Note

Plant Biotechnology, 17 (3), 247-252 (2000)

Identification of the gene encoding granule-bound starch synthase I in sweet potato (*Ipomoea batatas* (L.) Lam.)

Takashi KIMURA*[1], Osamu IDETA** and Akira SAITO*

*Kyushu National Agricultural Experiment Station, Kikuchi-gun, Kumamoto 861-1192, Japan
**Japan International Research Center for Agricultural Sciences, Ishigaki, Okinawa 907-0002, Japan
[1]Corresponding author E-mail address: tkimura@knaes.affrc.go.jp

Received 26 April 2000; accepted 9 June 2000

Abstract

The cDNA and genomic clones from sweet potato encoding granule-bound starch synthase I (GBSSI) were isolated and characterized. The sequence analysis of the cDNA shows that the sweet potato GBSSI mature protein is comprised of 531 amino acids and that the precursor has a transit peptide of 77 amino acids. A comparison of the cDNA and genomic sequences suggested that the sweet potato GBSSI gene has 14 exons and 13 introns, the first of which is located in the untranslated region. This gene is considered to be a low-copy gene based on genomic Southern analysis.

Starch granules usually consist of amylopectin, which is a α1,4-linked glucose polymer with frequent α1,6-branches, and amylose, a relatively unbranched α1,4-linked glucose polymer. The formation of amylose in a storage organ is catalyzed by granule-bound starch synthase I (GBSSI), also called the *Waxy* protein. Maddelein *et al.* reported that *Chlamydomonas* GBSSI, which synthesizes amylose, is also involved in the amylopectin synthesis (Maddelein *et al.*, 1994). Concerning this enzyme in higher plants, its possible participation in the synthesis of amylopectin has been pointed out (Denyer *et al.*, 1996).

The sequences of the GBSSI structural genes from several species have been published. These include maize (Klösgen *et al.*, 1986), rice (Wang *et al.*, 1990; Hirano and Sano, 1991), barley (Rohde *et al.*, 1988), wheat (Murai *et al.*, 1999) and potato (van der Leij *et al.*, 1991). In this study, we present the complete sequence of the sweet potato GBSSI gene.

The total RNA was isolated from the sweet potato (*Ipomoea batatas* (L.) Lam. cultivar Kokei 14) tuberous root using the SDS-phenol method. Poly(A)$^+$ RNA was prepared using Oligotex-dT30 (TaKaRa). A partial cDNA fragment (about 500bp) for the sweet potato GBSSI was cloned by RT-PCR amplification with degenerate primers. The degenerate primers (forward: 5'-GA(TC)CA(AG)TA (TC)AA(AG)GA(TC)GC(ACGT)TG, reverse: 5'-AT(ACGT)CC(ACGT)GC(CT)TTCATCCA(AG) TT) were designed by referring to the conserved amino acid sequences among the GBSSIs from other plants. A sweet potato tuberous cDNA library was constructed in λZAP II using a cDNA synthesis kit (Stratagene) as described in the instruction manual. The library (6×10^5 plaques) was screened with the partial GBSSI cDNA. Positive clones were purified and converted to a phagemid (pBluescript II) following the manufacturer's instructions (Stratagene). The largest positive clone was sequenced on both strands using the dideoxy chain termination method (Sanger *et al.*, 1977).

DNA was extracted from sweet potato (cultivar Kokei 14) leaves using the modified CTAB method. A sweet potato genomic library was constructed with the *Bam* HI arms of λEMBL3 (Stratagene). This library (4.1×10^5 plaques) was screened with the largest GBSSI cDNA. One of the positive clones, which hybridized with the fragments of both the 5' and 3' ends of the cDNA, was subcloned into pBluescript II and sequenced on both strands using the dideoxy chain termination method (Sanger *et al.*, 1977).

The largest GBSSI cDNA was also used for Southern hybridization. The digested DNA of the sweet potato (cultivar Kokei 14) was electrophoresed in agarose gels and blotted on nylon membranes as described by Sambrook *et al.* (1989). The probe labelling and detection were carried out using the Gene Images system (Amersham). Hybridization was done at 60 ℃ and the blot was washed under moderately stringent conditions (final washing at 60 ℃ in 0.2 × SSC, 0.1% SDS).

Nine of 190 cDNA positive clones were purified and analyzed. The length of largest cDNA clone

(clone 131) is 2211bp. The sequence of the sweet potato GBSSI cDNA was found from a search of the GenBank (accession no.U44126, unpublished) . The 5' untranslated region of clone 131 is 70bp longer than that of this cDNA. The cDNA clone 131 has a 608-amino acid open reading frame (ORF). The ORF starts at position 93 and ends at position 1916. A polyadenylation signal AATAAA was found for the cDNA (positions 2160-2165) upstream from the poly (A) tail. A cleavage site consensus sequence proposed for the chloroplast transit peptides is I/VXA/C ↓ A (Gavel and von Heijne, 1990). In potato and pea, the cleavage site sequence of GBSSI is IVC ↓ G (van der Leij *et al.*, 1991; Dry *et al.*, 1992). A similar sequence was identified in the sweet potato GBSSI as IVC ↓ K (**Fig.1** and **2**). Therefore, the lengths of the plastid transit peptide and mature protein appear to be 77 and 531 amino acids, respectively (**Fig. 1**). A comparison of the amino acid sequences of the sweet potato with that of other plants shows that there is a high similarity between the mature proteins, i.e., 85% homology with potato, 75% with pea, 72%with maize and 71% with rice (Klösgen *et al.*, 1986; Wang *et al.*, 1990; van der Leij *et al.*, 1991; Dry *et al.*, 1992). Three conserved boxes of the amino acid sequences of the bacteria glycogen synthase and the plant GBSSI (van der Leij *et al.*, 1991; Salehuzzaman *et al.*, 1993) are shown in **Fig.1**.

Thirteen positive genomic clones were isolated from the screening. The nucleotide sequences of the analyzed genomic clone are shown in **Fig. 2**. A comparison of the genomic and cDNA sequences suggested that the sweet potato GBSSI gene has 13 introns (**Fig. 3**) though the transcriptional start site is unknown. The maize, rice, and potato GBSSI genes have been reported to possess 13 introns (Klösgen *et al.*, 1986; van der Leij *et al.*, 1991; Wang *et al.*, 1995). All introns in the sweet potato GBSSI gene follow the universal GT-AG rule. The exon sequences are identical with that of the cDNA. In 11 of 13 introns, the third nucleotide of the 5' splice sites is A. In 9 of 13 introns, the nucleotide at position -3 of the 3' splice sites is C. The exons and introns have a total G+C content of 46% and 35%, respectively.

The putative TATA box TACAAAT occurs 46bp upstream of the cDNA 5' end. This sequence is identical with that of the potato GBSSI gene TATA box (van der Leij *et al.*, 1991). A 9-bp sequence (CCACGTGGC), which contains the G box (Schulze-Lefert *et al.*, 1989) or the ABA responsive element (Guiltinan *et al.*, 1990), is located between positions -85 and -77. This gene may respond to light and /or some environmental stresses.

The GBSSI genes of maize, rice, and diploid potato have been shown to be a single copy (Shure *et al.*, 1983; Okagaki *et al.*, 1988; Visser *et al.*, 1989). The result of the Southern blot analysis of the genomic DNA of sweet potato, which is a hexaploid plant, is shown in **Fig. 4**. Digestion with *Eco* RI, *Eco* RV and *Xba* I, each of which has a unique site in the genomic DNA of GBSSI (see **Fig. 3**), gave four to five restriction bands. On the other hand, digestion with *Sac* I and *Hin*d III, each of which cuts the 3' or 5' end of the gene (see **Fig. 3**), gave two restriction bands. Taken together, it is conceivable that there are two to three copies of GBSSI in the genome of sweet potato. It is unclear at present whether this result is due to the hexaploid and heterozygous nature of sweet potato. The complicated band pattern also has been seen in the analysis of the GBSSI gene of cassava, a tetraploid plant (Salehuzzaman *et al.*, 1993). It would be interesting to clarify the homology between/among these copies of GBSSI and whether they have the same strengths and patterns of expression.

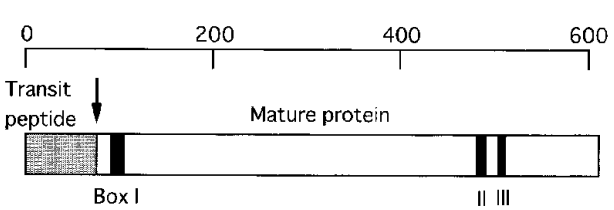

Fig. 1. Structure of the sweet potato granule-bound starch synthase I. The numbers in the scale refer to the amino acid residues. Three conserved boxes (positions 93-108,481-491, and 504-512) are indicated as Box I, II, and III, respectively. The arrowhead shows the putative processing site of the protein.

```
-435  aaaaagatggcgggacgtcacatgaaatgtcgtccgttctcacttctcaccgttcatctt
-375  tctggcgatctgaaagcgcgggggccccattgtcatctccaccagaaagtagtgtatttct
-315  ccttttgtcgcactgccatatttggagacaagagcggacggcccaaaaaaatctcgtcac
-255  gcaatttcaaataaggccctcaaacttggagcatattcccagttatcccccaaaagtttt
-195  caccgggccccacattcggggtcacaattgcaaagaagtacctcaaattcgtagcatatt
-135  cccactgacccagtggggccctctggggatccccatgaatcctccaccgcccacgtggca
 -75  cccctcgtttcccaagttgccaccattgctacaaattgtagccgctctctccatgcttct
 -15  tcttcatctctaacc
   0                  CGTGCTTCCACACTCTTGCAGTAGCTGCTATAACCGCCGTTGCTC
  46  AGCCGCGGAGTTTCCGGTGAAGCGgtgagtgccgttaccgttaccgttacgattcttcat
 106  cacttttgaacctcggttagtagtgataatagtaactgattttgagaaatttttctgcatt
 166  tctccggatcgcttcaatcttactctcttcccactcatacccaattcttttcatatcttc
 226  taatctcttatcccgtgtttttgcatggtatatattgatcttcccttcccctggagtagta
 286  tataagtacgcggtgatcctttctcttttgatcttctgtttcgattctacttatgatgct
 346  agtggcctagtgcatgcaaatcttgaatcttgcttttcgggttcttgattcaatctctttt
 406  gatgggttgttttctaaattctaaagcttgctgatttaaataaatggatttgttcttgaa
 466  aaaagctccagtcttgtctgtgtctgctactttctgatgatactattgagctttataaaa
 526  tgaggtgctaactgttccttattttctagGGTGTGGTGTGAGACTGTGAGTGATGGCAAC
                                                        M  A  T
 586  TATAACTGCCTCACACTTTGTTTCTCATGTCTGTGGGGGTGCCACTTCTGGAGAATCAAA
       I  T  A  S  H  F  V  S  H  V  C  G  G  A  T  S  G  E  S  K
 646  AGTGGGGTTGGGTCAATTAGCCCTGAGGAGTCAAGCTGTGACTCACAATGGGTTGAGACC
       V  G  L  G  Q  L  A  L  R  S  Q  A  V  T  H  N  G  L  R  P
 706  TGTGAACAAGATTGACATGTTACAATTGAGAACCAGTGCCAAGAAGCCCAGCAAAAATGG
       V  N  K  I  D  M  L  Q  L  R  T  S  A  K  K  P  S  K  N  G
 766  AAGGGAAAATGAGGGTGGAATGGCAGCAGGAACTATTGTGTGTAAACAACAAGGGATGAA
       R  E  N  E  G  G  M  A  A  G  T  I  V  C  K  Q  Q  G  M  N
                                                    ↑
 826  CTTGGTCTTTGTGGGATGTGAGGTGGGTCCCTGGTGCAAAACTGGTGGACTTGGAGATGT
       L  V  F  V  G  C  E  V  G  P  W  C  K  T  G  G  L  G  D  V
 886  TCTTGGAGGATTGCCACCAGCCTTGGCAgtaagtctcacacttcatttcatttctgattt
       L  G  G  L  P  P  A  L  A
 946  tacttttagtgtagtttctaaatatataaattttgtatattaatactaaacttaatatta
1006  tgaaattttgaattaaaaataactccagttaagcatcattatgctaatcgaatcgaatca
1066  ggcacgtaaaatgggatggagggaatatttagcttgtgcagttgtgggatagttgtccat
1126  tctacagtttcattagtcatacatgtgatggttgaattgaagaatgaatcgttgctaatt
1186  cagttgtgcgtacatccattttccagGCGCGCGGGCATAGAGTTATGACAGTGTGTCCCC
                                  A  R  G  H  R  V  M  T  V  C  P  R
1246  GTTATGATCAGTACAAAGATGCTTGGGATACCTGTGTGGTTGTTGAGgtaatcttctgcc
       Y  D  Q  Y  K  D  A  W  D  T  C  V  V  V  E
1306  atttttatatgtttgtgctaaatttcttctatgcaacaagtcaaagtattgagtgcttta
1366  tatgtatcttgcagCTACAAGTTGGAGACAGAATTGAACCTGTTCGTTTCTTCCATTCAT
```

```
                    L  Q  V  G  D  R  I  E  P  V  R  F  F  H  S  Y
1426  ACAAACGCGGAGTTGATCGCGTTTTTGTGGATCATCCTATGTTCCTGGAGAAGgttagta
        K  R  G  V  D  R  V  F  V  D  H  P  M  F  L  E  K
1486  tagagtatagactcatggattttccagagttatggtgtatgttaccagtgaattgtttga
1546  atggttataagctactaatcgacatgaatgctttcgatctgtgctatttagGTTTGGGGA
                                                        V  W  G
1606  AAAACTGGATCTATGCTCTATGGCCCCAAGGCTGGGAAAGATTACAAGGACAACCAGTTG
      K  T  G  S  M  L  Y  G  P  K  A  G  K  D  Y  K  D  N  Q  L
1666  CGGTTCAGTTTGTTGTGCCAAgtaatgcatagtgtctaatcgtttatcttaggcttcaca
      R  F  S  L  L  C  Q
1726  tgagtgaacattatttaacatttcacttgtgttggtttcagGCAGCACTTGAGGCACCGA
                                                  A  A  L  E  A  P  R
1786  GAGTTTTGAATCTTAACTCCAGCAACTACTTCAGTGGACCTTATGgtatttttcttcttc
        V  L  N  L  N  S  S  N  Y  F  S  G  P  Y  G
1846  tagaggttttggaagtacttgagattagagtgtggaaagaattaacataataatctttta
1906  tgaaattgtaaagGTGAGGATGTTGTTTTCGTTGCCAATGATTGGCACACTGCTCTCCTT
                        E  D  V  V  F  V  A  N  D  W  H  T  A  L  L
1966  CCATGCTATCTGAAAACCATGTACCAGTCGAGAGGAATCTACATGAACGCCAAGgtaatg
      P  C  Y  L  K  T  M  Y  Q  S  R  G  I  Y  M  N  A  K
2026  cctcttttggattggactggacggaagttttccacatttaatcctacctaatcacaagga
2086  ctatacttatatgtgttcccgctatttgctaaaatgttgccagGTTGCTTTCTGCATTCA
                                                    V  A  F  C  I  H
2146  CAACATTGCCTACCAAGGCAGATTCGCCTTTTCAGACTTTTCTCTTCTGAATCTGCCTGA
       N  I  A  Y  Q  G  R  F  A  F  S  D  F  S  L  L  N  L  P  D
2206  CGAGTACAAGGGTTCTTTTGATTTTATTGATGGgtaggaattagatgcttttagctaaga
       E  Y  K  G  S  F  D  F  I  D  G
2266  gcctaagattatgaattctgtatcttgattcttgctgcttgatctctgaattcaattcat
2326  tctcaattttgccagGTATGACAAGCCAGTGAAGGGGAGAAAGATAAACTGGATGAAAGC
                        Y  D  K  P  V  K  G  R  K  I  N  W  M  K  A
2386  TGGAATACGTGAAGCAGACAGGGTTTTTACTGTGAGCCCAAACTACGCCAAGGAGCTTGT
       G  I  R  E  A  D  R  V  F  T  V  S  P  N  Y  A  K  E  L  V
2446  TTCTTGCGTTTCGAAGGGTGTGGAATTGGACAACCACATCCGAGACTGCGGCATCACTGG
       S  C  V  S  K  G  V  E  L  D  N  H  I  R  D  C  G  I  T  G
2506  TATTTGTAATGGAATGGATACCCAAGAGTGGAACCCTGCAACTGACAAGTACCTTGCTGT
       I  C  N  G  M  D  T  Q  E  W  N  P  A  T  D  K  Y  L  A  V
2566  TAAATATGATATCACAACTgtaagatagtactttaatttgtgtctacattctcatttgat
       K  Y  D  I  T  T
2626  attgaatttagggagttttgctcattcggaattttatgcttaatagGTTATGCAAGCGAA
                                                    V  M  Q  A  K
2686  GCCCTTGTTGAAGGAAGCTCTTCAAGCGGCAGTTGGTTTGCCAGTTGACAGGAATATTCC
       P  L  L  K  E  A  L  Q  A  A  V  G  L  P  V  D  R  N  I  P
```

```
2746  ACTGATTGGTTTTATCGGCAGACTTGAAGAGCAGAAAGGCTCAGACATTCTTTATGCTGC
        L  I  G  F  I  G  R  L  E  E  Q  K  G  S  D  I  L  Y  A  A
2806  GATTTCTAAGTTCATTTCAATGGATGTTCAGATATTGATTCTCgtaagtgctatgtggac
        I  S  K  F  I  S  M  D  V  Q  I  L  I  L
2866  tcttataatgcacgcttttcttatgtccgtcccttgccaaaccgtggatctgaattgca
2926  atgatgaaatcaatcagGGAACTGGGAAGAAGAAATTTGAGCAGCAGATTGAGCAGCTCG
                          G  T  G  K  K  K  F  E  Q  Q  I  E  Q  L  E
2986  AAGTGATGTATCCGGACAAAGCTAGAGGAGTGGCAAAGTTCAACGTTCCTTTGGCTCACA
        V  M  Y  P  D  K  A  R  G  V  A  K  F  N  V  P  L  A  H  M
3046  TGATTACTGCTGGCGCTGATTTTATGTTGATCCCGAGCAGATTTGAGCCGTGTGGTCTCA
        I  T  A  G  A  D  F  M  L  I  P  S  R  F  E  P  C  G  L  I
3106  TTCAGTTGCATGCTATGCGATATGGAACAgtaagaaacccgacacttgaacctatccaaa
        Q  L  H  A  M  R  Y  G  T
3166  actctcctttcatgattcttcctagttaaagttctaattgtcaatataaatgtgtgcata
3226  tatcagCCGTGCATCTGTGCCTCAACTGGCGGACTCGTTGACACTGTGAAAGAAGGTTAT
              P  C  I  C  A  S  T  G  G  L  V  D  T  V  K  E  G  Y
3286  ACAGGGTTCCACATGGGGGCTTTCAACGTCGACgtatgtgattctcaacataatacattc
      T  G  F  H  M  G  A  F  N  V  D
3346  tttctgtctattaattatgttatctcataacaagaggtaacgaagttttgttgttgcttg
3406  tgctcagTGTGAAACTGTTGACCCAGAGGACGTGCTGAAGGTGATAACCACTGTTGGTAG
              C  E  T  V  D  P  E  D  V  L  K  V  I  T  T  V  G  R
3466  AGCACTTGCGATGTACGGAACCCTTGCATTCACTGAAATGATCAAGAACTGCATGTCACA
        A  L  A  M  Y  G  T  L  A  F  T  E  M  I  K  N  C  M  S  Q
3526  AGAGCTCTCGTGGAAGgtaggcattcgcccttgttgagcataaatataatatataaaacg
        E  L  S  W  K
3586  taaatgtgcagtccaactatcagcttaggcttttagttgagatggagcatgtttcaattt
3646  ggtatcagagccatgcaaaaagtcatggttcgaatcgtttgccctaccgggttcaattaa
3706  cagttctccctcgtctacagtttcaaagtcgctaactggttttcgttgcagGGACCTGCC
                                                          G  P  A
3766  AAGAATTGGGAAACAGTGCTGTTGAGCTTAGGAGTTGCTGGGAGCGAGCCCGGGGTTGAA
      K  N  W  E  T  V  L  L  S  L  G  V  A  G  S  E  P  G  V  E
3826  GGAGACGAAATTGCACCACTTGCAAAGGAAAACGTCGCCACTCCATGAAAACTATGGTTT
      G  D  E  I  A  P  L  A  K  E  N  V  A  T  P  *
3886  CATAAGCTGGCATATGAAACTAATAAAGTTTTATATATATGTTGAAAGAGCAGAGGCGAT
3946  TTGGTGCTCTTGAGCATTATGAGGTGATGAAAGAGCCAATGGGGGACTCTTTCTTTATTG
4006  TTTTTTGGTATGCCCAGGTGGTAGTAAATACAGAGTTTAAACTAACTAGTTTGGTGCTTT
4066  AAGACATTGGGAACTTGCAGCACTTCAGCAGGCTAAATGTATAATATAAATAAACAATGG
4126  CCTCTTGTGGTTTTGTGCTTTGAtttccagcaacttgtgatgattttgctatatatatct
4186  ggattcattctacctttagcacactgcatttagaatgctgatacttaagtcatagcattc
4246  attaggtatt
```

Fig. 2. Nucleotide and deduced amino acid sequences of the sweet potato granule-bound starch synthase I gene. Exons are shown as uppercase letters. Introns and flanking sequences are shown as lowercase letters. Position 1 corresponds to the 5' end of the longest cDNA clone. The TATA box and polyadenylation signal are underlined. The arrowhead shows the putative processing site of the protein. The translational termination codon is indicated by an asterisk.

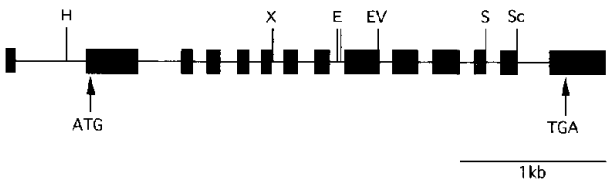

Fig. 3. Exon/Intron structure and restriction map of the sweet potato granule-bound starch synthase I gene. Boxes and lines represent exons and introns, respectively.
E, *Eco* RI; EV, *Eco* RV; H, *Hin*d III; S, *Sal* I; Sc, *Sac* I; X, *Xba* I.

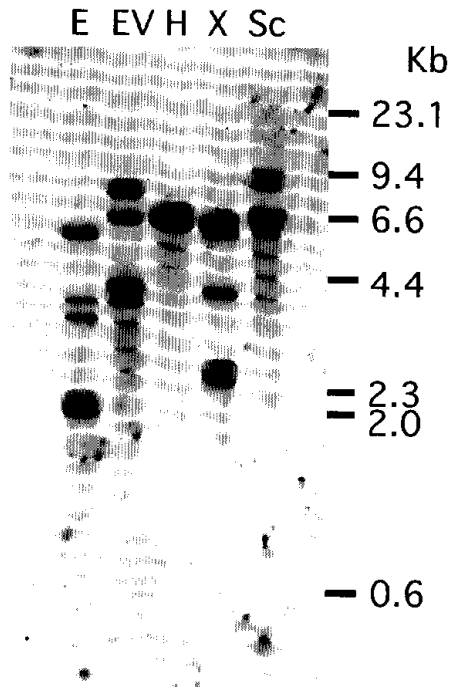

Fig. 4. Southern blot analysis of sweet potato DNA. Genomic DNA was digested with a restriction enzyme and allowed to hybridize to the sweet potato GBSSI cDNA. E, *Eco* RI; EV, *Eco* RV; H, *Hin*d III; X, *Xba* I; Sc, *Sac* I.

References

Denyer, K., Clarke, B., Hylton, C., Tatge, H., Smith, A.M., 1996. The elongation of amylose and amylopectin chains in isolated starch granules. Plant J., **10:** 1135-1143.

Dry, I., Smith, A., Edwards, A., Bhattacharyya, M., Dunn, P., Martin, C., 1992. Characterization of cDNAs encoding two isoforms of granule-bound starch synthase which show differential expression in developing storage organs of pea and potato. Plant J., **2:** 193-202.

Gavel, Y., von Heijne G., 1990. A conserved cleavage-site motif in chloroplast transit peptides. FEBS Lett., **261:** 455-458.

Guiltinan, M.J., Marcotte, W.R., Quatrano, R.S., 1990. A plant leucine zipper protein that recognizes an abscisic acid response element. Sci., **250:** 267-271.

Hirano, H.-Y., Sano, Y., 1991. Molecular characterization of the *waxy* locus of rice (*Oriza sativa*). Plant Cell Physiol., **32:** 989-997.

Klösgen, R.B., Gierl, A., Schwarz-Sommer, Z., Saedler, H., 1986. Molecular analysis of the *waxy* locus of *Zea mays*. Mol. Gen. Genet., **203:** 237-244.

Maddelein, M-L., Libessart, N., Bellanger, F., Delrue, B., D'Hulst, C., van den Koornhuyse, N., Fontaine, T., Wieruszeski, J-M., Decq, A., Ball, S., 1994. Toward an understanding of the biogenesis of the starch granule. J. Biol. Chem., **269:** 25150-25157.

Murai, J., Taira, T., Ohta, D., 1999. Isolation and characterization of the three Waxy genes encoding the granule-bound starch synthase in hexaploid wheat. Gene, **234:** 71-79.

Okagaki, R.J., Wessler, S.R., 1988. Comparison of non-mutant and mutant *waxy* genes in rice and maize. Genetics, **120:** 1137-1143.

Rohde, W., Becker, D., Salamini, F., 1988. Structural analysis of the *waxy* locus from *Hordeum vulgare*. Nucl. Acids Res., **16:** 7185-7186.

Salehuzzaman, S.N.I.M., Jacobsen, E., Visser, R.G.F., 1993. Isolation and characterization of a cDNA encoding granule-bound starch synthase in cassava (*Manihot esculenta* Crantz) and its antisense expression in potato. Plant. Mol. Biol., **23:** 947-962.

Sambrook, J., Fritsch, E. F., Maniatis, T., 1989. Molecular Cloning: A Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Sanger, F., Nicklen, S., Coulson, A.R., 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA, **74:** 5463-5467.

Schulze-Lefert, P., Dangl, J.L., Becker-André, M., Hahlbrock, K., Schulz, W., 1989. Inducible *in vivo* DNA footprints define sequences necessary for UV activation of the parsley chalcon synthase gene. EMBO J., **8:** 651-656.

Shure, M., Wessler, S., Fedoroff, N., 1983. Molecular identification and isolation of the *Waxy* locus in Maize. Cell, **35:** 225-233.

van der Leij, F.R., Visser, R.G.F., Ponstein, A.S., Jacobsen, E., Feenstra, W. J., 1991. Sequence of the structural gene for granule-bound starch synthase of potato (*Solanum tuberosum* L.) and evidence for a single point deletion in the *amf* allele. Mol. Gen. Genet., **228:** 240-248.

Visser, R.G.F., Hergersberg, M., van der Leij, F.R., Jacobsen, E., Witholt, B., Feenstra, W. J., 1989. Molecular cloning and partial characterization of the gene for granule-bound starch synthase from a wildtype and an amylose-free potato (*Solanum tuberosum* L.). Plant Sci., **64:** 185-192.

Wang, Z.-Y., Wu, Z.-L., Xing, Y.-Y., Zheng, F.-G., Guo, X.-L., Zhang, W.-G., Hong, M.-M., 1990. Nucleotide sequence of rice waxy gene. Nucl. Acids Res., **18:** 5898.

Wang, Z.-W., Zheng, F.-Q., Shen, G.-Z., Gao, J.-P., Snustad, D. P., Li, M.-G., Zhang, J.-L., Hong, M.-M., 1995. The amylose content in rice endosperm is related to the post-transcriptional regulation of the *waxy* gene. Plant J., **7:** 613-622.