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F
                 5
                    E
                      т.
                         N
                             D
                                N
 46 tttta
        tattcctacctacatacttgcaccggatgctccgaataag
           P
                  Y
                       т.
                          А
                             Ρ
                                D
 91 agtaggcatataggtttacctgataatgtacctacatgccctgtg
    SR
                       D
                         N
                             v
               G
136 ctagtgtttattaattctaaaagcggaggtcaacttggaggagaa
   LVF
              Ν
                  s
                    к
                       S
                         G
                             G
                                0
                                        G
181 cttcttcgtacatttcgccatcttcttaacaaatatcaggttttt
   LL
                  R
                    н
                       L
                         - L
                             Ν
226 gatttgggagaagaagctcctgatagtgttttacgtagagtgtac
    DL
        G
           E
                      D
                         S
              E
                 A
                    P
                            v
271 ttgaatatagaaaagctgaagagtggtggtgatcaatttgctgcc
   L N
           E
              K
                 L
                    KSG
                            G
                                D
                                   0
                                        A
        T
                                     F
316 aaaactgaagagagaatgagaataattgttgcaggtggagatggc
   КТ
               R
                    R
                       I
                                   G
                                      G
                                        D
361 acagetggetggettettggagttgtatetgateteaaactatet
         G
                    G
                       v
                          v
                             S
                                D
406 cagcctccaccaattgctacagtgcctttgggaactggaaataac
         P
                       v
                          P
                             L
                                G
                                      G
                                        N
                                   т
451 cttccatttgcttttggctggggaaagaagaacccaggaaccgac
                  G
                     W
                       G
                          к
                             К
                                N
                                        т
496 cttaactccgtcatctcatttttgaagcaagtaatgcaaaa
   L
                       L
                          к
                             0
                                v
                                      N
                                           K
                  s
541 gaaatgaagatggacagttggcacattcttatgaggatgagagca
   E
                       н
               D
                  s
                     M
                          т
                             т.
586 ccaaaaggtggttcttgtgatcctattgcaccgcttgaattgcct
   P
         G
            G
               s
                  С
                     D
                       P
                          I
                             А
631 cattetttgcatgcatttcaccgggtttctccatetga
                                        tgaactg
   H
           H
              A
                    н
                       R
                          v
                             s
676 aatgtggaaggtttccatacattccgtggaggtttctggaattac
   NVE
           G
              F
                  н
                      F
                         R
                             G
                                G
                                      W
                                        N
                                           Y
                    т
                                   F
721 ttcagcatggggatggatgcacaagtatcttatgcatttcactca
   F S M G M D A Q V S Y A F H S
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766 gagcgaaaaatgaacccagagaaattcaaaaaccaacttgtcaat
    ERKMNPEKFKNQLVN
 811 cagagtgcatatttaaagctaggatgtacacaaggatggtttttt
    OSA
           YL
                      G
                           т
                              QG
                                   W
                                     FF
                 K
                    L
                        С
 856 gcccctctcgttcatcgttcttcgaggaacatagctcaactgact
           v
              Н
                 R
                   S
                     S
                        R
                           N I
                                A
                                   0
                                     LT
 901 aaggttaagataatgagaaagcagggtgaatggcaagaccttcac
    K V K
           1
                 RKQ
                        GE
                             WQD
                                     L
946 atccctcacagtgtcaggtcaatcgtatgcctcaacttgcctagc
                        v c
           s
              v
                 R
                   S
                      1
 991 ttttccggtgg
                   tccttgggggaacaccaaatagcaacaaa
    F
                                    S
                                     N
                      W
                         G
                            т
                                 N
1036 cgccgatatagggacttgactcctccatttgtagacgatggcctt
              D
                    т
1081 cttgaggttgtcggattcagagatgcttggcacggattagtcctt
                                      VL
    LE
        v
            v
              G
                 F
                    R
                     D
                            W
                              н
                                G
                                   L
                        - A
1126 cttgctccaaaaggacatgggactcgtcttgcccaggcacataga
            к
              G
                 н
                   GTRLA
                                   A
                                      н
         Р
                                0
1171 atccgatttgagtttcacaagggtgcagctgaccatacatttatg
                 H
                    K
                      GA
                           A D
    I R
              F
                                H
1216 aggatagatggggaaccctggaagcaaccctacctgaagatgat
        DG
              E
                PWKQPL
                                P
                                      D
1261 gacaccgttgtggtagaaatttctcatcttggccaggttaagatg
    D T V V V E I S H L G Q V K M
1306 cttgctactcatgactgcagagccaaaagtatacacgatccctca
    LA
           н
              DCRAK
                           S
                                 н
                                    D
                                      P
                                         S
1351 agtcatttcaatcatgaagctgacgatgatgatgatgatgacgaa
    S H
            N
               Н
                 E
                    A
                      D
                         D
                            D
                              D
                                S
                                   D
                                      DE
1396 aactcggtcgatgaaggaaggaggaaatttggggcagcagacact
        V D
              E
                 GRR
                        K
                                   ADT
                           FGA
1441 ttcaaaattccagatgaggtcgatgtttctcgtctcagttaa 1482
            P
              DEVD
                        V S
                              R L S
    FK
        т
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**Supporting information figure S1:** The translated coding sequence of the tobacco *NtDGK5* gene. The identified nucleic acid sequence was deposited in GenBank under the reference # 1902348. It codes for a 493-amino acid-long protein (55.24kDa), having a predicted pl of 6.75. *In silico* translation was performed using the freeware ORF Finder available at the NCBI website (http://www.ncbi.nlm.nih.gov/gorf/gorf.html).

III	AtDGK5sv AtDGK5lv AtDGK6 LDGK1 SGN-U585112 SGN-U439985 SGN-U439984	482 CTCCAGTEGAC TCCTTTTTGCAACAAGTCTTGAAGGCAAAAGTCATGAAGATTGACAAT 482 CTCCAGTEGAC TCCTTTTTGCAACAAGTCTTGAAGGCAAAAGTCATGAAGATTGACAAT 503 CTTCCGTCGAC TCTTTTTTGCCTAAAGTCATCAATGCAAAGACATGAAGATAGACAATAGACAAT 488 ACTCCGTCATC TCTTTTTTGCAGCAAGTAATGAACGCAAAGGAAATGAAGATGAACAGT 872 ACTCTGTCATC TCCTTTTTGGAAGCAAGTAATGAATGCAAAGGAAATGAAGATGACAGT 577 ACTCCGTCATC TCATTTTGGAAGCAAGTAATGAATGCAAAGGAAATGAAGATGGACAGT 620 ACTCCGTTATC TCATTCATGGAAGCAAGTAATGGATGGAAAGAAAGAAATGAAGATGAACAGT	658       CTAAACAAGGAAGGCTGCCACACTTTTCGAGGAGGGTTCTGGAAT         658       CTAAACAAGGAAGGCTGCCACACTTTTCGAGGAGGGTTCTGGAAT         661       GAAATATGGAAGGTTACC
II	AtDGK3 AtDGK7 AtDGK4 SGN-U433298 SGN-U472030 SGN-U440091	650 CTGCTGTTAAAAGAACACTCCATCCGCAAGTATGGGTCCAGTTCCTAGACTTGACAGCT 659 CTGCTATGAAAAGAACATTACATCGAGCAACTTTAGGTTCCATTCCTCGATTAGATAGCT 647 CTCCGATAAAACGAACACTTCATAGAGCAAGTTTGCTCCTATAAGCCGTTTAGATAGCT 693 CAGCCACGAAAAGAATTCTTGACAGGGTTCCCAATGGTCCTATCTGCCGTCTAGATAGCT 503 CAGCCACGAAAAGAATTCTTGACAGGGTTCCCAATGGTCCTATCTGCCGTCTAGATAGCT 812 CAGCCACAAAAGAATTCTTGACAGGGTTCCAACGGTCCCAATTAACCATCTGGATAGTT	<ul> <li>795 CHTAATGIGGAAGGA TIGCT TACCACTTGCAAAGCATTCGIGGAGGATTCTGGAAT</li> <li>799 TTGCACGCGCGCAATTGATGCGCCCTCCACTTGCAAAGCTCTTATGAAGGAGTGTTTTCTACAAC</li> <li>811 TTGCATGCTGATGGAGAGGTCCCCCCACAAGCAAAGTCGTCTTATGAAGGAGTCTTCTACAAC</li> <li>796 TTGGAGATAGGACGGAGAGGACCCCCCCCACGACGAATTGCCAAGGAGGAGTCTTCTACAAT</li> <li>842 TTGGAAGTTGATGGTCAATTACCAGGAGAGTCGTCCTACTATCAAGGAGTATTTTACAAT</li> <li>652 TTGGAAGTTTATGGGCAATTACCAGGAGAGTCGTCCTACTATCAAGGAGTATTTTACAAT</li> </ul>
	AtDGK5sv AtDGK51v AtDGK6 LDGK1 SGN-U585112 SGN-U439985 SGN-U439984	542       GCACATACTTATGAGGATGAAAACTCCCAAAGAAGGTGGTTCTTGTGATCCTGTTCTC         542       GCACATACTTATGAGGATGAAAACTCCCAAAGAAGGTGGTTCTTGTGATCCTGTTCTC         563       GCACATACTTATGAGGATGAGCACCATCCCAAAGAAGGCTCTTGTGATCCTGTTCCAC         548       GCCACATTCTTATGAGGATGAGGGCACCAAAAGTTGGTTCTTGTGATCCTGTTCCAC         932       GCCACATTCTTATGAGGATGAGGGCACCAAAAGTTGGTTCTTGTGATCCTGTTCCAC         637       GCCACATTCTTATGAGGATGAGAGGCACCAAAAGTGGGTTCTTGTGATCCTATTCCAC         680       GCCACATTCTAATGAGGATGAGAGCACCAAAAGTGAGTGGTTCTTGTGATCCTATTCCAC	
II	AtDGK3 AtDGK7 AtDGK4 SGN-U433298 SGN-U472030 SGN-U440091	710       GGAAGATTTTAGTGTCAATG       CCA       TCTGGAGA       AG         719       GGAAGATTGTAGTGTCGATG       CCA       TCTGCAGA       AG         707       GGAATATTTTGATTACAATG       CCT       TCTGGTGA       AG         753       GGAATCTTGTAATATCAATG       CCA       CCTGGGGA       AA         753       GGAATCTTGTAATATCAATG       CCA       CCA       GCTGGGGA       AA         753       GGAATCTTGTAATATCAATG       CCA       GCTGGGGA       AG         872       GGAATCTTGTAATATCAATG       CCC       GCTGGGGA       AA	
	AtDGK5sv AtDGK51v AtDGK6 LDGK1 SGN-U585112 SGN-U439985 SGN-U439984	602CTCTTGACTTACCACATTCCTACATGCATTTCACCGTGTTT-CTCCAACTGATGAA602CTCTTGACTTACCACATTCCTACATGCATTTCACCGTGTTT-CTCCAACTGATGAA614CTCTTAACCTACCACATTCTCTTCCTCGTATTT-TCCCATCAGATCAA605CGCTTGAATTGCCTCATTCTTTCCATGCCTTTCACCGGGTTT-CTCCATCTGATGAA989TGCTTGAATTGCCTCATTCTTTCCATGCCTTTCACCGGGTTT-CTCCATCTGATGAA694CGCTTGAATTGCCTCATTCTTTCCATGCATTCACCGGGTTT-CTC-ATCTGATGAA737CACTGGATTGCCTCATTCTTCCATCGTTCCACGGGTTC-CTCCATCTGATGAA	
II	AtDGK3 AtDGK7 AtDGK4 SGN-U433298 SGN-U472030 SGN-U440091	743       TCGTAGATCCTCCTTATTCTTA-PAACCCGCTGAAGAAAAC-GAACTGGATCAGGCT         752       TCGTTGATCCTCCTTATTCTTA-PAGCCTACAATAGAGGAAACTGCACTGGATCAGGCT         740       TTGTGGATCCTCCTTATTCTTA-PAGCCTACA-CAAGAATGTACA-TGACCAGAAT         786       CTTTGGAGACACCCTTATTCTTTA-PAGCCTACACAAGAATGTTACA-TGACCAGAAT         786       CTTTGGAGACACCCTTATTCTTTA-PAGCCTACGGAGTCTCCATCTCTTGATCAGGAA         596       CTTTGGAGACACCCTTATTCTTA-AAACCTTCGGAGTCTCCATCTCTTGATGAGGAA         905       AACTGGAAACACCTCATTCTCTG-AAACCTACTGAGGATGCCTCTCTTGATCAGGAA	

**Supporting information figure S2**: Partial alignment of plant DGK coding sequences from clusters II and III. This figure shows the nucleic acid coding region used for miRNA design (squared in red), which is highly conserved among cluster III members and divergent with cluster II counterparts. Full-length nucleic acid sequences were aligned using version 2.0.10 of Clustal X (Larkin et al. 2007) and the resulting alignment was processed online at the BoxShade server (<a href="http://www.ch.embnet.org/software/BOX\_form.html">http://www.ch.embnet.org/software/BOX\_form.html</a>). Roman numbers (II and III) on the right of the alignment indicate DGK cluster.



**Supporting information figure S3**: Effects of inhibitors on the alkalization triggered by cryptogein. Cells were preincubated 15 min with inhibitors (or DMSO, solvent of the inhibitors) before cryptogein (50 nM) elicitation. Final concentrations of edelfosine, R59022 and R59949 were 25  $\mu$ M, 75  $\mu$ M and 75  $\mu$ M, respectively. R59949 is another DGK inhibitor. Extracellular pH alkalinization was measured and reported as the difference between initial and final (1 hour of cryptogein treatment) pH values. Means +/- SD.



Supporting information figure S4: Topology of the sixteen identified tobacco DGK proteins. Protein structure is depicted for the 3 phylogenetic clusters. DGK nucleic acid sequences were retrieved from the Solanacea Genomics Network (SGN) database (https://solgenomics.net/) using the BLAST algorithm and keyword search tool. Known Arabidopsis, rice and tomato DGKs were used as nucleic acid sequence queries. Upon in silico translation using ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html), the resulting polypeptides were compared to their Arabidopsis counterparts and the Conserved Domain Architecture Retrieval Tool (CDART, (Geer et al. 2002)) was used to predict domain location. Within a panel (corresponding to one protein cluster), the closest Arabidopsis relative is followed by orthologous tobacco translated sequences. The latter are referenced by their SGN numbers and the identified part of the protein is presented. The length of the corresponding contigs is also indicated in green. Abbreviations: AtDGK5lv, AtDGK5 long splice variant; AtDGK5sv, AtDGK5 short splice variant; C1, DAG binding domain; CBD, calmodulin-binding domain; DGKcat, DGK catalytic domain; DGKacc, DGK accessory domain. The grey rectangle localized to the N-terminal part of AtDGK1 represents a transmembrane domain.





**Supporting information figure S5:** Identification of the PA binding domain of NtRBOHD by alignment with the cognate domain of Arabidopsis isoform D. Full length primary amino-acid sequences of RBOHD from *Arabidopsis thaliana* (SwissProt:Q9FIJ0.1) and *Nicotiana tabacum* (GenBank:ABN58915) were aligned using version 2.0.10 of Clustal X (Larkin et al. 2007) and the resulting alignment was then processed online at the BoxShade server (<a href="http://www.ch.embnet.org/software/BOX\_form.html">http://www.ch.embnet.org/software/BOX\_form.html</a>). Stars indicate the two basic amino-acid residues between the two proteins.