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Classical Genetics and Traditional Breeding

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ABSTRACT

Much advancement has been made in traditional breeding and classical genetics of cucurbit crops, although most significant advancement have been related to qualitative traits. Significant improvement in many quantitative traits have been much harder to achieve, and typically result from several incremental advances that occur over a long period of time. Molecular techniques have the potential to overcome many of the obstacles presented by traditional breeding techniques, but it is imperative that the development and utilization of these new molecular technologies work in conjunction with traditional breeders who have the skill set necessary to evaluate the germplasm resulting from these new technologies.

3.1 Introduction

Cucurbit crops simultaneously bestow upon the breeder several advantages and disadvantages. As pointed out by Whitaker and Bohn (1950), cucurbit crops are easily grown, indeterminant plant types, which typically offer plenty of large flowers to work with over a fairly long period of time. Probably the greatest disadvantage is that cucurbit crops tend to be large plants that require abundant field space for proper examination of most agronomically important traits. Adding to the disadvantages, cucurbit

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crops must be hand-pollinated to prevent cross-pollination, and since most selections are for fruit qualities, pollinations must be made before selection of desired phenotype. Consequently, our genetic understanding has lagged behind other crops that can be more easily self-pollinated and properly evaluated in a smaller area, such as tomato.

The establishment of the Cucurbit Genetics Cooperative (CGC) and the annual publication of Cucurbit Genetics Cooperative Report has facilitated the dissemination of information on cucurbit genetics. For example, the number of identified genes in watermelon has grown from a total of 25 prior to the establishment of the CGC in 1978, to 60 genes and 111 isozyme markers in 2007, the most recent year in which the genes were reported for watermelon (Wehner 2007). This annual report is an excellent resource for cucurbit breeders since each crop's gene list (watermelon, melon, cucumber, squash and pumpkin, other genera and species) is updated every five years.

3.2 Classical Genetics

3.2.1 Inheritance of Traits

Classical genetics have increased our understanding of cucurbit crops and aided breeders in the development of new and improved varieties. Breeders in the past have been able to make improvement without understanding the genetic control of these traits, but improvement under these conditions are painstakingly slow. Understanding how different genes affect a variety of traits allow breeders to devote the proper resources needed to improve a particular trait. For example, if a breeder is selecting for a trait controlled by a single gene, the population size will likely be much smaller than if the trait is controlled by multiple genes with a large environmental influence. The application of Mendelian genetics using classical techniques has facilitated the discovery of a number of genes and their inheritance in cucurbit crops.

3.2.1.1 Watermelon

Watermelon is a useful crop species for genetic research because of its small genome size, and the many available gene mutants. The genome size of watermelon is 424 million base pairs (Arumuganathan and Earle 1991). Like some of the other cultivated cucurbits, watermelon has much genetic variability in seed and fruit traits. Genetic investigations have been made for some of those, including seed color, seed size, fruit shape, rind color, rind pattern, and flesh color.

Many watermelon fruit quality genes have been identified. Fruit shape is controlled by an incompletely dominant gene, resulting in elongate (*OO*), oval (*Oo*), or spherical (*oo*) fruit (Weetman 1937; Poole and Grimball 1945). A recessive gene (*f*) controls furrowed fruit surface (Poole 1944). Explosive rind (*e*) causes the fruit rind to burst or split when cut (Porter 1937); these fruit are easily crushed and are sometimes used as pollenizer cultivars not intended for harvest. Tough rind (*E*) improves shipping ability. The single recessive gene *su* (Chambliss et al. 1968) eliminates bitterness in *C. lanatus*, and is allelic to the dominant gene (*Su*) for bitter flavor in *Citrullus colocynthis*.

Watermelon flesh color is controlled by several genes to produce scarlet red, coral red, orange, salmon yellow, canary yellow, pale yellow, green, or white. Genes conditioning flesh colors are *B* (Shimotsuma 1963), *C* (Poole 1944), *Wf* (Shimotsuma 1963), *y* (Porter 1937), *y-o* (Henderson 1989; Henderson et al. 1998), and *py* (Bang et al. 2010). There is some confusion in the literature regarding flesh color inheritance, possibly due to the potential presence of two different "white flesh" phenotypes (Bang et al. 2010). *Wf* is reported to control white flesh in watermelon and is reported to be epistatic to *B*, where genotypes *Wf---* are white, *wfwfB-* are yellow and *wfwfbb* are red (Shimotsuma 1963). Henderson et al. (1998) reported two genes separate red flesh and canary yellow flesh, *C* and *i-C*, where a dominant *C* gives canary yellow flesh except in the presence of a dominant *i-C*, which would give red flesh. Bang et al. (2007) demonstrated a single gene distinguishing red and canary yellow flesh. They also showed a pale yellow phenotype can result between crosses of canary yellow and red (Bang et al. 2010). The recessive *py* gene seems to require the presence of a dominant *C* gene. This *py* mutant may have been confused with white flesh, which is caused by a dominant *Wf*. Interactions of *Wf*, *B*, *C*, and now *py* need further study for clarification.

The coral red gene (*Y*) is dominant to salmon yellow (*y*), and orange flesh (*y-o*) is a member of a multiple allelic system at that locus, where *Y* (*Coral red flesh*) is dominant to both *y-o* (*orange flesh*) and *y* (salmon yellow), and *y-o* (*orange flesh*) is dominant to *y* (salmon yellow). It is reported that a dominant *Scr* produces scarlet red flesh instead of the recessive coral red flesh (summarized in Wehner 2007). *Scr* is now believed to be part of the multiple allelic system at the *Y* locus where *Scr* is another allele at the *Y* locus (T Wehner, unpubl. data). More study is needed that includes classical genetics combined with biochemical and molecular data to fully understand the genes and inheritance of flesh color in this crop. This understanding is critical since flesh color is indicative of carotenoid content, which impacts the nutritional benefits of watermelon.

Several genes have been identified that affect the rind of watermelon. The gene *Sp* produces spotted fruit (Poole 1944). Golden yellow (*go*) is a

single recessive gene that causes fruit to turn yellow at maturity (Barham 1956). Intermittent stripes (*ins*) produces narrow dark stripes at the peduncle end of the fruit that become irregular in the middle and nearly absent at the blossom end of the fruit (Gusmini and Wehner 2006). Yellow belly, or ground spot, on "Black Diamond Yellow Belly" is controlled by a single dominant gene (*Yb*). Weetman (1937) proposed that three alleles at a single locus determined rind pattern. The allelic series was renamed to *G*, *gs*, and *g* by Poole (1944). The *g-s* gene produces a striped rind, but the stripe width (narrow, medium, and wide stripe patterns) has not been explained as yet. Porter (1937) found that dark green was completely dominant to light green (yellowish white, in his description). The watermelon gene *p* produces pencilled rind pattern (Robinson et al. 1976) and the *m* gene for mottled rind was first described by Weetman (1937).

3.2.1.2 Cucumber

Sex expression in cucumbers has played an important role in seed production as well as development of new fruit types. This trait is affected by several single-gene mutants. The *F* locus governs gynoecy, but is modified by other genes and the environment, and interacts with *a* and *m* (*androecious* and *andromonoecious*, respectively) (Rosa 1928; Tkachenko 1935; Galun 1961; Shifriss 1961; Wall 1967; Kubicki 1969a). The *F* gene may also be modified by an intensifier gene (*In-F*) which increases the femaleness (Kubicki 1969a). Other genes that affect sex expression are *gy* for *gynoecious*, *m-2* for *andromonoecious* (Kubicki 1974) and *Tr* for trimonoecious expression (Kubicki 1969b).

The discovery of parthenocarpy in cucumbers (Wellington and Hawthorn 1928) has led to the development of seedless fruit when combined with the gynoecious trait. There is dispute over the inheritance of parthenocarpy. Pike and Peterson (1969) suggested an incompletely dominant gene, *Pc*, affected by numerous modifiers. In contrast, de Ponti and Garretsen (1976) explained the inheritance by three major isomeric genes with additive action.

Bitterness in cucumbers can affect fruit quality, health potential, and insect resistance. Eliminating bitterness in this crop can be accomplished by selecting for the bitterfree allele (*bi*), which inhibits biosynthesis of cucurbitacin (Andeweg and De Bruyn 1959). Cucurbitacins can be toxic at high levels and they may act as an attractant for cucumber beetles, but have been shown to repel spider mites and aphids.

Disease resistance is an important trait in cucumber as diseases can reduce yield and quality. Currently there are 15 genes known to control disease resistance in *C. sativus*. Three of these genes condition virus resistance. Wasuwat and Walker (1961) found a single dominant gene

(*CMV*) for resistance to cucumber mosaic virus. However, others have reported more complex inheritance (Shifriss et al. 1942). Two genes have been found to condition resistance to papaya ringspot virus (Wang et al. 1984; Wai and Grumet 1995), and five different genes have been identified for resistance to watermelon mosaic virus II (Cohen et al. 1971; Wai et al. 1997). Resistance to zucchini yellow mosaic virus has also been identified (Provvidenti 1987; Kabelka et al. 1997).

Both resistance to scab and resistance to bacterial wilt are dominant and controlled by *Ccu* (Bailey and Burgess 1934; Andeweg 1956; Abul-Hayja et al. 1978) and *Bw* (Nuttall and Jasmin 1958; Robinson and Whitaker 1974), respectively. Other dominant genes providing resistance are: *Cca* for resistance to target leaf spot (Abul-Hayja et al. 1978), *Cm* for resistance to Corynespora blight (Shanmugasundarum et al. 1971), *Foc* for resistance to Fusarium wilt (Netzer et al. 1977) and *Ar* for resistance to anthracnose (Barnes and Epps 1952). In contrast, resistance to Anthracnose race 1 (Abul-Hayja et al. 1978) and angular leaf spot (Dessert et al. 1982) are conditioned by the recessive genes *cla* and *psl*, respectively.

Several reports have indicated that more than one gene controls resistance to powdery mildew with interactions occurring among loci. The resistance genes *pm-1* and *pm-2* were first reported by Hujieda and Akiya (1962) in a cultivar which they developed and named "Natsufushinari". Kooistra (1968) using this same cultivar, later confirmed their findings and identified one additional gene (*pm-3*) from USDA accessions PI200815 and PI200818. Shimizu et al. (1963) also supported three recessive genes, which are responsible for resistance of "Aojihai" over "Sagamihan".

Currently, one gene, *dm*, has been identified, which confers resistance to downy mildew (van Vliet and Meysing 1974). Inherited as a single recessive gene, it also appeared to be linked with *pm* (van Vliet and Meysing 1977). There are, however, indications that more than one gene may be involved (Jenkins 1946).

3.2.1.3 *Melon*

Most melon cultivars are andromonoecious, but other expression patterns are possible. Genes *a* (*andromonoecious*) and *g* (*gynomonoecious*) interact to influence sex expression: *a_g_* = monoecious; *a_gg* = gynomonoecious; *aa g_* = andromonoecious; and *aa gg* = hermaphrodite (Pitrat 2006). A third gene was identified that creates a much more stable gynomonoecious phenotype (*gy*), so that *a+_ gg gygy* = stable gynomonoecious.

Sterility is common in melon, as there are five different male-sterile genes and two total plant sterility genes (Pitrat 2006). However, since there are no seedling markers for these recessive male sterile genes, it is impossible to tell which plants are sterile without growing the plants to flowering. If

the purpose is to use the male-sterile trait for hybrid seed production, by the time you identify which plants are sterile, the fertile plants will have contaminated your hybrid seed.

Fruit quality in melon is polygenic, but there are several genes that have major effects. Some negative alleles for fruit quality include *Bif* for *Bitter fruit* (Parthasarathy and Sambandam 1981), *Me* for *Mealy flesh texture* (Ganesan 1988) and *So* for *Sour taste* (Kubicki 1962). Flesh color is dictated by single genes but the intensity of color is quantitative. Fruit shape is also influenced by genes for *oval fruit shape* (*O*), *sutures* (*s*) and *spherical fruit shape* (*sp*), but sex expression may also have an influence as perfect flowers tend to give round fruit while female flowers tend to give more elongated fruit (Lumsden 1914; Bains and Kang 1963; Wall 1967).

There are many loci in melon for disease resistance. Fusarium wilt resistance is provided by *Fom-1* and *Fom-3*, which are alleles for independent genes that provide resistance to races 0 and 2, and *Fom-2* gives resistance to races 0 and 1 (Risser 1973; Zink and Gubler 1985). Resistance to Alternaria is provided by *Ac* (Thomas et al. 1990), and there are up to six genes that provide a high to moderate level of resistance to gummy stem blight (Prasad and Norton 1967; Zuniga et al. 1999; Frantz and Jahn 2004). There are up to 17 different genes that govern resistance to powdery mildew, depending on the race/species involved (Jagger et al. 1938; Bohn and Whitaker 1964; Harwood and Markarian 1968a, b; Kenigsbuch and Cohen 1989; Epinat et al. 1993; Anagnostou et al. 2000; McCreight 2003). There are four genes reported for downy mildew resistance (Cohen et al. 1985; Thomas et al. 1988; Epinat and Pitrat 1989; Kenigsbunch and Cohen 1992), and a fifth gene is reported to act in combination with at least one other modifier gene (Angelov and Krasteva 2000). There are also resistance genes for papaya ringspot virus, zucchini yellow mosaic virus, and necrotic spot virus (See Pitrat 2006 for gene names.).

Melon also has resistance genes for several insects. Gene *Af* provides resistance to red pumpkin beetle (Vashistha and Choudhury 1974). Tolerance to melon aphid is provided by *Ag* (Bohn et al. 1973), and *Vat* provides resistance to viruses transmitted by aphids (Pitrat and Lecoq 1980). Melon fruit fly resistance is provided by two genes, *dc-1* and *dc-2* (Sambandam and Chelliah 1972). As with cucumber and squash, cucurbitacin content also influences insect resistance/susceptibility, which in the case of melon is governed by two genes, *Bi* and *cb* (Lee and Janick 1978; Nugent et al. 1984).

3.2.1.4 Cucurbita spp.

A single gene for gynoecious sex expression (*G*) has been identified in *C. foetidissima* (Fulks et al. 1979; Dossey et al. 1981), but the gene has not

yet been transferred to cultivated squash. There are four male sterile genes reported, two in each species, for *C. pepo* and *C. maxima* (Scott and Riner 1946; Eisa and Munger 1968; Superak 1987; Korzeniewska 1992), but as with most of the other cucurbit crops there are no morphological markers, which would allow them to be useful for seed production. There are two total plant sterility genes, one in *C. maxima* and one in *C. pepo* (Carle 1997).

The reduced internode length that gives a bush habit in *C. pepo* and *C. maxima* is governed by a single gene (*Bu*) (Shifriss 1947; Grebenščikov 1958; Decker-Walters and Munger 1963; Wu et al. 2007). This trait is greatly appreciated by gardeners with limited space. A unique gene found in the *Cucurbita* spp. is the naked seeded trait where seed lack a lignified seed coat and is typically used for the roasted pumpkin seed market (Schöniger 1952; Grebenščikov 1954; Xianglin 1987; Zraidi et al. 2003, 2007).

There are far fewer identified disease resistance genes in squash than the other major cultivated cucurbit crops. Resistance to powdery mildew (*PM*) in *C. okeechobeensis* and *C. lundelliana* is controlled by a single dominant allele and modifiers (Contin 1978; Paris and Cohen 2000; Cohen et al. 2003) and two PM-resistant genes to race 1 and race 2 were identified in *C. moschata* (Adeniji and Coyne 1983). A single gene has also been reported to provide resistance to zucchini yellow mosaic virus. There are three complementary dominant genes for resistance to *Phytophthora capsici* (Crown rot) (Padley et al. 2009). Known virus resistance consists of one recessive gene for cucumber mosaic virus (Brown et al. 2003), one recessive resistance gene to papaya ringspot virus (Brown et al. 2003), two watermelon mosaic virus resistance genes, one from *C. moschata* (Fulks et al. 1979; Brown et al. 2003), which may be linked with or identical to *Zym-1* and one from *C. ecuadorensis* (Shifriss 1989), and a total of six resistance genes and one modifier gene have been reported for zucchini yellow mosaic virus in *C. moschata*, *C. pepo*, and/or *C. ecuadorensis* (Mains 1950; Fulks et al. 1979; Paris et al. 1988; Robinson et al. 1988; Paris and Cohen 2000; Brown et al. 2003; Pachner and Lelley 2004).

There is one resistance gene reported for insects (*Fr*, for melon fruit fly resistance) (Nath et al. 1976), although the gene *cu* (Sharma and Hall 1971), which reduces foliar cucurbitacin content, will have a similar effect as for the other cucurbit crops by reducing cucumber beetle preference while making the plant more attractive to aphids and spider mites.

Fruit quality, shape and color are extremely diverse in the squash and pumpkin species and a thorough review of the genes involved was compiled by Paris and Kabelka (2009). A number of fruit color genes have been identified. The *B* gene that was found in an ornamental gourd can be used to give a yellow color and high vitamin A content. This same gene also has pleiotropic effects on fruit and foliage and is affected by several modifier genes (*Ep-1*, *Ep-2* and *Ses-B*). The *B* gene is also complementary to *L-2* to give intense orange flesh instead of light yellow flesh color which also enhances

the carotenoid content. There are up to five different fruit bitterness genes, but allelism tests have not been reported for all five genes.

3.2.1.5 Other Cucurbit spp.

There are a few reports on identified genes, linkages, and improvements for other cucurbit crops. What is known is summarized in Taja and Wehner (2008). In West Indian Gherkin (*Cucumis anguria*), four gene loci have been described—a single dominant gene produces bitter fruit (*Bt*) (Koch and Costa 1991); a dominant gene for resistance to cucumber green mottle mosaic virus (*Cgm*) (den Nijs 1982); and two loci that control fruit spininess (*S* and *P*) (Koch and Costa 1991). African horned cucumber (*Cucumis metuliferus*) has two identified genes: watermelon mosaic virus-1 resistance is controlled by a single dominant gene (*Wmv*) (Provvidenti and Robinson 1972), and a single dominant gene for resistance to papaya ringspot virus (*Prsv*) (Provvidenti and Gonsalves 1982). *Luffa* spp. have two reported genes: the gynoecious gene (*g*) (Choudhury and Thakur 1965) interacts with andromonoecious gene (*a*) to produce the phenotypes—monoecious or trimonoecious (*AA GG*), andromonoecious (*aa GG*), gynoecious (*AA gg*), or hermaphroditic (*aa gg*) plants. *Melothria medraspatana* has been reported to have a recessive gene for small seed size (*s*), and the gene *w* that controls white seed coat color if *ww* (Sing 1972). Bitter Melon (*Momordica charantia*) has four identified genes—light brown seed (*lbs*) (Ram et al. 2006) is a single recessive to dark brown; large seed (*ls*) is recessive to small seed size (Srivastava and Nath 1972); white immature fruit skin (*ww*) is recessive to green epicarp (Srivastava and Nath 1972); Ram et al. (2006) reported that gynoecism is governed by a single recessive gene (*gy-1*).

Genes identified for bottle gourd (*Lagenaria siceraria*) include red pumpkin beetle (*Aulacophora faveicollis*) resistance, a single dominant gene (*Af*) (Vashishta and Choudhury 1972); bottle-shaped fruit (*bb*) is recessive to disk-shaped fruit (*BB*); *rr* produces round fruit that is recessive to *RR*, disk-shaped fruit. The gene *db* interacts with *b* to produce an F_2 of 9 club: 3 round: 4 dumbbell-shaped fruit (Tyagi 1976). Dark green fruit color is controlled by *GG*, which is dominant to light green fruit (*gg*) (Tyagi 1976). Light brown seed coat (*lb*) is recessive to brown seed coat (*Lb*) (Tyagi 1976). The single dominant gene (*S*) is responsible for segmented leaf shape (Akhilesh and Ram 2006).

3.2.2 Classical Genetic Mapping Efforts

If one could select useful traits at the seedling stage, this would overcome the disadvantages of needing large field plots and having to perform large numbers of controlled pollinations. Complexity is encountered

when trying to select for quantitative traits that are heavily influenced by the environment, such as yield. Because of environmental influences on quantitative traits, large populations are needed to account for this variability, which adds to time and space requirements, and cost for proper evaluation. Breeders and geneticists have attempted to identify markers that are associated with agronomically important traits, with the hope that the marker can be: 1) identified at the seedling stage or shortly thereafter, and 2) have a small or no environmental influence. Traditional approaches to identify traits early include morphological and isozyme markers, both of which have been used to some degree in various cucurbit crops. It is likely that both these markers have been used to maintain cultivar identities, but there is little published information with regard to the extent that these markers are used. Most major cucurbit seed companies have routinely used isozyme markers to check hybrid purity (S. King, pers. comm.).

3.2.2.1 Watermelon

In watermelon 60 genes and 111 isozyme markers have been identified (Wehner 2007), but there is only one linkage map. It includes two genes (fruit bitterness and red flesh) and 22 isozymes that comprise seven linkage groups covering 354 cM (Navot et al. 1990). While it is valuable to have important fruit traits such as flesh bitterness and red flesh color linked to markers, the utility has been limited since most breeding work is conducted within germplasm that already lacks the bitter trait and is often conducted within red fleshed cultivars.

Much work was performed to identify morphological markers associated with genetic male-sterility in watermelon. This trait would be extremely useful for the production of hybrid cultivars. It is essential, during hybrid seed production, to identify which progeny contain the male-sterile trait, since segregating populations are the only means to maintain the genetic male-sterility trait. There are currently five genes for genic male-sterility reported in this crop. One gene (*gms*) is associated with glabrous foliage (Watts 1962, 1967; Ray and Sherman 1988), which makes for an excellent morphological marker since it is simple to identify the trait in young plants. A second male-sterility gene (*ms-dw*) is associated with reduced internode length, another easy morphological marker (Huang et al. 1998). However, these genes, as well as two other male-sterility genes, are also associated with reduced female-fertility, limiting their suitability for hybrid seed production. The fifth genetic male-sterility gene (*ms-2*) is not associated with a reduction of female fertility (Dyutin and Sokolov 1990), but there are currently no morphological markers associated with this gene, limiting its utility for hybrid seed production.

3.2.2.2 Cucumber

Since cucumber has just seven chromosome pairs and over 100 known genes, it would seem that linkage maps would be fairly complete by now. Unfortunately, we know of a few references reporting linkages of more than two gene loci. Some of the difficulties linking genes in cucumber are due to a portion of the nomenclature being unclear about possible duplication of gene names originating from studies using different parental lines. Additionally, some of the linkage relationships analyzed in previous studies did not involve specific single genes but involved multigenic traits, or if a single gene was involved, it was not specifically identified. Even with these limitations, Wehner (2005) was able to summarize the literature for linkages and describe the different linkage groups. This summary contained six linkage groups and an assortment of linked genes that could not be placed in any of the linkage groups. His work on cucumber linkages that include traits is included below with modifications. The order in which the genes were expressed in each group does not necessarily represent the order in which they may be found on the chromosome and a question mark "?" will follow each gene which has a questionable origin.

3.2.2.2.1 Linkage Group A

The largest linkage group in cucumber has 12 genes, composed of *watermelon mosaic virus-1 resistance* (*wmv-1-1*), *gynoecious* (*gy*), *glabrous* (*gl*), *delayed growth* (*dl*), *divided leaf* (*dvl*), *determinate habit* (*de*), *Female* (*F*), *male sterile-2* (*ms-2*), *glabrate* (*glb*), *bitterfree* (*bi*), *delayed flowering* (*df*), and *Black spine-3* (*B-3*) or *Black spine-4* (*B-4*). In contributing to this grouping, Whelan (1974) noted that *ms-2* is linked with *glb* (rf = 0.215+.029) and *de* (rf = 0.335+.042) while being independent of *bi*, *gl*, *yc-1*, *yc-2*, and *cr*. Gene *de* is linked with *F* (Odland and Groff 1963b; Owens and Peterson 1982), which in turn is linked with *B-3* or *B-4* (Cowen and Helsel 1983), *gy* (rf = 0.04) (Kubicki 1974), *bi* (rf = 0.375) and *df* (rf = 34.7) (Fanourakis 1984; Fanourakis and Simon 1987). Gene *de* is also weakly linked with *dl* (Miller and George 1979), strongly linked with *dvl* (Anon 1983), and independent of *cp* (Kauffman and Lower 1976). Gene *wmv-1-1* is linked with bitterfree (*bi*) but independent of *Ccu*, *B*, *F* or *pm*? (Wang et al. 1987).

Two reports show that *dvl* is weakly linked with *gl* (rf = 0.40) and independent of *bi* and *Ccu* (den Nijs and Boukema 1983), while Robinson (1978d) originally indicated that *gl* was linked to *yc* and independent of *B*, *m*, *l*, and *yg* as well as *bi* (den Nijs and Boukema 1983) and *sp* (den Nijs and Boukema 1985), but more recently Robinson indicated that *gl* was independent of *yc* (Robinson 1987a).

Completing linkage group A, Cowen and Helsel (1983) demonstrated that the spine color genes (*B-3* and *B-4*) were independent of the genes for bitterness, and Whelan (1973) found that *pl* was independent of *glb* and *bi*, while *glb* was independent of *gl*, *bi*, *ls*, *yc*, and *cr*, which further confirms that *gl* (*glabrous*) and *glb* (*glabrate*) must indeed be separate loci.

3.2.2.2.2 Linkage Group B

Group B is composed of nine genes, *negative geotropic peduncle response* (*n*), *protruding ovary* (*pr*), *locule number* (*l*), *andromonoecious* (*m*),*opposite leaf arrangement* (*opp*), *adromonoecious-2* (*m-2*), *Bacterial wilt resistance* (*Bw*), *spine size and frequency* (*s*?) and a male-sterile gene (*ms*?) unless *s*? (Robinson 1978b) is the same as *s* from Hutchins (1940) and Poole (1944). If these were the same, then linkage groups B and C will be joined for a total of 12 genes. Of the first seven, two pairs have been defined with recombination values. Youngner (1952) determined that *m* and *l* were linked with a recombination frequency of 0.326 + 0.014 and Robinson determined that *opp* was linked to both (Robinson 1987b). Iezzoni and Peterson (1979, 1980) found that *m* and *Bw* were separated by only one map unit (rf = 0.011+0.003). Iezzoni et al. (1982) also determined that *m-2* was closely linked with both *m* and *Bw*, and that *Bw* was independent of *F* from linkage group A (Iezzoni and Peterson 1980).

Robinson (1978b, c), and Youngner (1952) found that linkages existed between *m*, *l*, *n*, *pr* and spine number (*s*?) with the possibility of pleiotropy being responsible for the *m* / *pr* relationship. They also demonstrated that *B*, *yg*, and *pm*? were independent of the same genes (Youngner 1952; Robinson 1978b). Rounding out the linkage group is one of the male-sterility genes (*ms*?). Robinson (1978c) found that it was linked with both *m* and *l*, but did not identify which male-sterile gene it was.

3.2.2.2.3 Linkage Group C

Group C is the oldest and most mystifying linkage group. It is currently composed of *Red mature* (*R*) for red or orange mature fruit color, *Heavy netting of fruit* (*H*), *Black or brown spines* (*B*), *cream mature fruit color* (*c*), and *spine size and frequency* (*s*) (Strong 1931; Tkachenko 1935; Hutchins 1940; Poole 1944). However, there is speculation on the nature of this linkage group. Since very few recombinants of the *R*, *H*, *B* and *c*, *h*, *b* linkage groups have been reported, it is also felt that these characteristics may be the response of two alleles of a single pleiotropic gene. There is also speculation that *R* and *c* are different alleles located at the same locus (see earlier discussion).

Hutchins (1940) found that *s* was independent of *B* and *H* while linked with *R* and *c*. If he was correct, then pleiotropy of *H* and *B* with *R* and *c* is ruled out. His report also indicated that *B* and *s* were independent of *determinate habit* (*de*) as was *de* of *R*, *c* and *H*.

A possibility exists that this linkage group may be a continuation of group B through the *s* gene. Poole (1944) used the data of Hutchins (1940) to determine that *c* and *s* are linked with a recombination frequency of 0.163 + 0.065. The question that remains is whether *s* (Hutchins 1940; Poole 1944) is the same as the gene for spine number in the findings of Robinson (1978b). If Cowen and Helsel (1983) are correct in their finding that a linkage exists between *Female* (*F*) and *B* then groups A and C may be on the same chromosome. However, in this text they will remain separated based on conclusions of Fanourakis (1984), which indicate that errors may be common when attempting to distinguish linkages with *F* since classification of *F* is difficult. This difficulty may also explain many conflicting reports.

3.2.2.2.4 Linkage Group D

Twelve genes, *numerous spines* (*ns*), *small spines* (*ss*), *Tuberculate fruit* (*Tu*), *Parthenocarpy* (*Pc*), *Dull fruit skin* (*D*), *uniform immature fruit color* (*u*), *tender skin of fruit* (*te*), *compact* (*cp*), *downy mildew resistance* (*dm*), *Anthracnose resistance* (*Ar*), *Corynespora cassiicola resistance* (*Cca*) and *powdery mildew resistance expressed by the hypocotyl* (*pm*? or *pm-h*) are in group D, but the identity of the specific gene for powdery mildew resistance is elusive. Van Vliet and Meysing (1947, 1977) demonstrated that the gene for resistance to downy mildew (*dm*) was either linked or identical with a gene for resistance to powdery mildew (*pm*?), but because the linkage between *pm*? and *D* was broken while that of *dm* and *D* was not, *pm*? and *dm* must be separate genes. The problem lies in the lack of identity of *pm*? because Kooistra (1971) also found that a gene for powdery mildew resistance (*pm*?) was linked to *D*. Further complicating the identity of *pm*, Fanourakis (1984) found that *pm-h* was linked to *te* and *dm*, yet *cp*, which must be located at approximately the same locus, was independent of *te*. He suggested that there were either two linkage groups, *ns*, *ss*, *Tu*, *Pc*, *D*, *U*, *te* and *cp*, *dm*, *Ar*, located at distal ends of the same chromosome with *pm-h* at the center, or the two groups are located on different chromosomes with a translocation being responsible for apparent cross linkages. However, evidence for the latter which suggested that *Female* (*F*) was associated with the seven-gene segment is not probable since there are few other supportive linkages between genes of this segment and linkage group A. A more likely explanation is the occurrence of two or more genes conditioning resistance to powdery mildew being found on this chromosome.

Lane and Munger (1985) and Munger and Lane (1987) determined that a gene for resistance to powdery mildew (*pm*?) was also linked with *Cca* for susceptibility to target leaf spot but that linkage, though fairly tight, was breakable.

The last four genes in this group are *Tu*, *D*, *te* and *u* (Strong 1931). Until recently it was believed that each in the recessive form was pleiotropic and consistent with European type cucumbers and each in the dominant form was pleiotropic and consistent with American type cucumbers.

Fanourakis (1984) and Fanourakis and Simon (1987) reported that crossing-over (R = 23.7) occurred between *te* and the other three genes, which still appeared to be associated. However, using triple backcrosses they demonstrated that there is a definite order for *Tu*, *D* and *u* within their chromosome segment and that the *Tu* end is associated with the *ns* and *ss* end.

3.2.2.2.5 Linkage Group E

Group E is currently composed of three genes *long hypocotyl* (*lh*), *short petiole* (*sp*) and *umbrella leaf* (*ul*). The gene *sp* was strongly linked with *lh* and weakly linked with *ul* (Zijlstra and den Nijs 1986). However Zijlstra and den Nijs (1986) expressed concern for the accuracy of the *sp* and *ul* linkage data, since it was difficult to distinguish *ul* under their growing conditions.

3.2.2.2.6 Linkage Group F

Group F is comprised of two genes, *Fruit length* (*Fl*) and *Cladosporium cucumerinum resistance* (*Ccu*) which appear to be tightly associated. Wilson (1968) concluded that pleiotropy existed between scab resistance and fruit length because backcrossing scab resistance into commercial varieties consistently resulted in reduced fruit length. However, Munger and Wilkinson (1975) were able to break this linkage producing varieties with scab resistance and longer fruit (Tablegreen 65 and 66, Marketmore 70 and Poinsett 76). Now when these varieties are used to introduce scab resistance long fruit length is consistently associated.

3.2.2.2.7 Unaffiliated Genes

Independent assortment data are as important in developing linkage maps as direct linkage data and several researchers have made additional contributions in this area. However, like linkage data, independent assortment data care must be taken when utilizing them. For a complete list of cucumber unaffiliated genes, see Wehner (2005).

3.2.2.3 Melon

There are approximately 186 identified genes and isozyme markers in melon, making it the most saturated cucurbit crop in terms of identified genes (Pitrat 2006). Linkages have been identified between several agronomic and morphological traits. The linkages have been assigned to eight linkage groups (Pitrat 1991).

3.2.2.3.1 Linkage Group 1

One of the genes for *short internodes* (*si-1*) was found to be linked to *yellow virescence* (*yv-2*), which causes pale yellow cotyledons (Pitrat 1991).

3.2.2.3.2 Linkage Group 2

Pitrat and Lecoq (1982) described linkages between *virus aphid transmission* (*Vat*) and *flaccida necrosis* (*Fn*: wilting and necrosis in response to infection with the F pathotype of Zucchini yellow mosaic virus), and Pitrat (1991) added *resistance to powdery mildew* (*Pm-w*), and determined the order of linkage to be *Fn—Pm-w—Vat*.

3.2.2.3.3 Linkage Group 3

McCreight (1983) described linkages between the male sterile gene *ms-1* and *red stem* (*r*), which conditions red pigment under the epidermis of stems, particularly at the nodes. Pitrat (1991) was able to add the glabrous foliage gene (*gl*), and the chlorophyll deficient gene *pale green* (*pa*) to this linkage group, and determined the order of genes to be: *pa—gl—r—ms-1*.

3.2.2.3.4 Linkage Group 4

Pitrat and Lecoq (1984) described linkages between *andromonoecious* (*a*) and *Zucchini yellow mosaic virus resistance* (*Zym*), and Pitrat (1991) added *halo cotyledons* (*h*) and one gene for powdery mildew resistance, which was identified as *Pm-X*. The order of the genes was not determined (Pitrat 1991).

3.2.2.3.5 Linkage Group 5

One of the genes for resistance to Fusarium wilt races 0 and 2 (*Fom-1*) was found to be linked to resistance to papaya ringspot virus (*Prv*) along with a chlorophyll deficient marker (*yv-x*, which was later named *yv-2*, Pitrat et al. 1991). The exact order of the genes was not determined, but was reported as either: *Fom-1—Prv—yv-2*, or *Prv—Fom-1—yv-2* (Pitrat 1991).

3.2.2.3.6 Linkage Group 6

Linkages were found between a second Fusarium wilt resistance gene (*Fom-2*), reduced chlorophyll content in the yellow green gene (*yg*) and a male-sterile gene (*Ms-2*). The order of the genes was determined to be: *Fom-2—yg—Ms-2* (Pitrat 1991).

3.2.2.3.7 Linkage Group 7

Resistance to melon necrotic spot virus (*nsv*) was found to be linked to the *Pm-y* gene for powdery mildew resistance (Pitrat 1991).

3.2.2.3.8 Linkage Group 8

The chlorophyll deficient mutant *flava* (*f*) was found to be linked to the *lmi* gene for long main stem internodes.

Pitrat (1991) identified five additional morphological and agronomic trait genes that did not fit into any of the linkage groups and these have been assigned to linkage groups 9 through 13 by Pitrat (1994) as follows: Group 9 = *male sterile-4* (*ms-4*); Group 10 = *dissected leaf* (*dl*); Group 11 = *virescent* (*v*); Group 12 = *male sterile 3* (*ms-3*); Group 13 = *male sterile-5* (*ms-5*). *Acute leaf apex* (*Ala*) was linked with *Lobed leaf* (*L*) but was not assigned to a linkage group (Ganesan and Sambandam 1985).

3.2.2.3.9 Isozyme Markers

Staub et al. (1998) were able to identify 30 isozyme markers in melon. Eleven of these markers were assigned to two linkage groups (A and B). The resulting map spanned 98 cM and had a mean linkage distance of 9 cM. However, none of the isozyme markers were associated with agronomic traits during the creation of the isozyme map.

3.2.2.4 Cucurbita spp.

Sanjur et al. (2002) listed up to 13 species in the genus *Cucurbita* and Robinson and Decker-Walters (1997) suggested there are up to 15 species in this genus, all of which are believed to have 20 pairs of chromosomes. This genus has a total of 87 identified genes and 49 isozyme markers (Paris and Kabelka 2009). The majority of the identified genes are from *C. pepo* (70), followed by *C. moschata* (25) and *C. maxima* (19). The remaining genes are distributed across four wild species and interspecific crosses. The isozyme markers were useful for determining phylogenetic relationships, hybrid purity and cultivar identity (Loy 1972; Ignart and Weeden 1984; Kirkpatrick et al. 1985; Decker

and Wilson 1987). There are also reports of genetic linkage between genes, where *dark stem* (*D*) was found to be linked to mature *orange fruit* (*mo-2*) in *C. pepo* (Paris 1997), *mottled leaves* (*M*) were linked to *warty fruit* (*Wt*) in *C. pepo* (Paris et al. 2004), resistance to watermelon mosaic virus 2 (WMV-2) with a plastid-specific aldolase (*Aldo-p*) in *Cucurbita ecuadorensis*, and *bitter fruit* (*Bi*) was found to be linked to *lobed leaves* (*Lo-2*) in a *C. ecuadorensis* x *C. maxima* interspecific cross (Herrington and Brown 1988; Paris et al. 2004). While it is obvious how some of these linkages could be valuable in a breeding program (e.g., select against lobed leaves to eliminate bitter fruit), in practicality, three of these linkages have limited usage because there are multiple genes for the trait as well as other modifier effects which affect the phenotype which are not accounted for in the linkage.

3.2.3 Limitations of Classical Genetic Linkage Mapping and Potential of Molecular Mapping

The primary limitation of current morphological and isozyme maps has been the limited number of markers available, along with the relatively few economically important traits associated with the markers. While the maps have sometimes been useful to screen for hybrid purity during seed production, only in rare cases are they used during the development of new varieties. Morphological and isozyme markers are limited to expressed genes, and because of this there is also the potential for environmental influences, since gene expression patterns can be influenced by the environment. The full potential of molecular maps can only be fully exploited when the entire genome can be visualized on a map and important traits can be associated with the map. Modern DNA based markers and their associated molecular maps have the potential for overcoming the obstacles of morphological and isozyme maps.

3.3 Traditional Breeding

3.3.1 Traditional Breeding Objectives and Achievements

Major goals for breeding programs are to develop high yielding cultivars that have high quality fruit. Methods for achieving these goals differ among breeders, as does his/her definition of quality fruit. Cucurbit crops are naturally outcrossing, but often do not show heterosis in hybrid combinations, and when there is heterosis, it usually is not as great as it is for other outcrossing crops, such as onion or maize (Wehner 1999). Cucurbit crops do not usually exhibit inbreeding depression, a factor that may be related to the reduced heterosis (Rubino and Wehner 1986). Studies in cucumber, melon, and squash indicate that in general, there

is little or no inbreeding depression but there is significant heterosis in certain combinations (Robinson and Decker-Walters 1997; Whitaker and Davis 1962). Robinson (1999) states that significant heterosis for earliness and yield has been reported for cucurbits, including *Benincasa, Lagenaria, Luffa, Momordica,* and *Trichosanthes.* He goes on to state that inbreeding depression is not an important factor for producing seed of most hybrid cucurbits cultivars.

3.3.1.1 Watermelon

Watermelon is unique among the cucurbit crops in that a significant portion of current cultivars are seedless triploids, especially in the American market. Triploid hybrids are produced by crossing tetraploid female with diploid male inbred lines. Improving seedless watermelon involves selecting the best diploid and tetraploid line, then testing them in hybrid combinations. This method creates a new level of complexity for breeders since both diploid and tetraploid lines must be managed. Initial breeding efforts on tetraploids simply involved selecting the best diploids and using these to create tetraploid parent lines. While this method has provided many current triploid cultivars in the market, it has limitations since most diploids do not make good tetraploid parents for triploid seed production. Fertility in the tetraploid is an extremely important trait that has limited the use of many tetraploid parents. Additionally, breeding within tetraploids is often more complex than breeding diploids.

Watermelon cultivars are often monoecious, with older cultivars and many wild accessions being andromonoecious. Watermelon is naturally cross-pollinated like maize. However, there is little inbreeding depression and heterosis in watermelon. It has been suggested that the lack of inbreeding depression is due to the small population sizes used by farmers during the domestication of the species, which forced out deleterious recessive alleles. Therefore, even with monoecious sex expression and insect-pollinated flowers, there would have been considerable inbreeding among the few plants representing the population. Since there is little inbreeding depression in watermelon, inbred lines are developed using self-pollination with little loss of vigor from the parental population.

In studies of heterosis in watermelon, some estimates have shown a 10% advantage of the hybrid over the high parent, but only for some parental combinations (Wehner 1999). The small amount of heterosis observed in watermelon makes it possible for growers to compete in the seeded market using less expensive open-pollinated lines. However, hybrid varieties are useful for combining multiple dominant traits from different parents. Examples of such traits include red or canary yellow flesh, resistance to Fusarium wilt and anthracnose, and resistance to powdery mildew. Hybrids

also protect proprietary breeding lines from unauthorized use. One of the most important uses of hybrids is the production of seedless varieties.

Watermelon breeders today are less interested in studying heterosis or measuring general (GCA) and specific (SCA) combining ability, because hybrids have an advantage for protection of the proprietary breeding lines. Furthermore, seedless cultivars are in high demand and can be produced only as triploid hybrids. However, in the future it might be possible to develop transgenic diploid seedless watermelons. In that case, the advantage of using heterotic hybrids vs. inbred cultivars will again be questioned.

Environmental factors such as water availability may be important in contrasting inbred cultivar vs. hybrid yields. A Florida study observed that watermelon hybrids out-yielded inbred cultivars only in irrigated fields, but quality was higher among the inbred cultivars in dry conditions (Rhodes 1985).

Disease resistance has been, and continues to be a high priority of most watermelon breeding programs. Resistance to Fusarium wilt has been studied since the early 1900s (Orton 1911), and most modern cultivars have resistance to most Fusarium races today (Henderson et al. 1970), although the fungus continues to evolve in response to host plant resistance (Zhou et al. 2010), necessitating continued breeding efforts. Resistance genes are also used to provide protection from anthracnose (Layton 1937; Winstead et al. 1959), papaya ringspot virus (Guner et al. 2008), and zucchini yellow mosaic virus (Provvidenti 1991; Xu et al. 2004). Gummy stem blight remains a high priority for watermelon research (King and Davis 2007), but despite potential resistance in germplasm, protection does not hold up in all locations.

Quality traits have been selected in watermelon for thousands of years. These traits include size, shape, shelf-life, color, sugar content, and total nutrient content. Watermelon is a dynamic plant with great potential for alteration of quality traits. However, what is perceived as quality depends on the country, demographics, and personal preference. Some people prefer the mini melons, around 5 pounds, whereas others want giant watermelons over 100 pounds. There are unsweet, firm, white watermelons used for pickling and preserves, and dark crimson watermelon with brix up to 14, even 15%. More recently, breeders have been interested in phytonutrient content, and breeding programs have focused on increasing total carotenoids, lycopene, and improving citrulline contents (personal communication with watermelon breeders).

3.3.1.2 Cucumber

Early studies on cucumber report considerable heterosis and/or inbreeding depression within this crop, so long as the parents are not closely related

(Hayes and Jones 1916; Hutchins 1938; Ghaderi and Lower 1979a, b). However, Rubino and Wehner (1986) indicated that inbreeding depression was not important in cucumber and that midparent heterosis was noted for most traits in many hybrids obtained from crossing S_6 lines with a gynoecious inbred line. In a similar but larger study, Cramer and Wehner (1999) demonstrated in one out of four crosses, heterosis for fruit yield was associated with a decreased correlation between percentage of fruit set and fruit weight, an increased negative correlation between percentage of fruit set and both the number of branches per plant and the percentage of pistillate nodes, and an increased negative correlation between the number of nodes per branch and total fruit weight. Inbreeding depression was associated with a weakening of the strong negative correlations between percentage of fruit set and the number of branches per plant, and between the number of nodes per branch and total fruit weight. Those correlations were associated with high-parent heterosis and inbreeding depression only for the one cross, and would not necessarily apply to future crosses in which heterosis may be observed for yield. More recently, Munshi et al. (2006) suggest from their results that heterosis breeding is important for effective utilization of non-additive gene actions; and Godoy et al. (2008) showed both positive heterosis of hybrids over parent lines.

Cucumber breeders have combined several of the sex expression and fruit quality genes to create improved varieties. It was discovered that gynoecious sex expression created an earlier maturity, since there are no male flowers. Many pickling cucumber hybrids were created that combined gynoecious varieties with a monoecious variety to create a blended hybrid composed of two distinct varieties. The monoecious variety provides pollen to create fruit set in the gynoecious variety.

Gynoecious sex expression has also been combined with parthenocarpy as a way to set fruit without pollen. It has been discovered that parthenocarpic fruit has an improved shelf life and quality, and when combined with the bitterfree allele the resultant fruit are of high quality. The combination of gynoecious sex expression along with parthenocarpy is responsible for the development of the extensive greenhouse cucumber industry, since this eliminates the need for pollination and produces a superior quality fruit. Gynoecious sex expression can also be used for hybrid seed production since the female plants do not produce male flowers. Since it has been found that sex expression can easily be changed with growth regulators, maintaining the female line is relatively straight forward.

Cucumber breeders have been able to create varieties with multiple disease resistances. Resistance to angular leaf spot, Anthracnose, downy mildew, powdery mildew scab and target leaf spot are common in the market. There are also varieties with multiple virus resistance available, including cucumber mosaic virus, papaya ringspot virus, watermelon

mosaic virus and zucchini yellows mosaic virus. These disease resistant varieties were created by screening thousands of segregating plants in multiple generations using tedious inoculations.

3.3.1.3 Melon

Early reports differed in their analysis of whether melon showed heterosis in yield and quality traits. The cumulative data suggests that well chosen parental lines can produce hybrids with improved quality and yield (See Robinson 1999 for a complete review of this subject in melons). More recent findings demonstrated that in snake melon (*C. melo* L. var. *flexuosus*) heterosis increased ascorbic acid and carotenoid content (Pandey et al. 2010), and heterosis could alter fruit shape in *C. melo* (Fernández-Silva et al. 2009). An elegant study by Luan et al. (2010) demonstrated dramatic performance differences between parents from diverse geographic origins and among F_1 hybrid progeny, a strong relationship between genetic distance (determined using molecular techniques) and heterotic effects was not consistently detected.

Breeders have created a diversity of types of melon, with well over a dozen distinct forms currently on the market in various regions around the world. The different types included a variety of shape, skin and flesh color and texture. Breeders have also successfully combined a number of quantitative traits such as sweetness and level of aromatic compounds, despite the lack of efficient markers for these quantitative traits.

The discovery of gynoecious melons created a lot of excitement in the seed industry, since the ability to produce gynoecious inbreds should be an advantage for seed production in the same way it is for cucumber; however, our experience has been that the gynoecious trait is influenced by genetic background so that occasional perfect flowers may sometimes develop. When a strong gynomonoecious genotype is identified, converting its sex expression with hormones is much more difficult than with cucumber (S King, unpubl. data).

Breeders have also created multiple disease resistant varieties of melon. Resistance to Fusarium wilt and powdery mildew are common, but the pathogens for these diseases continue to evolve making continued breeding efforts necessary.

3.3.1.4 Cucurbita spp.

Many studies have demonstrated that *C. pepo* and *C. maxima* hybrids can have superior yields (summarized in: Robinson 1999; see also Firpo et al. 1998; Ahmed et al. 2003; López Anido et al. 2004). Stephenson et al. (2001) report that sporophytic vigor (e.g., flower and fruit production) increased with the

level of heterozygosity and that the level of heterozygosity of the sporophyte affects the *in vitro* and *in vivo* performance of the microgametophytes it produces. In addition to yield, Gwanama et al. (2001) demonstrated that heterosis in tropical pumpkin can increase soluble solids content.

As with the other cucurbit crops, there is a wide diversity of fruit types within this group. The Cucurbita spp. are somewhat unique in that there are different plant types and harvest stages for the fruit, some being harvested as immature fruit while others are harvested as mature fruit.

Breeders have utilized the gene for bush habit found in Cucurbita spp., and all modern summer squash varieties currently have the bush habit. Recently, parthenocarpic summer squash varieties have come on the market, with the potential for greenhouse production. While this market is currently limited, the full potential is currently unknown.

There is a limited number of resistance genes currently available in Cucurbita spp., so there are few resistant varieties available. Resistance to powdery mildew is available in some varieties, and breeders have utilized the precocious yellow gene, which prevents the expression of mottling symptoms as a way to reduce damage caused by virus infection. There are also a number of virus resistance genes which have been used in various summer squash varieties, but their utility has been somewhat limited since there are a number of different viruses that can affect squash, and each resistance gene is specific for a particular virus. Virus resistant squash has also been created using genetic engineering, which has the advantage that multiple resistance can be stacked on a single construct so that resistance segregates as a single gene, making breeding much less difficult.

3.3.2 Limitations of Traditional Breeding and Rationale for Molecular Breeding

Traditional breeding has relied, either directly or indirectly, on morphological markers to identify the trait of interest for selection in a segregating population. Traditional methods include a direct measure of the phenotype (e.g., flesh color), or an association of one phenotype with another (e.g., lobed leaves with bitter fruit). Traditional breeding has been extremely effective for making qualitative changes in cucurbit crops. Traits such as lycopene containing watermelon (linked to red flesh), β-carotene containing melon (linked to orange flesh) and parthenocarpy (linked to seedless cucumbers) are examples of selection using phenotypic markers. In addition to improvement in qualitative traits, there have been huge changes in important quantitative traits through traditional breeding. These include seed germination, seedling vigor, fruit yield, early maturity, fruit size, sugar content, and freedom from defects.

Further improvement in quantitative traits using traditional strategies will be time consuming. For example, selecting for higher carotenoid concentration will be difficult using visual appearance as was done in the past. It is fairly straight forward to select watermelon genotypes containing lycopene from a segregating population, but it is difficult to distinguish levels of lycopene based on color. Likewise, selecting orange fleshed fruits in squash has led to squash fruits that contain carotenoids, but it has been shown that quantitative differences in carotenoid content is controlled by the complex interaction of alleles at many genes, some of which have modifier effects (Paris 1994).

The most effective method for selecting a multiple allele trait is to utilize multiple markers to identify a majority of the alleles. This is especially true of the cucurbit crops, which require large amounts of space to evaluate. However, morphological markers will have an intrinsic disadvantage if the trait is influenced by the environment. The potential for morphological markers is also limited by probability, since only coding regions of the genome can be used as potential markers. The total genes, including isozymes, available in cucurbit crops, are approximately 661, including watermelon (171, Wehner 2007), cucumber (168, Wehner 2005), melon (186, Pitrat 2006), and *Cucurbita* spp. (136, Paris and Kabelka 2009). Considering the volume of traits and how few genes are identified in each species, the potential for a morphological or isozyme tightly associated with any particular trait of interest is quite low.

Breeding for disease resistance is often challenging for cucurbit crops. Many disease resistance traits are quantitative, expression is often affected by environment, a complex inoculation procedure may be required, and in some diseases, reliable resistance has yet to be found. Gummy stem blight (GSB) is a good example of a disease where developing new cultivars with resistance has thus far proven difficult. GSB is a serious disease of watermelon, leading to substantial yield losses (Keinath and Duthie 1998), and has been identified by US watermelon producers as the number one problem needing further research (King and Davis 2007). Host plant resistance should be an effective method for control of GSB. However, despite numerous attempts, resistant cultivars are not currently available for this disease in watermelon. Several resistant sources have been identified (Sowell and Pointer 1962; Sowell 1975; Gusmini et al. 2005), and "resistant" cultivars have been released (Norton et al. 1986), but these cultivars do not withstand current disease pressure in multiple locations (Hall and Sumner 1999; S. King and T. Wehner unpubl. data). Resistance has been tracked using conventional screening methodology which for GSB has proven unreliable, probably because of the significant number of escapes using the current screening procedure. Progress in breeding GSB resistant cultivars will only be achieved when a reliable method to track resistance gene(s) is achieved.

Molecular markers have the potential for overcoming the limitations associated with traditional selection strategies, since they are non-destructive, eliminate the environmental variation associated with disease resistance and can be evaluated for multiple traits simultaneously.

However, the use of molecular markers require the development of breeding populations, which segregate for the trait of interest, and the trait must be properly identified during marker identification. It is extremely important that populations used for marker development be properly identified. Mistakes made phenotyping plants used for marker development will delay development and may cause potentially useful markers to be missed. This is an important consideration since many of the traits that would be most useful for marker development are traits that are highly influenced by the environment or have some other high degree of variability associated with them.

There are two important issues to consider regarding molecular markers: time and cost. Although molecular breeding and the development of molecular markers has great potential, it may take a significant amount of time to properly develop the marker and thoroughly test the marker in multiple populations. Along with this time is the expense of marker development, which is significant. The potential for molecular markers to save money is in their long-term utilization in combination with multiple markers for a wide variety of traits; this will allow breeders to select for multiple traits from large populations in a manner that is not currently possible.

Another aspect of molecular breeding approaches includes the use of genetically-modified organisms (GMO). Inserting a gene that does not naturally occur in cucurbits can sometimes have dramatic effects on crop performance. In fact, the second commercial GMO crop in the US was a cucurbit crop, and variations of this GMO crop are still on the market today (virus resistant squash), proving the success of this strategy. However, genetically-modified cucurbits as well as other vegetables (other than virus resistant squash) have thus far mostly been limited to research. The increased use of grafting in cucurbit crops does present an avenue where GMOs may impact this family of plants in the short-term. Rootstocks can be genetically engineered to resist a host of biotic and abiotic stresses and have the trait transferred to the scion through grafting rather than direct transformation. It remains to be seen whether public opinion will influence the development of GMO rootstocks.

3.4 Conclusion

Traditional genetics and plant breeding have made great strides in our understanding and improvement of cucurbit crops. We have used classical

genetics to enhance our understanding of taxonomy and phylogenetic relationships in the Cucurbitaceae; plant breeders have identified a number of genes associated with commercially important traits and used this information to create superior cultivars with greatly improved yield and quality. However, previous advances were typically through adding single traits with high heritability to adapted germplasm. Advancement of some traits using traditional techniques is difficult and time consuming since the complexity increases with each added trait. Molecular breeding offers an avenue to overcome many of the problems associated with traditional breeding and genetics. In fact, molecular breeding has already made contributions to our understanding of cucurbit genetics, and has been directly responsible for some of the improvements made in modern cucurbit cultivars.

Molecular breeding offers avenues for crop improvement not otherwise available to traditional approaches. Classical breeders have said, "Anything is possible using traditional approaches; it is just that the world is not large enough to hold the populations needed to find the variation required for some traits." Molecular breeding provides a tool to search for traits and combinations of traits that are otherwise not feasible using traditional approaches. As we move forward with molecular breeding in cucurbit crops, it is important that we understand the need to maintain traditional breeding programs, and that the skill set required for classical breeding is not lost. Developing molecular markers requires traditional populations with traits identified using traditional methods, at least in the initial stages. Also, the time and expense of molecular marker development is significant, and will often take time and money away from traditional breeding. If we want to fully exploit the potential for molecular breeding, it is imperative that we maintain a balance between molecular and traditional approaches. Chapter 4 of this book delves into the advances made in cucurbit breeding regardless of the breeding technique used.

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