified by its high affinity to calmodulin (Williams *et al*., 1990). The overall results of common finding in functional locations of Ca^{2+}-ATPase gene in other related organisms suggested

>TOTAL_CA

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GCGGCGCGGCTGCGGCGGTACGGGCCCAACGAGCTGGAGCGGCACGCAGCGCCGTCGGTGTGGAAGCTGGTG 72
A  A  R  L  R  R  Y  G  P  N  E  L  E  R  H  A  A  P  S  V  W  K  L  V
CTCGAGCAGTTCGACGACACGCTCGTGCGCATCCTCCTCGCCGCCGCCGTGGTGTCGTTCGTGCTCGCGCTC 144
L  E  Q  F  D  D  T  L  V  R  I  L  L  A  A  A  V  V  S  F  V  L  A  L
TACGACGGTGCGGAGGGCGGCAAGGTCGGGGCGACCGCCTTCGTGGAGCCCCTCGTCATCTTCCTCATCCTG 216
Y  D  G  A  E  G  G  K  V  G  A  T  A  F  V  E  P  L  V  I  F  L  I  L
ATAGTGAACGCCGTCGTCGGGGTCTGGCAGGAAAGCAACGCCGAGAAGGCGCTCGAGGCGCTGAAGGAGATC 288
I  V  N  A  V  V  G  V  W  Q  E  S  N  A  E  K  A  L  E  A  L  K  E  I
CAGTCCGAGCACGCAACCGTCAAGCGCGACGGCCGGTGGTCCCATGGGCTTCCGGCGCGCGACCTCGTCCCC 360
Q  S  E  H  A  T  V  K  R  D  G  R  W  S  H  G  L  P  A  R  D  L  V  P
GGAGACATCGTGGAGCTCCGTGTTGGCGACAAGGTCCCGGCAGACATGCGTGTGCTCCAGCTTATCAGCTCC 432
G  D  I  V  E  L  R  V  G  D  K  V  P  A  D  M  R  V  L  Q  L  I  S  S
ACCCTCCGTGTCGAGCAGGGTTCCCTCACTGGTGAGACTGCTTCGGTTAACAAGACCAGCCATAAAATCGAG 504
T  L  R  V  E  Q  G  S  L  T  G  E  T  A  S  V  N  K  T  S  H  K  I  E
CTGGAGGACACAGACATCCAGGGAAAGGAGTGTATGGTTTTTGCTGGCACTACAATTGTCAACGGCAGCGCT 576
L  E  D  T  D  I  Q  G  K  E  C  M  V  F  A  G  T  T  I  V  N  G  S  A
GTCTGTGTTGTGACGGGCACTGGAATGGACACTGAAATAGGCAAGATCCATGCCCAGATCCAGGAAGCCTCA 648
V  C  V  V  T  G  T  G  M  D  T  E  I  G  K  I  H  A  Q  I  Q  E  A  S
CAAGAGGAAGATGACACGCCACTGAAAAAGAAGCTCAATGAGTTTGGGGAGGCACTGACAGCCATAATTGGG 720
Q  E  E  D  D  T  P  L  K  K  K  L  N  E  F  G  E  A  L  T  A  I  I  G
GTGATCTGTGCCTTGGTTTGGCTTATCAACGTTAAGTATTTCCTCACCTGGGAGTATGTGGATGGGTGGCCC 792
V  I  C  A  L  V  W  L  I  N  V  K  Y  F  L  T  W  E  Y  V  D  G  W  P
AGGAATTTTAAGTTCTCATTTGAGAAGTGCACATATTACTTTGAGATTGCAGTGGCACTGGCTGTTGCAGCA 864
R  N  F  K  F  S  F  E  K  C  T  Y  Y  F  E  I  A  V  A  L  A  V  A  A
ATTCCAGAGGGCCTGCCTGCTGTTATCACCACATGCTTGGCGCTCGGCACCAGGAAGATGGCACAAAAGAAT 936
I  P  E  G  L  P  A  V  I  T  T  C  L  A  L  G  T  R  K  M  A  Q  K  N
GCACTTGTGAGGAAGTTGCCAAGTGTTGAGACACTGGGTTGCACTACAGTGATTTGCTCCGATAAGACAGGA 1008
A  L  V  R  K  L  P  S  V  E  T  L  G  C  T  T  V  I  C  S  D  K  T  G
ACATTGACCACCAACCAGATGTCAGCGGTGAAGCTTGTGGCAATTGGGAGGTGGCCTGATACACTTAGGAGC 1080
T  L  T  T  N  Q  M  S  A  V  K  L  V  A  I  G  R  W  P  D  T  L  R  S
TTTAAGGTTGATGGAACCACTTATGATCCAAGTGACGGGAAGATAAACGAGTGGCCAAGTTTGAGCATGGAT 1152
F  K  V  D  G  T  T  Y  D  P  S  D  G  K  I  N  E  W  P  S  L  S  M  D
GAAAATCTCCAGATGATTGCTAAGATTGCTGCAGTTTGCAATGATGCAAGTATTGCTCACTCTGAGCATCAG 1224
E  N  L  Q  M  I  A  K  I  A  A  V  C  N  D  A  S  I  A  H  S  E  H  Q
TATGTTGCCACTGGGGTGCCCACAGAGGCTGCTCTGAAGGTTTTGGTTGAGAAAATGGGCCTTCCTGGTGGA 1296
Y  V  A  T  G  V  P  T  E  A  A  L  K  V  L  V  E  K  M  G  L  P  G  G
TATACTCCATCCCTGGATTCATCTGATTTGCTAAGGTGCTGTCAATGGTGGAACAATGCTGCTAAGAGAGTA 1368
Y  T  P  S  L  D  S  S  D  L  L  R  C  C  Q  W  W  N  N  A  A  K  R  V
GCTACTCTGGAGTTCGATCGGACTAGAAAATCAATGGGAGTTATTGTAAAAAAAGCAGACTCTGGAAAGAAT 1440
A  T  L  E  F  D  R  T  R  K  S  M  G  V  I  V  K  K  A  D  S  G  K  N
TTGCTGCTTGTCAAGGGAGCTGTAGAAAATTTGCTAGAAAGGAGTGGCTATATTCAGTTGCTTGATGGATCT 1512
L  L  L  V  K  G  A  V  E  N  L  L  E  R  S  G  Y  I  Q  L  L  D  G  S
GTTGTGCTCCTGGATGAAGGTGCCAAGGCGCTCATATTATCAACACTTCGTGAAATGTCTGCTAGTGCATTG 1584
V  V  L  L  D  E  G  A  K  A  L  I  L  S  T  L  R  E  M  S  A  S  A  L
CGATGTTTGGGTTTTGCGTACAAGGAGGATTTGGCGGAATTTGCAACATATGATGGTGAAGAGCATGCAGCT 1656
R  C  L  G  F  A  Y  K  E  D  L  A  E  F  A  T  Y  D  G  E  E  H  A  A
CACAAATATCTACTTGATCCTTCATACTACTCTTCCATAGAGAGTAATTTGATATTTTGTGGTTTTGTTGGT 1728
H  K  Y  L  L  D  P  S  Y  Y  S  S  I  E  S  N  L  I  F  C  G  F  V  G
CTAAGGGATCCTCCCCGAGAAGAAGTCCACAAAGCAATTGAAGACTGTAGAGCTGCTGGAATACGTGTTATG 1800
L  R  D  P  P  R  E  E  V  H  K  A  I  E  D  C  R  A  A  G  I  R  V  M
GTGATTACTGGTGATAACAAAGAAACAGCAGAGGCGATATGCCGTGAGATTGGAGTTTTTGGTTCCACGGAA 1872
V  I  T  G  D  N  K  E  T  A  E  A  I  C  R  E  I  G  V  F  G  S  T  E
GATATTAGTTCAAAGAGCTTCACAGGGAAGGAATTCATGTCTCTTTCAGATAAGAAGAAACTCTTAAGGCAA 1944
D  I  S  S  K  S  F  T  G  K  E  F  M  S  L  S  D  K  K  K  L  L  R  Q
ACAGGTGGCCTTCTCTTCTCTAGGGCTGAGCCGAAACATAAGCAGGAGATAGTTAGATTGCTCAAAGAAGAT 2016
T  G  G  L  L  F  S  R  A  E  P  K  H  K  Q  E  I  V  R  L  L  K  E  D
GGTGAAGTGGTTGCTATGACTGGTGATGGAGTGAATGATGCACCAGCTCTGAAGTTGGCTGACATTGGGGTT 2088
G  E  V  V  A  M  T  G  D  G  V  N  D  A  P  A  L  K  L  A  D  I  G  V
GCTATGGGCATTACAGGGACTGAGGTTGCAAAAGAAGCTTCAGATATGGTTCTTGCAGATGATAACTTCAGT 2160
A  M  G  I  T  G  T  E  V  A  K  E  A  S  D  M  V  L  A  D  D  N  F  S
ACAATAGTCGCAGCTGTTGGTGAAGGGAGGTCAATCTACGACAATATGAAGGCATTTATAAGATATATGATC 2232
T  I  V  A  A  V  G  E  G  R  S  I  Y  D  N  M  K  A  F  I  R  Y  M  I
```

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TCTTCAAACATCGGGGAAGTTGCTTCCATATTTCTAACGTCAGCTTTAGGTATTCCTGAAGGTCTCATCCCT 2304
S  S  N  I  G  E  V  A  S  I  F  L  T  S  A  L  G  I  P  E  G  L  I  P
GTGCAACTTCTGTGGGTCAATCTTGTTACAGATGGTCCTCCTGCAACGGCTTTGGGGTTCAACCCACCTGAT 2376
V  Q  L  L  W  V  N  L  V  T  D  G  P  P  A  T  A  L  G  F  N  P  P  D
AAAGATATCATGAAGAAACCTCCACGAAGGAGTGATGACTCGTTGATCACTCCATGGATCCTATTCCGTTAT 2448
K  D  I  M  K  K  P  P  R  R  S  D  D  S  L  I  T  P  W  I  L  F  R  Y
ATGGTCATTGGGATGTACGTTGGGATTGCCACAGTGGGAGTGTTTATCATTTGGTACACACATGGCTCTTTC 2520
M  V  I  G  M  Y  V  G  I  A  T  V  G  V  F  I  I  W  Y  T  H  G  S  F
CTGGGAATTGACCTGGCTGGTGATGGTCACTCTCTTGTCTCGTACTCCCAGCTCTCAAACTGGGGCCAGTGC 2592
L  G  I  D  L  A  G  D  G  H  S  L  V  S  Y  S  Q  L  S  N  W  G  Q  C
TCCTCATGGGAGGGTTTCAAGGTGTCACCATTCACAGCAGGTGCCCGTACATTCAACTTCGATGCGAACCCA 2664
S  S  W  E  G  F  K  V  S  P  F  T  A  G  A  R  T  F  N  F  D  A  N  P
TGTGATTACTTCCAGGGCGGCAAAATTAAAGCGACAACCCTCTCCCTGTCTGTCTTGGTGGCCATTGAGATG 2736
C  D  Y  F  Q  G  G  K  I  K  A  T  T  L  S  L  S  V  L  V  A  I  E  M
TTCAACTCGCTGAATGCGCTGTCGGAGGATGGTAGCCTTCTGAGCATGCCTCCGTGGGTTAACCCTTGGCTT 2808
F  N  S  L  N  A  L  S  E  D  G  S  L  L  S  M  P  P  W  V  N  P  W  L
CTTCTGGCAATGTCGGTTTCATTCGGGCTGCATTTCTTGATCCTCTACGTGCCATTCCTTGCCCAAGTCTTT 2880
L  L  A  M  S  V  S  F  G  L  H  F  L  I  L  Y  V  P  F  L  A  Q  V  F
GGTATTGTGCCCCTAAGCTTCAATGAATGGCTTTTGGTGATAGCAGTTGCTTTCCCAGTGGTTCTCATCGAC 2952
G  I  V  P  L  S  F  N  E  W  L  L  V  I  A  V  A  F  P  V  V  L  I  D
GAGGTCCTTAAATTTGTGGGGCGGTGCTTGACAGCCCGTGCCAGAAAACAATCAGGAAAGCGGAAGGAAGAT 3024
E  V  L  K  F  V  G  R  C  L  T  A  R  A  R  K  Q  S  G  K  R  K  E  D
TAGATGCTAGTCATCAGTGTTTTAGATTTCTAGTTAAGTCACGAGGAACCAACACCTGATGTAGCACAAACA 3096
*
TTATTTTTTCTCGAGTTCCAAAGGCTCTCGTTCTTTGTTGAGAGAATGTTTGCGAACATGACTATGCAGCGA 3168
ACTTAGGTACTCTATAGAACTCATAATCAGGCAGCGACACAGACCGCATTTATTCATTGTAACTAAGATTGT 3240
TATTTGTGAATGTGCTGGAGAGACAGCATGTTTGCCTGCTCAACTGCTAAAAAAAAAAAAAAAAAAAAAAG 3312
TACTAGTCGACGCGTGGCC                                                       3331
```

Figure 6 Ca^{2+}-ATPase cDNA sequences of KDML105 rice showing all 3,331 bp. The deduced 1,008 amino acid sequence is indicated below the codons of ORF. The nucleotides in 3′-UTR region is underlined.

that Ca^{2+}-ATPase gene of KDML105 was likely to be a type IIA which embedded itself in the endoplasmic reticulum (ER type IIA) of the cell.

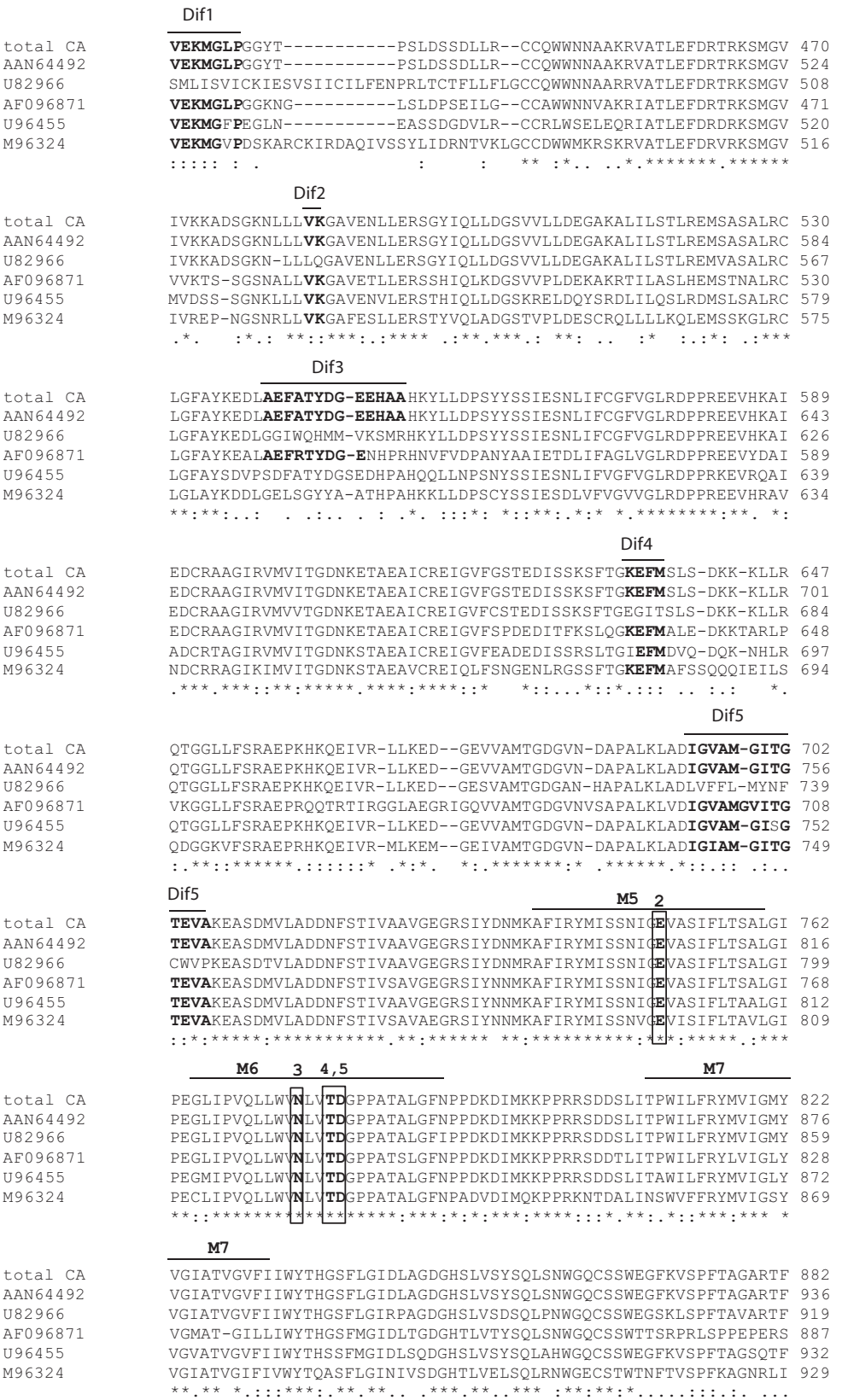
CONCLUSION

Base sequences of Ca^{2+}- ATPase gene in KDML105 were determined using three approaches. Upon using mRNA from the leaves as template to synthesize cDNA by RT-PCR, ten primers, i.e., CA1-CA10 were designed from the conserved regions of *L. esculentum* and *A. thaliana* as reported in the GenBank. Seven combinations of these primers gave seven overlapping segments of amplified PCR products which were combined to construct a continuous DNA fragment (fragment A) of 2,480 bp. This fragment was found to be 99% homology to the putative Ca^{2+}-ATPase gene of *Oryza sativa* (Japonica cultivar-group) cv. Nipponbare.

The second approach was done at 3′-end of the gene using 3′RACE technique. Three primers, i.e., 3′GSP1, 3′GSP2, and 3′ UAP were designed for this purpose. The amplified product was found to be 933 bp long which had an overlapping region with fragment A. Combining these two DNA segments gave a continuous fragment B of 2,944 bp showing 99% homology to the putative Ca^{2+}- ATPase gene of *Oryza sativa* (Japonica cultivar-group) cv. Nipponbare. The third approach was done at 5′-end of the gene using 5′RACE technique. Four designed primers, 5′ GSP1, 5′GSP2, 5′AP, and 5′ UAP were used. The amplified product was found to be 473 bp long which had an overlapping region with fragment B. Combining these two DNA segments gave a continuous total CA of 3,331 bp showing 99% homology to the putative Ca^{2+}- ATPase gene of

CLUSTAL W (1.82) multiple sequence alignment

```
total CA        ------------------------------------------------------AARLRR  6
AAN64492        MGKGGQEEGKRRDGSDASGSEPAAAAFPAWARTPSECLAELGVAADRGLSSEEAAARLRR 60
U82966          ----------------------MEVKRFGRSSEEVLN-IGVN-ETGLSIDTVKCRKER 34
AF096871        ------------------------------------------------------MPAQA  5
U96455          ---MGKGSEDLVKKESLNSTPVNSDTFPAWAKDVAECEEHFVVSREKGLSSDEVLKRHQI 57
M96324          ----------------------MEEKPFPAWSWSVDQCLKEYQVKLEKGLSTYEVDKRRER 39
                                                                       :  .

                                          M1                            M2
total CA        YGPNELERHAAPSVWKLVLEQFDDTLVRILLAAAVVSFVLALYDGAEGGKVGATAFVEPL 66
AAN64492        YGPNELERHAAPSVWKLVLEQFDDTLVRILLAAAVVSFVLALYDGAEGGEVGATAFVEPL 120
U82966          YGLNELFFEEGKTVRSLVLEQFHDILVKILLNAAYISFVLAYIE---EGEAGFTAYVEPI 91
AF096871        HNTSNLALISSDERGTRLLDQFEDTLVRVLVAAAAVSFLLALSSS--AGTLTLAAFVEPL 63
U96455          YGLNELEKPEGTSIFKLILEQFNDTLVRILLAAAVISFVLAFFDGDEGGEMGITAFVEPL 117
M96324          YGLNELEKEKGKPLWRLVLEQFDDTLVKILLGAAFISFVLAYVNQDETGESGFEAYVEPL 99
                :: ::*.      :* :.*:** *:**.:*:.**  **:**       *. : .*.****

                     M2
total CA        VIFLILIVNAVVGVWQESNAEKALEALKEIQSEHATVKRDGRWSHGLP-ARDLVPGDIVE 125
AAN64492        VIFLILIVNAVVGVWQESNAEKALEALKEIQSEHATVKRDGRWSHGLP-ARDLVPGDIVE 179
U82966          VIFLILIVNPVVGVWQESNAEKALEALKEIQSEHATVKRDGRWSHGLP-ARDLVPGDIVE 150
AF096871        VIFLILVVNAAVGVWQEANAERRVDALREIQSHHAAVLRDARCVPRAPLARDLVPGDVVQ 123
U96455          VIFLILIVNAIVGIWQETNAEKALEALKEIQSQQATVMRDGTKVSSLP-AKELVPGDIVE 176
M96324          VILWILVLNAIVGVWQESNAEKALEALKEMQGESAKVLRDGYLVPDFP-AKELVPGDIVE 158
                **::**.:*: **:***.***::::**:*:*:..*.* **:.    .* *..*****:*:

total CA        LRVGDKVPADMRVLQLISSTLRVE-QGSLTGETASVNKTSHKIELEDTDIQGKECMVFAG 184
AAN64492        LRVGDKVPADMRVLQLISSTLRVE-QGSLTGETASVNKTSHKIELEDTDIQGKECMVFAG 238
U82966          LRVGDKVPADMRVLQLISSTLRVE-QGSLTGETASVNKTSHKIELEDTDIQGKECMVFAG 209
AF096871        LRVGAKVPADMRVPASRAPPSSASSRASLTGETASVNKTSRALPLEDADIQAKDCMVFAG 183
U96455          LRVGDKVPADMRVVALISSTLRVE-QGSLTGESEAVSKTTKHVD-ENADIQGKKCMVFAG 234
M96324          LRVGDKVPADMRVATLKSSTLRVE-QSSLTGESMPVTKSTDFLATDDCELQAKENMVFAG 217
                ************* :.:::::::: :.*****...*.*:.    .:: ::* *.:*****

                                                          M3
total CA        TTIVNGSAVCVVTGTGMDTEIGKIHAQIQEASQEEDDTPLKKKLNEFGEALTAIIGVICA 244
AAN64492        TTIVNGSAVCVVTGTGMDTEIGKIHAQIQEASQEEDDTPLKKKLNEFGEALTAIIGVICA 298
U82966          TTIVNGSAVCVVTGTGMDTEIGKIHAQIQEASQEEDDTPLKKKLNEFGEALTAIIGVICA 269
AF096871        TTVVNGAALCIVARTGMDTEIGAIHAQIHQASQEDDDTPLKKKLNEFGEALTKIIGLICA 243
U96455          TTVVNGNCICLVTDTGMNTEIGRVHSQIQEAAQHEEDTPLKKKLNEFGEVLTMIIGLICA 294
M96324          TTVVNGSCICIVVNTGMCTEIGKIQRQIHDASMEESDTPLKKKLDEFGNRLTFAIGVVCL 277
                **:*** .:*:*. *** **** :: **::*: .:.********:***:.** :**::*:

                                  M4          1
total CA        LVWLINVKYFLTWEYVDGWP-RNFKFSFEKCTYYFEIAVALAVAAIPEGLPAVITTCLAL 303
AAN64492        LVWLINVKYFLTWEYVDGWP-RNFKFSFEKCTYYFEIAVALAVAAIPEGLPAVITTCLAL 357
U82966          LVWLINVKYFLTWEYVDGWP-RNFKFSFEKCTYYFEIAVALAVAAIPEGLPAVITTCLAL 328
AF096871        LVWLINCKYFLTFDLQGGWVPRNITFSFEKCTYYFEIAVALAVAAIPEGLPAVITTCLAL 303
U96455          LVWLINVKYFLSWEYVDGWP-RNFKFSFEKCTYYFEIAVALAVAAIPEGLPAVITTCLAL 353
M96324          VVWAINYKYFLSWEVVDDWP-SDFRFSFEKCAYYFKIAVALAVAAIPEGLPSVITTCLAL 336
                :**:**.****:::.:::*: :::.******:***:**********:***:********

                                  P
total CA        GTRKMAQKNALVRKLPSVETLGCTTVICSDKTGTLTTNQMSAVKLVAIGRWPDTLRSFKV 363
AAN64492        GTRKMAQKNALVRKLPSVETLGCTTVICSDKTGTLTTNQMSAVKLVAIGRWPDTLRSFKV 417
U82966          ATRKMSPKNALVRKLPSVETLGCTTVICSDKTGTLTTNQMSAVKLVAIGRWPDTLRSFKV 388
AF096871        GTRKMAAKNALVRKLPSVETLGCTTVICSDKTGTLTSNKMSVAKLVAVGDSSQEVRTFKV 363
U96455          GTRKMAQKNALVRKLPSVETLGCTTVICSDKTGTLTTNQMAVSKLVAMGSRIGTLRSFNV 413
M96324          GTRKMAQKNAIVRKLQSVETLGCTTVICSDKTGTLTTNQMSVSEFFTLGRKTTACRVFGV 396
                .****:.***:****:**********************:*:*:. ::.::*.     *.*.*

                                                                  Dif1
total CA        DGTTYDPSDGKINEWPSLSMDENLQMIAKIAAVCNDASIAHSEHQYVATGVPTEAALKVL 423
AAN64492        DGTTYDPSDGKINEWPSLSMDENLQMIAKIAAVCNDASIAHSEHQYVATGMPTEAALKVL 477
U82966          DGTTYDPSDGKINEWPSLSMDENLQMIAKIAAVCNDASIAHSEHQYVATGMPTEAASDLL 448
AF096871        DGTTYDPRDGKIHDWPAGSIDANLETIAKVAAVCNDANVAHSSHQYVATGMPTEAALKVL 423
U96455          EGTSFDPRDGKIEDWPMGRMDANLQMIAKIAAICNDANVEQSDQQFVSRGMPTEAALKVL 473
M96324          EGTTYDPKDGGIMNWNCCKMDANLLLMAEICAICNDAGVFCDGRLFKATGLPTEAALKVL 456
                :**::** **:* :*:  .:* **  :*::.*:****.:..: ::.:::*.*****:::*
```


Dif1
total CA VEKMGLPGGYT----------PSLDSSDLLR--CCQWWNNAAKRVATLEFDRTRKSMGV 470
AAN64492 VEKMGLPGGYT----------PSLDSSDLLR--CCQWWNNAAKRVATLEFDRTRKSMGV 524
U82966 SMLISVICKIESVSIICILFENPRLTCTFLLFLGCCQWWNNAARRVATLEFDRTRKSMGV 508
AF096871 VEKMGLPGGKNG----------LSLDPSEILG--CCAWWNNVAKRIATLEFDRTRKSMGV 471
U96455 VEKMGFPEGLN----------EASSDGDVLR--CCRLWSELEQRIATLEFDRDRKSMGV 520
M96324 VEKMGVPDSKARCKIRDAQIVSSYLIDRNTVKLGCCDWWMKRSKRVATLEFDRVRKSMGV 516
Dif2
total CA IVKKADSGKNLLLVKGAVENLLERSGYIQLLDGSVVLLDEGAKALILSTLREMSASALRC 530
AAN64492 IVKKADSGKNLLLVKGAVENLLERSGYIQLLDGSVVLLDEGAKALILSTLREMSASALRC 584
U82966 IVKKADSGKN-LLLQGAVENLLERSGYIQLLDGSVVLLDEGAKALILSTLREMVASALRC 567
AF096871 VVKTS-SGSNALLVKGAVETLLERSSHIQLKDGSVVPLDEKAKRTILASLHEMSTNALRC 530
U96455 MVDSS-SGNKLLLVKGAVENVLERSTHIQLLDGSKRELDQYSRDLILQSLRDMSLSALRC 579
M96324 IVREP-NGSNRLLVKGAFESLLERSTYVQLADGSTVPLDESCRQLLLLKQLEMSSKGLRC 575
Dif3
total CA LGFAYKEDLAEFATYDG-EEHAAHKYLLDPSYYSSIESNLIFCGFVGLRDPPREEVHKAI 589
AAN64492 LGFAYKEDLAEFATYDG-EEHAAHKYLLDPSYYSSIESNLIFCGFVGLRDPPREEVHKAI 643
U82966 LGFAYKEDLGGIWQHMM-VKSMRHKYLLDPSYYSSIESNLIFCGFVGLRDPPREEVHKAI 626
AF096871 LGFAYKEALAEFRTYDG-ENHPRHNVFVDPANYAAIETDLIFAGLVGLRDPPREEVYDAI 589
U96455 LGFAYSDVPSDFATYDGSEDHPAHQQLLNPSNYSSIESNLIFVGFVGLRDPPRKEVRQAI 639
M96324 LGLAYKDDLGELSGYYA-ATHPAHKKLLDPSCYSSIESDLVFVGVVGLRDPPREEVHRAV 634
Dif4
total CA EDCRAAGIRVMVITGDNKETAEAICREIGVFGSTEDISSKSFTGKEFMSLS-DKK-KLLR 647
AAN64492 EDCRAAGIRVMVITGDNKETAEAICREIGVFGSTEDISSKSFTGKEFMSLS-DKK-KLLR 701
U82966 EDCRAAGIRVMVVTGDNKETAEAICREIGVFCSTEDISSKSFTGEGITSLS-DKK-KLLR 684
AF096871 EDCRAAGIRVMVITGDNKETAEAICREIGVFSPDEDITFKSLQGKEFMALE-DKKTARLP 648
U96455 ADCRTAGIRVMVITGDNKSTAEAICREIGVFEADEDISSRSLTGIEFMDVQ-DQK-NHLR 697
M96324 NDCRRAGIKIMVITGDNKSTAEAVCREIQLFSNGENLRGSSFTGKEFMAFSSQQQIEILS 694
Dif5
total CA QTGGLLFSRAEPKHKQEIVR-LLKED--GEVVAMTGDGVN-DAPALKLADIGVAM-GITG 702
AAN64492 QTGGLLFSRAEPKHKQEIVR-LLKED--GEVVAMTGDGVN-DAPALKLADIGVAM-GITG 756
U82966 QTGGLLFSRAEPKHKQEIVR-LLKED--GESVAMTGDGAN-HAPALKLADLVFFL-MYNF 739
AF096871 VKGGLLFSRAEPRQQTRTIRGGLAEGRIGQVVAMTGDGVNVSAPALKLVDIGVAMGVITG 708
U96455 QTGGLLFSRAEPKHKQEIVR-LLKED--GEVVAMTGDGVN-DAPALKLADIGVAM-GISG 752
M96324 QDGGKVFSRAEPRHKQEIVR-MLKEM--GEIVAMTGDGVN-DAPALKLADIGIAM-GITG 749
Dif5
M5 2
total CA TEVAKEASDMVLADDNFSTIVAAVGEGRSIYDNMKAFIRYMISSNIGEVASIFLTSALGI 762
AAN64492 TEVAKEASDMVLADDNFSTIVAAVGEGRSIYDNMKAFIRYMISSNIGEVASIFLTSALGI 816
U82966 CWVPKEASDTVLADDNFSTIVAAVGEGRSIYDNMRAFIRYMISSNIGEVASIFLTSALGI 799
AF096871 TEVAKEASDMVLADDNFSTIVSAVGEGRSIYNNMKAFIRYMISSNIGEVASIFLTSALGI 768
U96455 TEVAKEASDMVLADDNFSTIVAAVGEGRSIYNNMKAFIRYMISSNIGEVASIFLTAALGI 812
M96324 TEVAKEASDMVLADDNFSTIVSAVAEGRSIYNNMKAFIRYMISSNVGEVISIFLTAVLGI 809
M6 3 4,5
M7
total CA PEGLIPVQLLWVNLVTDGPPATALGFNPPDKDIMKKPPRRSDDSLITPWILFRYMVIGMY 822
AAN64492 PEGLIPVQLLWVNLVTDGPPATALGFNPPDKDIMKKPPRRSDDSLITPWILFRYMVIGMY 876
U82966 PEGLIPVQLLWVNLVTDGPPATALGFIPPDKDIMKKPPRRSDDSLITPWILFRYMVIGMY 859
AF096871 PEGLIPVQLLWVNLVTDGPPATSLGFNPPDKDIMKKPPRRSDDTLITPWILFRYLVIGLY 828
U96455 PEGMIPVQLLWVNLVTDGPPATALGFNPPDKDIMKKPPRRSDDSLITAWILFRYMVIGLY 872
M96324 PECLIPVQLLWVNLVTDGPPATALGFNPADVDIMQKPPRKNTDALINSWVFFRYMVIGSY 869
M7
total CA VGIATVGVFIIWYTHGSFLGIDLAGDGHSLVSYSQLSNWGQCSSWEGFKVSPFTAGARTF 882
AAN64492 VGIATVGVFIIWYTHGSFLGIDLAGDGHSLVSYSQLSNWGQCSSWEGFKVSPFTAGARTF 936
U82966 VGIATVGVFIIWYTHGSFLGIRPAGDGHSLVSDSQLPNWGQCSSWEGSKLSPFTAVARTF 919
AF096871 VGMAT-GILLIWYTHGSFMGIDLTGDGHTLVTYSQLSNWGQCSSWTTSRPRLSPPEPERS 887
U96455 VGVATVGVFIIWYTHSSFMGIDLSQDGHSLVSYSQLAHWGQCSSWEGFKVSPFTAGSQTF 932
M96324 VGIATVGIFIVWYTQASFLGINIVSDGHTLVELSQLRNWGECSTWTNFTVSPFKAGNRLI 929

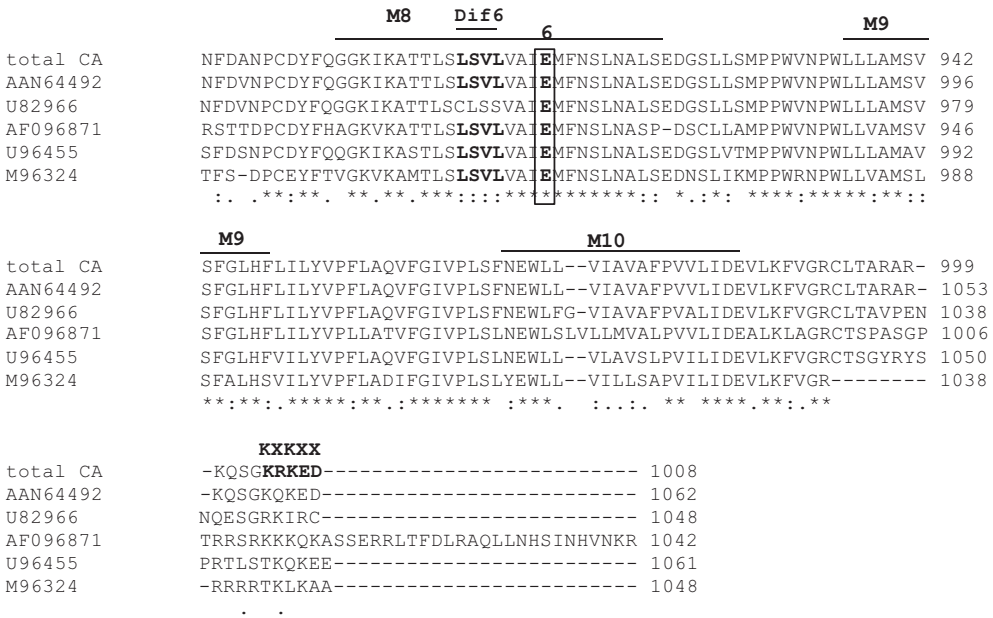


Figure 7 Multiple amino acid sequence alignment of Ca^{2+}-ATPases gene of KDML105 (total CA) with higher plant type IIA Ca^{2+}-ATPases. Deduced amino acid sequences were aligned by ClustalW method of www.ebi.ac.uk. The ten putative transmembrane domains (M1-M10) are overlined. The potential phosphorylation site is marked with P. Six amino acid residues essential for calcium ion transport are boxed and numbered. The position of putative ER retention signals with the consensus motif (K/X)(K/X)KXX-stop is indicated with KXKXX. Six different regions between KDML105 rice with *Oryza sativa* cv.. IR36 Ca^{2+}-ATPase gene (Dif1-6) are overlined. AAN64492: *Oryza sativa* (Japonica cultivar-group) putative calcium ATPase; U82966: *Oryza sativa* Ca^{2+}-ATPase gene, complete cds.; U96455: *Arabidopsis thaliana* ER-type Ca^{2+}-pumping ATPase (ECA1) mRNA, complete cds.; AF096871: *Zea mays* calcium ATPase gene; M96324: *Lycopersicon esculentum* Ca^{2+}- ATPase gene, complete cds.

Oryza sativa (Japonica cultivar-group) cv. Nipponbare. Analysis of the amino acid sequences of Ca^{2+}- ATPase gene indicated that this gene in KDML105 most likely belonged to ER type IIA group.

ACKNOWLEDGEMENTS

This work was financially supported by the Rockefeller Foundation (grants#98001), and Graduate School, Kasetsart University. We would also like to thank Pathumthani Rice Research Center for supplying rice KDML105.

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