The Genetic Improvement of Apple

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When we look at the evolution of apple varieties mass marketed at the wholesale and retail level in the United States, we started with a red apple, either Red Delicious or McIntosh, depending on the geographic area. Red apples were followed by the introduction and acceptance of a yellow-fruited variety, Golden Delicious. Next, a distinctive green apple, Granny Smith, became popular for its color as well as for its distinctive taste. While other varieties also fit into the red and yellow categories, the main varieties stayed the same.

The acceptance of the bi-colored Gala with a spicy flavor and the "ugly" yet very sweet Fuji may have been aided by their distinctiveness. Now we see Pink Lady and its marketing tied to its pink color and the significance of pink as a symbol of women's health issues. The importance of good names in marketing has become apparent with the success of Honeycrisp, a name that successfully describes the unique sensory attributes of this apple.

In trying to develop distinctive new varieties we are in a "Catch 22" dilemma. Breeders want to use well-characterized, good quality parents to impart quality traits to their seedlings, yet would like those seedlings to have distinctive characteristics that set them apart from their parents for easier marketing. However, in many cases "the apple doesn't fall far from the tree." Sometimes chance seedlings such as Cameo resemble a probable parent, in Cameo's case, Delicious. Cameo resembles Delicious in appearance and in having a mild flavor. Many of the new apple cultivars released by New Zealand have predominately Gala and Splendour as parents. Pacific Rose, Pacific Beauty and others in the Pacific series are similar to the parents in some attributes and also have some of the faults of the parents, such as russetting or cracking.

THE NEED FOR DISTINCTIVE VARIETIES

In developing new apple cultivars we need to ask whether this new cultivar is different, distinctive, better, more nutritious, disease resistant or of superior quality.

Genetic Diversity in Apple Breeding

When the genetic diversity of parents being used in apple breeding was examined, 64% of the 439 cultivars studied were descended from only five founding clones: Cox's Orange Pippin, Golden Delicious, Delicious, Jonathan and McIntosh (Noiton and Alspach, 1996). The genetic base of cultivated apples is very narrow and could be improved by expanding the number and types of parents being used in breeding.

> *. . . We need to ensure that the new cultivars being produced are different, distinctive, better, more nutritious, disease resistant or of superior quality.*

The Need for Non-Russetting Yellow-Fruited Cultivars

Golden Delicious and other cultivars with Golden Delicious in their pedigree are very prone to surface russetting of the fruit when grown in New York. The problem is that many are not as high quality as Golden Delicious. There is still room for good non-russetting Golden Delicious types and also for a good quality, blushed Golden Delicious. Some Golden types being tested include Silken from the Summerland, British Columbia, program and Delblush (Tentation) from France and Suncrisp from Rutgers University. Delblush is prone to russet and Suncrisp, while of good quality, is prone to many diseases and to soft scald. These and other cultivars are being tested by researchers in the US and Canada as part of NE-183 regional project "Multidisciplinary evaluation of apple varieties." Performance information and disease and insect susceptibility information at different sites are available on-line *(www.virtualorchard.com).*

Russeted Cultivars

Apple fruit surface russeting can range from slight to extensive. It may be localized to the stem cavity or occur in other areas. Full surface russetting occurs with cultivars like Hudson's Golden Gem. Ironically when we made some crosses to study the genetics of russeting and to produce new, high quality russeted cultivars, some of the progenies had fruits that were russet-free. At the same time, in certain progeny of non-russetting parents (such as Braeburn x Honeygold) fully russeted offspring occurred at low frequencies. A russeted cultivar would have a unique market niche for its appearance and for the different and distinctive texture and flavor.

Green-Skinned Cultivars

The development of green-skinned cultivars adapted to New York's shorter growing season is complicated by the scarcity of green-fruited cultivars to use as parents. Granny Smith is the obvious choice as a parent for green color and is the parent in many new green cultivars such as Baujade from the INRA program in France and Greenstar from Belgium. Unfortunately, Granny Smith ripens too late in New York State and is susceptible to fire blight, powdery mildew and storage scald. In addition, Granny Smith can develop a blush under our growing conditions so progeny may also have some fruit coloration.

While Mutsu (Crispin) could be a source for green color, Mutsu is a triploid and if used in breeding will produce many aneuploid seedlings, seedlings with the loss of certain chromosomes needed for adequate productivity, fruit quality and performance. Mutsu is also highly susceptible to blister spot caused by the bacteria *Pseudomonas syringae.*

Orin, also from Japan, is another cultivar for use in breeding green-fruited cultivars, as is Shamrock, a cultivar developed at Summerland, British Columbia. Ginger Gold produces green offspring when used in crosses with green or yellow cultivars.

PARENTS USED IN BREEDING

If we examine popular cultivars being used in breeding today we see extensive use of Gala x Splendour both in New Zealand and in Summerland, Canada. Fuji, Braeburn, Elstar, Clivia (used quite a bit in Germany) and Honeycrisp also are being used extensively. Parents in apple breeding can be grouped into two categories, mild and intensely flavored.

Parents with Mild Flavor and/or Acidity

This category includes Ambrosia, Huaguan, Cameo and Honeycrisp. There are consumers who prefer mild flavor. The dominance of Delicious in the market means that future consumers will continue to like low acid, mild apples since studies have shown that adult preferences for apples are for varieties similar to those a person had been exposed to in youth.

Honeycrisp as a Parent

When Honeycrisp is used as a parent many of the positive attributes such as the crispness and juiciness are transmitted to its offspring. Unfortunately, many of the faults of Honeycrisp are also transmitted. Many offspring of Honeycrisp are prone to a clustering habit, have variable maturity within a tree and are susceptible to bitter pit and powdery mildew. Several Honeycrisp offspring have also developed soft scald in storage. Therefore, we need to evaluate carefully such selections for consistency of quality and determine the cultural challenges, such as calcium sprays for management of bitter pit.

Honeycrisp is heterozygous for color, it has a dominant gene for red color and a recessive gene for yellow, so that one-quarter of the progeny will be yellow-fruited when Honeycrisp is crossed with another cultivar that is heterozygous. Interestingly, Honeycrisp has a mild flavor, but surprisingly high acidity (~0.76 malic acid), so this acidity must be balanced with high sugars in genetic improvement. Some offspring are much too acidic.

Parents with Intense Flavor or Acidity

This is my preference for parents because strongly flavored cultivars may become weaker in flavor or acidity after storage, yet maintain acceptable flavor, whereas mild cultivars may become too bland following storage. Braeburn, Cox's Orange Pippin, Elstar and Corail (Pinova) are in this intense flavor grouping. Breeders in Europe tend to emphasize fully flavored parents.

GENETIC TOOLS—MOLECULAR MARKERS

To develop a molecular marker, the primers (based on DNA sequences) must produce bands on a gel that are present when a trait is present and absent when the trait is absent. In this case the presence of the band is linked to the trait and the laboratory tests can be used to "genotype" seedlings or know what genes they possess. This is reviewed in Brown and Maloney (2003). Markers being used in apples include RAPDs (Randomly Amplified Polymorphic DNA) (Conner et al., 1997; Maliepaard et al., 1998), AFLPs (Amplified Fragment Length Polymorphisms) (Tignon et al., 2000), SSRs (Simple Sequence Repeats) (Liebhard et al., 2003) and ISSRs (Inter Simple Sequence Repeats) (Goulao and Oliveira, 2001).

Many markers have been discovered in apple including those for fruit color (Cheng et al., 1996) and self-incompatibility groups (allowing us to determine which crosses are compatible and what cultivars can be used for cross-pollination) (Broothaerts, 2003). Markers for plant form attributes and resistance to disease are discussed below. There are also markers for resistance to rosy apple aphid and woolly apple aphid. Currently we do not have molecular tools to differentiate among genetic sports, but "SNP" technology (Single Nucleotide Polymorphism) may give us the means to differentiate on the basis of single nucleotide differences.

ESTs or expressed sequence tags are being generated from cDNA libraries prepared from fruits of varying degrees of development. These offer great prospects for enhanced understanding of genes involved in flowering, fruiting and vegetative development (Gardiner et al., 2003).

Linkage Maps (Genome Mapping)

The first genetic linkage maps of apple (Rome Beauty and White Angel) were made at Cornell (Hemmat et al., 1994). Linkage maps now exist for several different cultivars, including Wijcik McIntosh (the reduced-branching mutation of McIntosh) and two scab-resistant advanced breeding selections from Cornell (Conner et al., 1997), Prima and Fiesta (Maliepaard et al., 1998), Iduna and selection A679-2 (Gianfranceschi et al., 1998) and Discovery. Liebhard et al. (2003) created a saturated reference map that will aid research on genetic improvement. Many additional cultivar maps have been developed or are being developed.

Self-Incompatibility

When an apple cultivar is named, growers usually have little information about which cultivars are pollen compatible and can be used for pollination. Seventeen S incompatibility alleles govern the incompatibility reaction in apple. Many cultivars share the same incompatibility alleles and are then cross compatible (Broothaerts, 2003). This is due to the common genetic background of many of our most popular cultivars. Further research may help in the development of self-fertile apple trees.

Plant Architecture

Laurens (1999) surveyed apple breeders and found that genetic improvement of plant form is a major goal of many programs, especially for spur type, columnar (reduced branching) and tip bearing. This work will be enhanced by markers developed or being developed for these traits. Lauri et al. (1997) raised important questions on the role of morphological traits in selection of non-biennial bearing. More research is needed on how different genes for plant form interact, which are dominant and which are subject to environmental effects. There are markers for columnar or reduced branching habit, weeping habit, spur habit, tip-bearing and compact types.

Breeding for Resistance to Apple Scab (*Venturia inaequalis*)

Similar to disease susceptible cultivars, the genetic base in scab resistance breeding is restricted, with 90% of the scab resistant cultivars relying on the V_f gene for resistance from *Malus floribunda* 821 (Crosby et al., 1992). Prima, Liberty and Florina are commonly used as parents. Unfortunately two new races, race 6 and 7, have been identified that are virulent on cultivars with the V_f gene (Benaouf and Parisi, 2000). In considering the development of disease-resistant cultivars we need to consider the source of scab resistance and the level of resistance to powdery mildew, moldy core, fire blight, rusts and frog-eye leaf spot.

For example, the scab resistant cultivar Scarlet O'Hara has excellent firmness and storage life, but its use as a parent is a concern due to its high rate of moldy core and extreme susceptibility to fire blight. An open calyx is necessary for moldy core and parents with open calyxes, such as Scarlet O'Hara, tend to transmit this attribute to a great majority of their offspring. Multiple resistance is a goal of many programs, including the PRI program, the DARE program and the Dresden/Pillnitz program in Germany (Fischer, 2000).

Disease Resistance and Marker-Assisted Selection

There are at least 8 major genes for resistance to apple scab. Many researchers have found markers for the V_f gene from *Malus floribunda* 821. This gene was mapped and cloned (Patocchi et al., 1999; Xu and Korban, 2002). The V_f gene has been introduced into susceptible cultivars and is conferring resistance to apple scab (Barbieri et al., 2003).

Markers have also been developed for the V_m gene for resistance that is susceptible to race 5 of scab (Cheng et al., 1998). Two distinct genes, V_r and V_x, from Russian seedling R12740-7A have also been mapped and markers developed (Hemmat et al., 2002). Recently, markers have been developed for V_a from Antonovka and V_b from Hansen's baccata #2 (Hemmat et al., 2003). This will enable breeders to pyramid sources of breeding. Research on V_f was aided by the European project emphasizing Durable Apple Resistance (DARE) (Lespinasse et al., 2000). The DARE group is emphasizing the study of polygenic (many genes) resistance that appears in older cultivars possessing good "field" resistance to disease (Lateur et al., 1999).

For resistance to the bacterial pathogen fire blight (*Erwinia amylovora*) we need to ensure that resistance is characterized as to stage of growth. Material with resistance to shoot infection may still be susceptible to blossom infection. Transgenic approaches hold promise in this area.

Markers also exist for many sources of resistance to the fungal disease powdery mildew (*Podosphaera leucotricha*) (Dunemann et al., 1999; Phillips et al., 2000).

ENHANCED APPLE QUALITY

When we talk of fruit quality we may be talking of quite different attributes, depending on the individual, the circumstances, the perception and the subjective nature of characterizing quality components. Fruit quality definitions differ greatly so we need to define the parameters, develop quantitative (non-subjective) methods of assessment, evaluate the methods and see how they relate to sensory perception of the trait.

This is a major area of study in many breeding programs, in transgenic approaches to improving apple, in postharvest physiology research and in market analyses.

Flavor, texture, firmness and juiciness are the primary traits being emphasized. As with any quality trait, there is a need for consistency. Too firm, too hard, too coarse and too spongy textures are all possible. Some apples break into chunks when chewed and present a potential choking hazard. Crossing cultivars for enhanced firmness is complicated by our inability to characterize these different types of textures, but advances are being made.

For juiciness, the viscosity of the juice has an influence on the "mouth feel" or perception of juiciness. We found that acidity could have an effect on perception of juiciness. Goldrush is low in total juice, yet the acidity of the fruit stimulates saliva production resulting in

Goldrush being perceived as juicy in sensory testing (Boyden, 2002).

Sensory testing has become an important, integral part of many breeding programs with descriptive sensory analysis and correspondence analysis used to select apples for fresh and processing markets in Canada (Deslauriers et al., 1999; Hampson et al., 2000). Regional differences in consumer preference for visual, flavor and texture of apple cultivars were also evaluated in certain regions of Canada (Cliff et al., 1999). Harker et al. (2002a, b) examined the sensory interpretation of instrumental measurements of the texture of the apple fruit and the sweet and acid taste of apple fruit. These studies will help to suggest appropriate measurements and threshold values for quality and its perception.

Genetic Sports

Mutations for fruit surface color and for spur-type plant form have been commonplace in apple and often occur in orchards that have been pruned intensively or subjected to very severe freezes. Improved coloring sports of Delicious and McIntosh are well known, and increasingly sports of Gala, Fuji and Braeburn are also being selected. Yet Empire, a hybrid of McIntosh (with many sports) and Delicious (with many sports), has had only three improved coloring sports identified.

New discoveries in genetics may provide insight to these occurrences. In apple the existence of transposons has been determined (Tignon et al., 2001). These "turn on" or "turn off" certain genes depending on their location. In the future we may be able to activate transposons for enhanced color or spur-habit. Studies of transposons and their effects may allow us to further examine the negative effect of enhanced pigmentation on fruit aromatics (Fellman et al., 2000).

Transgenic Approaches

Transgenic approaches that allow researchers to maintain cultivar identity while changing one or more key characteristics are very desirable in apple due to the established marketing for existing cultivars and name recognition by consumers. Greensleeves was the first apple cultivar to be transformed and to have the transmission of its transgenes to its offspring demonstrated (James et al., 1989, 1996). However, Gala and its sports lead the list of apple cultivars transformed. Transgenic Gala exist with just selectable markers (Yao et al., 1995; 1999a), some transgenic Gala have lytic peptides for resistance to fire blight (Ko et al., 2002), sam-k for reduction of ethylene (Bommineni et al., 2000) and ACC and PG for reduced softening (Hrazdina et al., 2003).

Other cultivars transformed include Jonagold with antimicrobial peptides for resistance to disease (Broothaerts et al., 2000), McIntosh with endochitinase for disease resistance (Bolar et al, 1999, 2001), Delicious and Pink Lady (Sriskandarajah and Goodwin, 1998), Golden Delicious and Elstar (Puite and Schaart, 1996), Florina (Chevreau et al., 2001) and Fuji (Song et al., 2001). The Japanese apple cultivar Orin has the most unique transgenes including sorbitol-6-phosphate dehydrogenase, chitinase, glucanase and scarotoxin (Soejima et al., 2000). Non-browning of the flesh is also a goal (Murata et al. 2000).

Expression of the transgene must be sufficient to provide the resistance or attributes required without adversely affecting plant performance. Bolar et al. (2000) found that while endochitinase expression increased resistance to apple scab, certain levels resulted in stunted plants of very low vigor. Overexpression of polygalacturonase (involved in firmness) in transgenic apple resulted in leaf abnormalities that influenced plant performance (Atkinson et al., 2002).

Therefore expression levels of the gene must be balanced with any adverse effects on the plant. We need tissue-specific promoters and stress- or wound-inducible promoters that will "turn on" or activate a gene only when it is needed or only in certain tissues. These promoters are being developed for apple (Gittens et al., 2001). We must also ensure that any pathogenesis-related proteins native to apple or being used as transgenes from other organisms do not enhance allergenicity in apple. The promoter for the major allergen in apple (Mal d1) can be induced when challenged by stress or pathogens (Puhringer et al., 2000).

Intellectual Property Issues

Increasingly we see new cultivars being controlled and marketed under a club or franchise arrangement where only certain nurseries or growers/packers are allowed to provide trees or grow and pack fruit. There is a fee to belong to the club, there can be a per hectare charge and a certain amount (3 to 11%) goes to support marketing of the new cultivar. Pink Lady is one of the first apple cultivars to be marketed in this way, followed by Delblush (Tentation) and now for Pacific Rose and Jazz. Many nurseries belong to more than one cultivar marketing group.

In transgenic approaches intellectual property rights are also very important. Agreements must be reached with the owners of the cultivar being transformed, the selectable markers, the genes of interest and the promoters. If licensing fees for each component are added to the royalties, will transgenic apples be affordable?

Apetalous and Seedless

Apple blossoms without petals (termed apetalous) have been noted to occur in certain apple cultivars such as Spencer Seedless since the early 1900s. In advertisements, seedless apples were touted as a chance to "revolutionize the apple industry." Apetalous apple cultivars set fruits with little to no seeds. The apetalous gene is recessive meaning that two parents with normal blossoms must carry this "hidden gene" and, when crossed, only 25% of the seedlings will have this trait (Tobutt, 1994).

When researchers examined the genetic sequence of seedless apples in contrast to the sequence for the model plant *Arabidopsis*, they found that apetalous cultivars have mutations that block the function of a gene (*Pi*) that is not blocked in seeded cultivars (Yao et al., 2001).

Genes Involved in Flowering and Fruit and Shoot Development in Apple

Many genes involved in flowering and fruiting were first identified in model crops such as *Arabidopsis*. This information was then used to search for similar genes in unrelated crops. Yao et al. (1999b) found seven MADS-box genes expressed in different parts of the apple fruit. Kotoda et al. (2000) was able to show the expression pattern of homologues of floral meristem identity genes *LFY* and *AP1* during flower development in apple. Kotoda et al. (2002) then found that overexpression of MdMADS5, an APETALA1-like gene of apple, causes early flowering in transgenic *Arabidopsis*. Such research is key to learning more about complex floral and vegetative traits.

Fruit Ripening and Storage

Aminocyclopropane-1-carboxylate (ACC) synthase, ACC oxidase and polygalacturonase have been shown to play a role in apple ripening and storage. Dong et al. (1991) cloned a cDNA encoding 1-aminocyclopropane-1-carboxylate synthase and demonstrated expression of its mRNA in ripening apple fruit. Atkinson et al. (1998) examined ripening specific gene expression of apple ACC-oxidase and polygalacturonase in tomato. In 2000, Harada et al. found that an allele (form) of the ACC synthase gene (Md-ACS1) accounts for the low level of ethylene production in climacteric fruits of some apple cultivars. Research in this area holds promise for the improvement of shelf and storage life.

Fruit Nutrition

The health benefits of apples have received greater attention lately. Examples include the use of apples in a weight reduction program sponsored by a national gym as part of a promotion. The campaign suggests eating three apples a day for weight loss. The importance of antioxidants in apple has also received attention (Eberhardt et al., 2000).

Great variability exists for ascorbic acid content in apple and this has and will be improved by traditional breeding. We also have the ability to genetically modify and increase specific antioxidants. Non-browning flesh can be achieved by breeding cultivars with high vitamin C and low levels of the polyphenol oxidase enzyme that contributes to flesh browning.

Gene Discovery

There are many examples of traits in apple that are controlled by single genes and that could be used in genetic improvement (Alston et al., 2000; Brown, 1992). Examples include russeting, tip bearing and dwarfing. There are many ornamental apple cultivars that offer genes for resistance or attributes such as red flesh or high tannins for cider production. Studies on cold hardiness (Luby et al., 1999) and characterization and utilization of germplasm from the center of origin promises to diversify apple improvement (Hokanson et al., 1997).

Self-fertility and self-thinning are also promising areas for further progress. Quality and its characterization will require multi-institution and multi-disciplinary research, but genetic components will be identified as exemplified by King et al. (2000) and their work on genetic components of apple texture. Studies of transgene expression, transgene silencing and inheritance of transgenes offer prospects of better understanding gene structure and function in apple. More detailed genetic studies of quality and postharvest attributes are needed, such as genetic control of storage scald and use of mapping populations to answer questions about the mechanisms. New discoveries will offer additional tools for the genetic improvement of apple.

SUMMARY

Apple breeders have many new tools and techniques to genetically improve apple, and discoveries are being made at a rapid rate that will enhance our ability to manipulate characters of interest. In producing new cultivars by traditional breeding or transgenic approaches, we need to ensure that the new cultivars being produced are different, distinctive, better, more nutritious, disease resistant or of superior quality. These new tools will aid us in the development of unique fruits well suited to marketing that will provide the consumer with something new and of high quality.

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