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Improved Agronomic and Quality Traits in Transgenic Crops: Recent Advances

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Improved agronomic and quality traits in transgenic crops: recent advances

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SUMMARY

The potential for genetic engineering of plants to produce new and useful traits in crops has now been confirmed. Although numerous examples exist in the literature of agronomically important traits, the examples of quality improvements are rare but increasing. This article describes several new agronomic and quality traits produced at the Monsanto Plant Science Division. Tomatoes have been produced that have reduced ethylene levels which result in delayed fruit ripening. Delayed ripening contributes to an extended shelf life of the tomatoes. Higher starch potatoes add value both by the increased yield of starch and by decreasing oil absorption during frying. Cotton that expresses the *Bacillus thuringiensis* gene controls lepidopteran insect feeding damage as effectively as chemical insecticides. These genetically improved plants are valuable additions to modern agriculture and represent clear demonstrations of the types of improvements that are likely in the future.

1. IMPROVING TOMATO QUALITY BY CONTROLLING FRUIT RIPENING

The average annual per capita consumption of fresh tomatoes is eighteen pounds and is increasing at approximately 2% per year. Retail and food service sales of fresh tomatoes have surpassed those of potatoes and lettuce and are valued at greater than $3.5 billion per year. However, the quality of the average store bought tomato still fails to meet consumer expectations and typically is described as having poor flavour and a mealy texture.

The inferior flavour of current fresh tomatoes is a consequence of the stage of fruit development at which time the fruit are harvested. Flavour development correlates to length of time on the vine (Kader *et al.* 1977). To provide tomato fruit year round, agricultural practices were modified to incorporate mature green harvested tomato fruit. Tomato fruit are harvested at the mature green stage and treated with ethylene to facilitate ripening off the vine. Mature green fruit possess the physical properties needed within a system designed to efficiently handle large volumes of tomatoes. Harvesting at a mature green stage also provides a fruit with sufficient shelf life (length of time which fruit are marketable) to enable processing and distribution of fruit over large geographic areas. Mature green fruit, however, do not obtain optimum flavour and when the fruit are allowed to develop full flavour by remaining on the vine longer, they lose the handling properties required for cost effective distribution of the tomato fruit. An additional problem with harvesting mature green fruit pertains to the difficulty in distinguishing a mature green fruit from an immature green fruit. As a result, a typical tomato harvest contains up to 60% immature green fruit (S. Sargent, personal communication). Immature green fruit are far inferior in flavour even compared to the ripened mature green product.

An ideal fresh tomato would produce fruit which can be allowed to remain on the vine long enough to develop full flavour while still maintaining the appropriate firmness and shelf life properties to permit cost effective production of the tomato. We reasoned that, to accomplish this, the process of fruit maturation must be delayed.

The phytohormone ethylene is known to regulate tomato fruit development (Reid 1987). Increases in the level of ethylene correlate with tomato fruit ripening and inhibitors of ethylene synthesis or perception delay fruit ripening. Ethylene is synthesized from methionine via the intermediate 1-aminocyclopropane-1-carboxylate (ACC; McKeon & Yang 1987). We proposed to reduce levels of ethylene and thereby delay fruit development through the selective degradation of ACC. Previous studies have identified a *Pseudomonas* enzyme, ACC deaminase, which selectively degrades ACC to α-ketobutyrate and ammonia (Honma & Shimomura 1978). To obtain a similar enzyme a screening program was established to identify microorganisms which were capable of utilizing ACC as a sole nitrogen source. Several bacterium were identified using this procedure and the gene encoding the enzyme responsible for the ACC degradation was subcloned. Biochemical analyses showed this enzyme to be ACC deaminase (Klee *et al.* 1991).

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The ACC deaminase gene was engineered for plant expression using the cauliflower mosaic virus 35*S* promoter (Klee *et al.* 1991). This promoter will direct expression of the ACC deaminase protein essentially throughout the whole plant. Greenhouse grown, transgenic tomato plants were evaluated to determine the level of ethylene in leaves. Several lines were identified which contained significantly lower levels of ethylene. The ethylene level in leaves of tomato line 5673 was only 5% of that present in the leaves of non-transformed control plants (Klee *et al.* 1991). No obvious phenotypic effect was observed during the development of the ACC deaminase transgenic plants.

Transgenic and control tomato fruit showed similar profiles for ethylene synthesis throughout fruit development, however, the absolute levels of ethylene in the transgenic fruit were 90% lower than observed for the control (Klee *et al.* 1991). The time to ripen while on the vine was not affected at this level of ethylene synthesis. However, there was a significant reduction in overripening for transgenic fruit left on the vine. When fruit were picked at the breaker stage (first sign of colour change) the transgenic fruit showed significant increases in the length of time to develop full red colour, as compared to non-transformed controls, and in some cases transgenic fruit never developed full red colour. In addition, these transgenic fruit maintained firmness, 'on the shelf', for an extended period of time.

We have demonstrated that expressing ACC deaminase in tomato plants significantly reduces the level of ethylene. Transgenic tomato fruit, picked at a breaker stage, show a delay in time to red and a delay in overripening. We believe the ACC deaminase technology will serve as a powerful tool in modulating tomato fruit ripening and allow the development of fresh tomato varieties with both ideal flavour and handling attributes.

2. INCREASED STARCH SYNTHESIS IN POTATO

Potato represents a major staple food in many parts of the world, providing a rich source of dietary carbohydrate. The total market for potato grown in the U.S. alone is well over $2 billion annually, with the value determined predominantly by the food processing industry. The high value processing qualities include dry matter content, cold storage properties, disease tolerance, and chipping and french fry qualities. The most important determinant for these qualities is the dry matter content. A high solids potato processes better due to lower water content (which also reduces transportation and cooking costs), leading to increased processed-product recovery and reduced oil absorption during cooking that results in a product with reduced calories.

The major contributor to dry matter content is starch (Bajaj 1987), comprising about 70% of the dry matter of the mature tuber. Breeding efforts have been unsuccessful in making large gains in starch content in commercial cultivars, mostly because of the recalcitrant nature of potato breeding due to tetrasomic inheritance of characters, male sterility and self-incompatibility in many cultivars, and to high heterozygosity (Wenzel 1980). However, potato has proved amenable to manipulation through transgenic technology (Kaniewski *et al.* 1990; Rocha-Rosa *et al.* 1989), and this could provide a route for increasing starch content and improving dry matter yield in transgenic tubers.

Starch biosynthesis in plants (and glycogen biosynthesis in bacteria) requires the participation of the enzymes ADPglucose pyrophosphorylase (ADPGPP), starch (or glycogen) synthase, and branching enzyme (Preiss 1988, 1991). The first enzyme in the pathway, ADPGPP, utilizes glucose-1-phosphate and ATP to form ADPglucose, which subsequently serves as the substrate for starch synthase. The linear α-1,4-glucan polymer formed by the synthase may be subsequently branched by the activity of branching enzyme(s). In view of its sensitivity to allosteric effectors, ADPGPP has been suggested to be pivotal in plant starch biosynthesis, as it is in the bacterial pathway for glycogen biosynthesis. Therefore it was of interest to determine if enhanced ADPGPP activity would lead to increased starch biosynthesis in plants.

Plant ADPGPPs are tetramers that contain two distinct subunits and are regulated by 3-phosphoglyceric acid (PGA) and P_i as positive and negative effectors, respectively (Preiss 1988, 1991). Although both large and small subunits (Anderson *et al.* 1989; Olive *et al.* 1989; Bhave *et al.* 1990; Muller *et al.* 1990) show homology to the *E. coli* ADPGPP enzyme, the individual subunits do not appear to be catalytically active as evidenced by the absence of significant enzyme activity of the maize and *Arabidopsis* ADPGPP mutants (Bajaj 1987; Lin *et al.* 1988). In contrast, the *E. coli* ADPGPP, encoded by the *glgC* gene (Okita *et al.* 1981), is a regulated homotetrameric enzyme which is activated by fructose 1,6-bisphosphate (FBP) (Preiss *et al.* 1966). FBP increases the catalytic activity (V_{max}), reduces the K_m for the substrates, and modulates the sensitivity of the enzyme to the inhibitors 5′-AMP and inorganic phosphate (P_i) (Preiss *et al.* 1966; Gentner & Preiss 1968). A mutant *E. coli* K12 strain, 618, accumulates approximately 33% higher quantities of glycogen than its wild-type parent due to an alteration in the regulatory properties of ADPGPP (Cattaneo *et al.* 1969; Creuzet-Sigal *et al.* 1972). The mutant enzyme is less dependent on the activator, FBP, and is less sensitive to inhibition by the inhibitor, 5′-AMP, and this is due to a substitution of aspartic acid for glycine at position 336 in the ADPGPP enzyme (Leung *et al.* 1986; Lee *et al.* 1987). In view of the difficulties involved in coordinating the expression of two distinct genes, we chose to express in transgenic plants an *E. coli* ADPGPP enzyme to probe the regulation of metabolic flux by ADPGPP. Additionally, to minimize interference by the complex allosteric regulation of the wild-type *E. coli* gene, we used the mutant *glgC16* gene.

We introduced the *glgC16* gene fused with a transit peptide coding region (CTP) into Russet Burbank potato plants under the control of a tuber-specific patatin promoter (Bevan *et al.* 1986). The use of the transit peptide was required to direct the enzyme into

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the plastid, which is the site of starch biosynthesis. We recovered numerous transgenic plants which were vegetatively identical to the Russet Burbank parent. Subsequent analysis of transgenic plants showed that tubers expressing the patatin-CTP-*glgC16* gene contain, on average, 35% more starch than control tubers (Stark *et al.* 1992). Some transgenic potato lines produced tubers with nearly 60% more starch than controls. Therefore, tissue-specific expression of the CTP-*glgC16* gene using a patatin promoter results in significant increases in starch content without adverse effects on plant growth or development. Similar results recently were obtained in a small scale field trial (Stark *et al.* 1991), indicating that ADPGPP activity is rate-limiting for starch biosynthesis under conditions of potato cultivation.

Although the presence of the CTP-*glgC16* gene product was absolutely required for increased starch production, the degree of increase in starch content did not totally correlate with the level of expression of GlgC16 in all potato tubers (Stark *et al.* 1992). This suggests that relatively low levels of expression of the *E. coli* ADPGPP gene are sufficient to overcome the limitation of ADPglucose availability for starch biosynthesis. Whether the poor correlation with expression levels is due to substrate availability for the ADPGPP reaction, or to an unrelated reaction in the starch biosynthetic pathway remains to be established.

Our results show that ADPGPP activity is rate-limiting in starch biosynthesis. However, previous studies have shown that the activity of starch synthase is only one-twentieth that of ADPGPP and the branching enzymes (Preiss 1988, 1991; Robinson & Preiss 1987). These results imply that starch synthase is the rate-limiting reaction in starch biosynthesis. In view of our finding that enhanced ADPGPP activity increases the starch content of plant cells, we reasoned that allosteric regulation may play a significant role in modulating ADPGPP activity under *in vivo* conditions. To evaluate the importance of allosteric regulation in the control of ADPGPP activity and thus starch content, we expressed the wild-type *E. coli* ADPGPP gene in potato tubers in a manner similar to that of the *glgC16* gene. Unlike the GlgC16 enzyme, wild-type GlgC is fully subject to allosteric regulation (Okita *et al.* 1981), although the primary effector molecules differ between the plant and bacterial enzymes. Thus, depending upon the relative concentrations of these effectors *in vivo*, expression of the CTP-*glgC* gene may also result in increased ADPGPP activity and starch content in a plant cell environment. However, analysis of potato tubers showed that expression of CTP-*glgC* did not result in a noticeable increase in starch content, even though expression levels were equivalent to those in tubers expressing CTP-*glgC16* (Stark *et al.* 1992). We conclude that CTP-*glgC* expression may lead to a slight, but not significant, level of starch increase in plant tissues. The kinetic characteristics of the GlgC and GlgC16 enzymes have been previously reported (Cattaneo *et al.* 1969; Creuzet-Sigal *et al.* 1972; Leung *et al.* 1986; Lee *et al.* 1987), and differ primarily in allosteric regulation. The V_{max} of each enzyme is the same under fully activated conditions. Together, these results indicate that ADPGPP activity is rate-limiting in starch biosynthesis, and it is the regulatory properties of ADPGPP that make it rate-limiting and not the amount of enzyme protein.

The increased starch content in tubers expressing *E. coli* ADPGPP not only supports the role of ADPGPP activity in tuber sink modulation, as previously postulated (Rocha-Rosa *et al.* 1989; Preiss 1991; Lin *et al.* 1988), but presents evidence that modulation of source–sink relationships in the direction of increasing sink strength may prove to be an effective and powerful method of increasing dry matter content in storage organs of many different crops.

3. INSECT CONTROL IN COTTON

Cotton is an important, high value crop. An estimated 85 million acres are grown worldwide with over 12 million acres grown in the United States annually. Insect damage is a serious problem in the intensively managed cotton growing areas of the U.S. causing an estimated $645 million dollars per year in yield losses and insect control costs (Suguiyama *et al.* 1988). The lepidopteran insects, particularly *Helicoverpa zea* (cotton bollworm) and *Heliothis virescens* (tobacco budworm) are responsible for a large amount of insect damage on the high value acres of the Mississippi Delta Region.

Chemical insecticides, particularly pyrethroids, have been an effective means of controlling these insects. This control can be expensive in years of heavy infestation such as 1992, requiring scouting of the fields, timing of applications, and escalating rates of application because of decreasing effectiveness. Biological insecticide alternatives such as *Bacillus thuringiensis*, the bacterium that produces an insect control protein which does have activity against these pests (Aronson *et al.* 1986) has not been adopted for widespread use. These treatments are expensive, have a relatively short duration of protection and are not targeted to protect the productive parts of the plants such as the squares and bolls from insect damage.

Genes encoding the insect control proteins of *Bacillus thuringiensis* have been cloned, characterized and expressed in plants by several groups (Vaeck *et al.* 1987; Fischhoff *et al.* 1987). These plants exhibited insect control in the most sensitive lepidopteran insects but were limited by their low levels of expression of the insect control protein. Modified insect control protein genes, encoding proteins identical in amino acid sequence to the native genes of *Bacillus thuringiensis* have been more efficiently expressed in plants (Perlak *et al.* 1990, 1991). Among the factors dictating the modifications in the coding sequence of these genes are multiple sequence motifs that are not common in the coding regions of plant genes. These include localized regions of A+T nucleotide richness resembling plant introns, potential plant polyadenylylation signal sequences, ATTTA sequences which have been shown to destabilize mRNA in other systems and codons rarely used in plants.

The levels of expression of these modified genes in cotton (Perlak *et al.* 1990) have proven to be adequate

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first in greenhouse assays and then in the field tests that have been reported (Wilson *et al.* 1992) to control key economically important lepidopteran insects such as the tobacco budworm, cotton bollworm and pink bollworm (*Pectinophora gossypiella*). Extensive field test experimentation is currently underway to define the levels of control that these plants can offer the cotton grower and preliminary results are very encouraging. The success of these plants in controlling economically important cotton pests will offer the cotton grower an increased number of options to address his pest problems through more flexible integrated pest management programs. It could result in reduced production costs, reduced grower and environmental exposure to chemical insecticides and increase the effectiveness of the chemical applications that are utilized to manage crop production. This product will require a change in some agricultural practices in growing cotton and innovative crop management to recognize the full value of cotton protected from lepidopteran insect attack. These steps will make growers more productive and efficient in continuing to provide products of value to the world.

4. CONCLUSIONS

The above examples of transgenic crops indicate how new traits can improve the way crops are grown and utilized as well as improving their quality. The use of cotton plants that are naturally resistant to insect feeding reduces the amount of insecticides needed to grow the crop. Tomatoes with delayed ripening characteristics reduce the amount of spoilage after harvest and might provide a better tasting tomato to the grocery store. High starch potatoes might provide a healthful benefit by absorbing less oil during frying, resulting in less fat and less calories in the french fry or potato chip. These types of improved crop traits are only the beginning of the advances modern genetic engineering will bring to agriculture.

REFERENCES

Anderson, J.M., Okita, T.W. & Preiss, J. 1990 Enhancing carbon flow into starch: the role of ADPglucose pyrophosphorylase. In *The molecular and cellular biology of the potato* (ed. M. E. Vayda & W. D. Park), pp. 159–180. Wallingford: C.A.B. International.

Aronson, A.I., Beckman, W. & Dunn, P. 1986 *Bacillus thuringiensis* and related insect pathogens. *Microbiol. Rev.* **50**, 1–24.

Bajaj, Y.P.S. 1987 Biotechnology and the 21st century potato. In *Biotechnology in agriculture and forestry*, vol. 3. (ed. Y. P. S. Bajaj), pp. 3–22. Berlin and Heidelberg: Springer-Verlag.

Bevan, M., Barker, R., Goldsbrough, A., Jarvis, M., Kavanagh, T. & Iturriaga, G. 1986 The structure and transcription start site of a major tuber protein gene. *Nucl. Acids Res.* **14**, 4625.

Bhave, M.R., Lawrence, S., Barton, C. & Hannah, L.C. 1990 Identification and molecular characterization of *Shrunken-2* cDNA clones of maize. *Pl. Cell* **2**, 581.

Cattaneo, J., Damotte, M, Sigal, N., Sanchez-Medina, F. & Puig, J. 1969 Genetic studies of *Escherichia coli* K12 mutants with alterations in glycogenesis and properties of an altered adenosine-diphosphate-glucose-pyrophosphorylase. *Biochem. biophys. Res. Commun.* **34**, 694–701.

Creuzet-Sigal, N., Latil-Damotte, M. & Puig, J. 1972 Genetic analysis and biochemical characterization of mutants impairing glycogen metabolism in *Escherichia coli* k12. In *Biochemistry of the glycosidic linkage* (ed. R. Piras & H. G. Pontis), vol. 2, pp. 647–680. The Pan-American Association of Biochemical Societies Symposium. New York: Academic Press.

Gentner, N. & Preiss, J. 1968 Biosynthesis of bacterial glycogen. VI. Differences in the kinetic properties of the *Escherichia coli* B adenosine diphosphate glucose pyrophosphorylase depending on whether Mg+2 or Mn+2 serves as divalent cation. *J. biol. Chem.* **243**, 5882.

Honma, M. & Shimomura, T. 1978 Metabolism of 1-amino-cyclopropane-1-carboxylic Acid. *Agric. biol. Chem.* **42**, 1825–1831.

Kader, A., Stevens, M.A., Albright-Holton, M., Morris, L. & Algazi, M. 1977 Effect of fruit ripeness when picked on flavor and composition in fresh market tomatoes. *J. Am. Soc. Hort. Sci.* **102**, 724–731.

Kaniewski, W., Lawson, C., Sammons,B. *et al.* 1990 Field resistance of transgenic Russet Burbank potato to effects of infection by potato virus X and potato virus Y. *BioTechnology* **8**, 750–754.

Klee, H., Hayford, M., Kretzmer, K., Barry, G. & Kishore, G. 1991 Control of ethylene synthesis by expression of a bacterial enzyme in transgenic tomato plants. *Pl. Cell* **3**, 1187–1193.

Lee, Y.M., Kumar, A. & Preiss, J. 1987 Amino acid sequence of an *Escherichia coli* ADPglucose synthetase allosteric mutant as deduced from the DNA sequence of the *glgC* gene. *Nucl. Acids Res.* **15**, 10603.

Leung, P., Lee, Y.-M., Greenberg, E., Esch, K., Boylan, S. & Preiss, J. 1986 Cloning and expression of the *Escherichia coli glgC* gene from a mutant containing an ADPglucose pyrophosphorylase with altered allosteric properties. *J. Bacteriol.* **167**, 82–88.

Lin, T.P., Caspar, T., Somerville, C. & Preiss, J. 1988 Isolation and characterization of a starchless mutant of *Arabidopsis thaliana* (L) Henyh lacking ADPglucose pyrophosphorylase activity. *Pl. Physiol.* **86**, 1131–1135.

McKeon, T. & Yang, S.F. 1987 Biosynthesis and metabolism of ethylene. In *Plant hormones and their role in plant growth and development* (ed. P. J. Davies), pp. 94–112. Martinus Nijhoff Publishers/Kluwer Academic.

Muller, B.T., Koßmann, J., Hannah, L.C., Willmitzer, L. & Sonnewald, U. 1990 One of two different ADP-glucose pyrophosphorylase genes from potato responds strongly to elevated levels of sucrose. *Molec. Gen. Genet.* **224**, 136–146.

Okita, T.W., Rodriguez, R.L. & Preiss, J. 1981 Biosynthesis of bacterial glycogen: cloning of the glycogen biosynthetic enzyme structural genes of *Escherichia coli*. *J. biol. Chem.* **256**, 6944.

Olive, M.R., Ellis, R.J. & Schuch, W.W. 1989 Isolation and nucleotide sequences of cDNA clones encoding ADP-glucose pyrophosphorylase peptides from wheat leaf and endosperm. *Pl. molec. Biol.* **12**, 525.

Preiss, J. 1988 Biosynthesis of starch and its regulation. In *The biochemistry of plants* (ed. J. Preiss), vol. 14, pp. 181–254. San Diego: Academic Press.

Preiss, J. 1991 Biology and molecular biology of starch synthesis and its regulation. In *Oxford surveys of plant molecular and cell biology*, vol. 7 (ed. B. Miflin), pp. 59–114. Oxford University Press.

Preiss, J., Shen, L., Greenberg, E. & Genter, N. 1966 Biosynthesis of bacterial glycogen. IV. Activation and inhibition of the adenosine diphosphate glucose pyrophosphorylase of *Escherichia coli* B. *Biochemistry* **5**, 1833.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Reid, M. 1987 Ethylene in plant growth, development and senescence. In *Plant hormones and their role in plant growth and development* (ed. P. J. Davies), pp. 257–279. Martinus Nijhoff Publishers/Kluwer Academic.

Robinson, N.L. & Preiss, J. 1987 Localization of carbohydrate metabolizing enzymes in guard cells of *Commelina communis*. *Pl. Physiol.* **85**, 360.

Rocha-Rosa, M., Sonnewald, U., Frommer, W., Stratmann, M., Schell, J. & Willmitzer, L. 1989 Both developmental and metabolic signals activate the promoter of a class I patatin gene. *EMBO J.* **8**, 23–31.

Stark, D.M., Barry, G.F., Muskopf, Y.M., Timmerman, K.P., Kishore, G.M. 1991 Increased starch and dry matter deposition in transgenic russet burbank potato tubers. Paper presented at the Third International Congress of Plant Molecular Biology, Tucson, Arizona, 6–11 October 1991, abstract 714.

Stark, D.M., Timmerman, K.P., Barry, G.F., Preiss, J. & Kishore, G.M. 1992 Regulation of the amount of starch in plant tissues by ADPglucose pyrophosphorylase. *Science, Wash.* **258**, 287.

Suguiyama, L. & Osteen, C. 1988 The economic importance of cotton insects and mites. United States Department of Agriculture Economic Research Service, Agricultural Economic Report Number 599.

Vaeck, M., Reynaerts, A., Hofte, H. *et al.* 1987 Transgenic plants protected from insect attack. *Nature, Lond.* **328**, 33–37.

Von Scheele, C. 1937 Die Bestimmung des Starkghelts und der Trockensubstanz der Kartoffel mit hilfe des Specifischen gewichte.*Landw Ver Stn* **127**, 67–96.

Wenzel, G. 1980 The potentials and limits of classical genetics in plant breeding. In *Plant cell cultures: results and perspectives* (ed. F. Sala, B. Paris, R. Cella & O. Cifferri), pp. 33–47. Elsevier.

Wilson, F.D., Flint, H.M., Deaton, W.R. *et al.* 1992 Resistance of cotton lines containing a *Bacillus thuringiensis* toxin to Pink Bollworm (Lepidoptera: Gelechiidae) and other insects. *J. econ. Entomol.* **85**, 1516–1521.

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